Hematology – Biochemistry

(Numbering is according to the 6th set of slides in **Alra'ed**)

ONLY EXTRA NOTES

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- Generation of energy (ATP) is very critical for keeping the ionic balance (Na^+/K^+ pump) to avoid premature hemolysis and decrease in RBCs lifespan.

- Notice the pentose phosphate pathway - PPP (also known as the hexose monophosphate shunt - HMS pathway), 10% of glucose in the RBCs enters this pathway and that shows the significance of it. The first two irreversible steps of PPP that include the conversion of G6P into 6-Phosphoglucoronic acid (6-PG) by the enzyme G6PD and then conversion into ribose sugars by 6-PGD enzyme. These two reactions are exclusively responsible for the production of NADPH molecules in the RBCs, and those NADPH molecules are important for keeping glutathione reduced for anti-oxidant mechanisms.

- Notice the NADH molecules that result from the conversion of Glyceraldehyde-3-P into 1,3-Bisphosphoglycerate. This NADH is important for the function of cyt-b5 reductase enzyme that keeps ferrous reduced to avoid the production of methemoglobin. It's the only NADH used for anti-oxidant pathways because NADPH is the main agent used for this.

Slide 8

- The alternative pathways (like Krebs cycle) for NADPH regeneration are present in other cells, but unfortunately this is not the case in RBCs (e.g. NADP-dependent Malic enzyme which is a cytoplasmic enzyme is not present in RBCs). So, PPP is the only pathway responsible for regeneration of NADPH in RBCs.

Slide 6

- Reduced glutathione (GSH) detoxifies free radicals that are formed by oxidative stress (drugs, infections, food … etc).

Slide 5

- In the oxidation of ferrous into ferric (that naturally occurs in minor amounts), a superoxide ion is formed. This superoxide ion can be converted into hydrogen peroxide by superoxide dismutase enzyme, and the latter is then converted (by Fenton's reaction) into hydroxyl free radical. This hydroxyl radical is a very deadly compound that has huge damaging effects. To avoid all of this, we need those electron carriers (especially NADPH) to get rid of such dangerous radicals and prevent their premature hemolytic effects.

- ROS, if produced (as a result of oxidative stress), oxidize the SH- portion in Hb to create disulfide bonds that cause the Hb to precipitate and form Heinz-bodies. These aggregates destroy the membranes of RBCs.

- As we said earlier, we cannot use methylene blue dye to treat methemoglobin accumulation in patients with G6PD deficiency; basically because methylene blue uses NADPH as a source of electrons to reduce the ferric of methemoglobin into ferrous, and in G6PD deficiency, NADPH levels are already reduced.

Slide 7

- G6PD gene is located on X chromosome.

- Mediterranean areas also include Greece and parts of Italy.

- Clinical symptoms and severity depend upon the degree of G6PD deficiency.

Slide 4

- All oxidant drugs as well as other precipitating factors must be prophylactically prohibited because different people show different responses to each agent without a precise underlying cause.

- Infections are considered the biggest oxidative stress factor; they involve NADPH oxidase enzyme (in WBCs) activity that generates superoxide ion during respiratory burst, and that ion is converted into hydrogen peroxide > hydroxyl radical > destruction of antigens membranes and damage to some other normal WBCs.

- High incidents rates of neonatal jaundice are found in people with G6PD deficiency.

- In the old days, laboratories have been using simple biochemical tests (Km for enzymes for example) and differentiations in symptoms and clinical presentations between people with normal G6PD enzyme and others with deficient enzyme levels. So, we have definitely got huge numbers of different variants for the disease (about 400 variants) while being inaccurate at the same time. However, recent molecular biology techniques (DNA sequencing … etc) made it easier and helped in precisely determining the variants, so their numbers are decreased to about 160 variants.

- Almost all the mutations are missense ones with no large deletions/frameshifts. The reason is simply because such dangerous mutations may cause complete deficiency in the enzyme, and we cannot even survive with the absence of this very important G6PD (considered a housekeeping enzyme) to be able to pass such mutations to our offspring.

- Normal G6PD is given the symbol (B).

- Mediterranean variant is the most common (60-70% of deficiencies are of this variant) and it's named (B^-). The substitution involves the mentioned nucleotide and results in transformation of 188 Ser into Phe. This Phe is not much different than Ser in terms of charge (both are not ionized) so their migration in electrophoresis is similar. So the (B^-) variant is a deficiency but with properties similar to normal.

- A^- variant stands for (Africans/black Americans) because it's common there. It involves two variations; one with transformation of Asn into Asp that is negatively-charged, so it will migrate toward the anode faster (if this substitution happens alone, it's called A^+ because it only differs in electrophoresis without abnormal activity or clinical symptoms). The second substitution is Val > Met. Both substitutions if happen together cause deficiency in the enzyme.

Slide 3

- Oxidative stress makes any categorized variant happen with worse hemolytic effects.

- Some mutations without effect cause no harm at all (class IV).

- If an African (with A^- variant) patient comes to you suffering from hemolytic anemia, then his RBCs are suspected to be relatively new (because of the continuous hemolysis effect). If you measure the enzyme levels there, you will find them in relatively normal concentrations, so you will have to repeat the test after a period of time (1 month for example) in order to let the RBCs age, and after that you will find that the levels are drastically reduced and that he really suffers from G6PD deficiency.

Slide 2

- Every enzyme in glycolytic pathway has actually been reported to be deficient (Glucose-6-phosphate isomerase (PGI) constitutes 4% of cases). However, 95% of the cases are PK deficiency.

- A very accurate PK levels test is actually needed. This is due to the following reason: WBCs have 70x activity levels of this

PK enzyme more than RBCs, so any little contamination of RBCs with WBCs will absolutely give wrong results.

- This deficiency is responsible for decreasing ATP levels as well as RBCs lifespan. On the other hand, the increase in 2,3-BPG levels is basically because it lies prior to the deficient enzyme in the glycolytic pathway. This BPG acts as an allosteric regulator of Hb that causes a shift-to-right, and subsequently, decrease in its affinity for oxygen and increase in delivery, so this increase of oxygen delivery will partially overcome the anemia caused by ATP depletion.

Slide 1

- After substitution of Glu > val, a cleavage site is missing in sickle-cell beta-chain's gene. When we do cleavage by certain enzymes in the laboratory, we will definitely get an extra fragment of the sickle-cell gene because it's cleaved fewer times than the normal gene. Thus and after electrophoresis, this extra piece will cause the migration to be slower than normal.