◉Sheet ○Slides

Subject :	(G6PD)deficiency
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Glucose - 6 - Phosphate Dehydrogenase **(G6PD)**deficiency

SLIDE 1

G6PD deficiency is a common hereditary disease that results in hemolytic anemia & affects large number of people worldwide (200-400 millions) . There is a higher prevalence in the Middle east, S.E Asia & Mediterranean region. It's X-linked inheritance (that's why it's more common in males). There are many mutations (>400 mutations worldwide have been identified).Deficiency in this enzyme provides resistance to falciparum malaria (that's why populations in which malaria is common usually have higher prevalence of G6PD Deficiency because the individual who is G6PD deficient has an advantage in that he is more resistant to malaria).

SLIDE 2-3

Why do cells require G6PD ? Why do RBCs require G6PD ?
(that will result in hemolytic anemia if it is insufficient)

G6PD is important because diminished G6PD activity impairs the ability of the cell to form NADPH that is essential for the maintenance of the G-SH pool. G-SH(reduced glutathione) can chemically detoxify H2O2(one of the reactive oxygen species) in a reaction that is catalyzed by the selenium-containing glutathione peroxidase to form water and G-S-S-G (oxidized gluthathione) which no longer has protective properties unless it is reduced again. The reduction of oxidized gluthathione using NADPH in a reaction catalyzed by the enzyme glutathione reductase regenerates reduced gluthathione that can be used again to reduce hydrogen peroxide (H2O2) again. G-SH also helps maintain the reduced states of sulfhydryl groups in proteins, oxidation of those sulfhydryl groups leads to the formation of denatured proteins and rigidity of the cells.
So:
- Gluthathione is oxidized to GSSG (2 molecules joined together)
- Gluthathion reductase is used to regenerate the reduced Gluthathione (NADPH —> NADP+)
- Continuous supply of NADPH requires G6PD.

RBCs obtain NADPH only from pentose phosphate pathway, while in other adult type of cells/tissues, there are other sources for NADPH, but these sources depend on the presence of mitochondria(for e.g the pathway that uses NADP+ dependent malate dehydrogenase(malic enzyme)).RBCs do not have mitochondria and don't have alternative pathways to produce NAPDH, so they depend completely on pentose phosphate pathway to get NADPH (there are no other sources for the production of NADPH).

If there is oxidative stress (it occurs sometimes in the individual after taking some drugs or having infections), in these cases, the production of hydrogen peroxide (H_2O_2) will increase. And, as the production of peroxide (H_2O_2) increases, there will not be an adequate amount of NADPH to help detoxify H_2O_2.

- in other cases, when the production of peroxide is moderate or at a low level, even though the G6PD is partially deficient, it still can produce enough NADPH to take care of the H_2O_2.
- But, on the other hand, if the production of H_2O_2 greatly increases, G6PD that is producing NADPH will no longer be able to produce enough NADPH due to the deficiency.

To summarize this, most individuals who have inherited one of the G6PD mutations do not show clinical manifestations (they are asymptomatic), however some patients with G6PD deficiency develop hemolytic anemia if they are in oxidative stress situations, in certain rare mutations the individual has chronic hemolytic anemia.

So:

Why GSH is needed?

- it helps maintain the SH group in the protein (in reduced state). GSH is not only used to reduce the peroxide but also to maintain the SH group in the protein (in the RBC), otherwise proteins can react together and be oxidized (2 molecules that have SH together can combine, then oxidized. If they are oxidized, they will be denatured at the end). Oxidation of protein will cause denaturation of protein and it increase the rigidity of cells. It's important for the RBCs to be flexible to be able for them to squeeze & move around the blood vessels. If it is rigid, it will be destroyed.

Which group of RBCs will be destroyed?
The old ones. The new ones still have G6PD. The level of the enzyme (G6PD) decreases in the old RBCs. RBCs cannot regenerate its enzymes because there is an absence of nucleus, mitochondria and ribosomes. So, there is no protein synthesis in the cells. The molecule which is lost will not be replaced. The old RBC will suffer more due to deficiency in G6PD. The half life of RBCs is 120 days, so the old RBCs will be susceptible for hemolysis.

Precipitating Factors in G6PD Deficiency

They are the factors that cause Oxidative Stress & increase the production of peroxide (H_2O_2)

-RBC cannot take care of these oxidizing agents in some patients with G6PD deficiency resulting in hemolytic anemia.
These factors include:
-Oxidant drugs: they can be remembered by the mnemonic AAA which stands for

1. antibiotics (for example, sulfamethoxazole and chloramphenicol).
2. antimalarials (for example,primaquine).
3. antipyretics (for example, acetanilide but not acetaminophen).

-Favism: some forms of G6PD Deficiency (e.g the Mediterranean variant) are susceptible to hemolytic effect of fava bean consumption.
-Infection: the inflammatory response to infection results in generation of free radicals in macrophages, which can diffuse into the RBC and cause oxidative damage. Infection is the most common precipitating factor of hemolysis in G6PD Deficiency.

Note: Neonatal jaundice: a clinical manifestation of G6PD deficiency that typically results from increased production of unconjugated biliruben, it occurs in many infants but more cases of neonatal jaundice occur in those who are G6PD Deficient.

SLIDE 4
As we said previously, there are many variants of G6PD, and some of the variants include:

-The wild type is type B.
-The Mediterranean Variant (B-) . According to the classification, it is Class 2 (II)
What is the mutation?
Only one base (563) on the DNA is changed from C to T (C --> T) that leads to the problem in the enzyme.
-African Variant (A-) (classIII)
There are 2 point mutations (less severe than B-).
-African Variant (A)
It has normal activity (80%) but the activity will decrease with time.
-The very severe deficiency is in class I.

The majority of mutations that result in G6PD Deficiency are missence mutations or point mutations. Some mutations are large deletion of DNA (removed) or frameshift mutations, but these are not observed in G6PD deficient individuals and they give us a very abnormal enzyme that is not compatible with life.

G6PD deficiency/mutation occurs in all cells of the affected individual, but cells other than RBCs still produce NADPH though there is deficiency in G6PD through other reactions (alternatives).

SLIDE 5

There are 4 main classes for G6PD deficiency (I, II, III, IV)

- Class 1 is very severe. The residual enzyme activity is (< 2%). Affected individuals have chronic nonspherocytic hemolytic anemia.
- Class 2 is severe. The residual enzyme activity is (< 10%),the Mediterranean variant is in this class.
- Class 3 is moderate . The residual enzyme activity is (10 - 50%),the African A- variant is an example.
- Class 4 apparently is normal but the enzyme activity is low in the old RBC.

SLIDE 6 - Graph the activity of the enzyme G6PD against the age of erythrocyte (RBC)

This graph demonstrates that the activity of erythrocyte G6PD declines with aging of the cell.

In the normal enzyme (black line),the activity declines as the cells age, but despite that, the cells will have a sufficient level of activity even after the 120 days,at that time the enzyme still has an activity of about 40%, which is enough.

In African A- type (green line), at 50/60 days age of RBC, the activity is almost zero, but in this variant the activity in the young cells is almost normal(the activity is about 80%) and then it rapidly declines and this tells us that the enzyme in this case is not stable, and here we have a clinical application which is: if a patient came to you right after he had suffered hemolysis, and you suspected that he has G6PD Deficiency and you ordered a test for the activity of G6PD in his RBCs, the results might show that the activity is normal, but this doesn't necessarily mean that he is not G6PD Deficient because there is a possibility that what happened is that hemolysis destroyed his old RBCs (which are more prone to destruction) and the RBCs that are left are the young ones, which in the case of the A- variant have normal G6PD activity, so we have to do another test after a month ,for example, to know exactly whether he has a normal G6PD or G6PD A-.

In Mediterranean type (red line), the activity of enzyme decrease to zero only after 30 days, in this variant the activity starts low and then declines.

SLIDE 7

-NADPH is important in phagocytosis (a process of WBC to kill/ ingest the bacteria). The killing in phagocytosis can be oxygen-dependent or oxygen-independent.

Pathway of Microbial Killing by WBC

First, the immune system will recognize the bacterium and will produce antibodies (IgG) that will bind to the bacterium. After binding of the bacterium with the IgG, there will be binding of the bacterium(coated with IgG) to the receptor on phagocytic cells, which will lead to invagination/ phagocytosis. By invagination/ internalization of the membrane, phagosomes will be produced.The phagosomes will fuse with the lysosomes and become phagolysosomes.

How the destruction of bacterium occurs and what is the role of NADPH? There is an enzyme called NADPH oxidase. NADPH oxidase will reduce O2 into superoxide (free radical - an oxidizing agent). The enzyme superoxide dismutase can convert superoxide into Hydrogen peroxide (H2O2). Hydroxyl radical is the 3rd free radical which can be produced from H2O2 by a reaction called Fenton reaction that uses Fe+2.

H2O2 with chloride in the presence of the enzyme myeloperoxidase will produce hypochlorus acid (can kill the bacteria), H2O2 can also be converted to hydroxyl radicals that can kill the bacteria. Superoxide also can kill the bacteria. Superoxide radical, hydrochlorous acid and hydroxyl radical can kill the bacteria. Superoxides are toxic substances but they can be used in the cells to kill the foreign substance.

Respiratory Burst
-When NADPH oxidase is activated, the consumption of oxygen increases greatly in short amount of time and that will result in superoxide formation. This is referred to as the respiratory burst.

SLIDE 8 - NO & RNOS

- NO (nitric oxide) is considered a free radical. It can diffuse readily through the membrane. It's essential for life but it is toxic. It acts as a neurotransmitter in the brain and vasodilator in the smooth muscles in the blood vessels. It prevents platelet aggregation. It's a signal molecule (like hormones) that acts locally. It has very short life span (3-10 seconds).
- At high concentrations, it combines with O2 or superoxide to form RNOS (Reactive Nitrogen Oxygen Species)
- RNOS are involved in neurodegenerative diseases and inflammatory diseases.

SLIDE 9-10 - NO (Nitric Oxide) Synthesis

- Synthesis of NO is from Arginine, an amino acid, that is converted to Citruline by the enzyme NO Synthase using NADPH which is converted to NADP+. Citruline is an amino acid that is not found in our proteins. It can be converted again into Arginine.
- Arginine is a precursor for the NO, converting it to Citruline and requiring NADPH.

NO Synthase
- There are 3 isoforms of this enzyme that have been identified (nNOS, eNOS, iNOS)

nNOS(isoform I) & eNOS(isoform III)
-n is from neural, e from endothelial
-both of them are constitutive enzymes, meaning that they are always at a constant level
(enzymes which are produced on demand are inducible)

iNOS(isoform II)
-inducible (it is induced by signals/hormones)
-transcription of DNA of enzyme is induced by certain signals (hormones / signal molecules)
-in many cells, as the enzyme is stimulated, the level of the enzyme increases to kill the invading bacteria.

SLIDE 11 - Action Of NO on the Vascular Endothelium

- It acts as a muscle relaxant
- Endothelial cells synthesize the NO and it can act on the smooth muscle (endothelial of blood vessels)
- In the presence of NO in smooth muscle cells, a cytosolic form of the enzyme guanylate cyclase will convert GTP into cGMP (cyclic GMP). This will activate protein kinase G.
- Protein Kinase G will cause the phosphorylation of calcium channels. Once these channels are phosphorylated, the channels will be blocked ,causing decreased entry of Ca+2 into smooth muscle cells, so the calcium level will decrease in the cell. As a result, contraction of muscle decreases & relaxation occurs.
- cGMP is deactivated by converting it to GMP, by phosphodiesterase enzyme (called so due to the presence of phosphate with two esters).

SLIDE 12

- NO causes relaxation of smooth muscle, prevents platelet aggregation, functions as a neurotransmitter in the brain, mediates tumoricidal and bactericidal actions of macrophages.

SLIDE 13 - 15 (Metabolism of Alcohol)

- Ethanol can be considered as a fuel (energy stores). Ethanol can provide 7kcal/g.
- Ethanol will be metabolized mainly in the liver.
- The first step of alcohol metabolism is by alcohol dehydrogenase. It converts ethanol into acetaldehyde in the cytosol. Acetaldehyde is toxic because it can react with protein.
- The acetaldehyde is oxidized again in the mitochondria into acetate (acetic acid)
- Acetate is released into the blood, reaching the organs including muscles and it is converted into acetyl CoA (by enzyme Acetyl CoA synthetase).if the resulting Acetyl CoA is in the cytoplasm it will be used in cholesterol and fatty acid synthesis,but if it is in the mitochondria it will enter the TCA cycle.

Metabolism of Alcohol : Oxidation in the liver —> Oxidation in the Mitochondria —> Activation of Acetic Acid into Acetyl CoA.

-Ingestion of alcohol leads to high NADH/NAD+ ratio. As the level of NADH increases, lactate dehydrogenase will convert more pyruvate into lactate/lactic acid (there will be a shift in equilibrium). Lactic acidosis will occur, there will be inhibition of gluconeogenesis (that will cause hypoglycemia) and inhibition of fatty acid oxidation will occur.

Oxidation of Alcohol by Microsomal Oxidizing Systems (MEOS)
- about 10 to 20% of ingested ethanol is metabolized by this system
- it consumes NADPH and converts it into NADP+.

SLIDE 16

Cytochrome P450 (CYP2E1 - a subtype of P450)

- it is characterized by high Km for ethanol.
- Because of the high Km, only a little amount of ethanol is metabolized by it.
- If the level of ethanol is very high, metabolism will increase through this pathway.
- It is inducible by ethanol (it is increased in response to the ethanol).

- In chronic drinkers of alcohol, the activity of enzyme cytochrome P450 reductase is high.
- it converts NADPH into NADP+.

SLIDE 17 - Formation and Uses of Glucuronate

- Glucuronate / Glucuronic Acid is oxidized at carbon no 6.
- Glucuronate is an oxidized form of glucose in which the hydroxyl group on carbon no 6(C-OH) is oxidized to a carboxyl group(COO-)
- UDP-glucuronate is the activated sugar of glucuronate, it is produced by converting UDP-glucose to UDP-glucuronate in a reaction that is catalyzed by UDP-glucose dehydrogenase, which also produces NADH.

How can oxidation occur ?
When UDP-glucose is formed in glycogen metabolism, we can use it to form UDP-glucuronate.

- Glucuronic acid is more soluble than glucose (due to the presence of a carboxyl group). UDP-glucuronate is used in many pathways, mainly to form GAGs or other glycosylated molecules, and it is used to increase the solubility of other molecules (e.g: xenobiotics and drugs) to increase their excretion.

SLIDE 18 - Bilirubin Diglucuronide

- billirubin combines with glucuronates to increase its solubility(polarity)to increase its excretion.
- billirubin is insoluble, and it is toxic for the body.
- in newborn, there is jaundice because of the high production rate of bilirubin, and the activity of the enzyme that conjugates 2 glucuronate molecules to bilirubin producing bilirubin diglucuronide is low at birth.

SLIDE 19

UDP-Glucose Metabolism
- it can be converted into UDP-galactose by epimerization (then UDP-galactose can be used to make lactose)
- it can be used in producing polysaccharides such as proteoglycans, glycoproteins and glycolipids (proteoglycans are very soluble in the water)
-it can be converted to UDP-glucuronate.

-the glucose part of UDP-glucose can be added to the hydroxyl group of Ser,Thr or hydroxylysine residues in a protein (forming an O-glycosidic bond) or to an Asn residue(forming an N-glycosidic bond) in a reaction catalyzed by glycosyltransferase.

Break a leg !