Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Thalassemias

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Slide 1:
Hello, my name is Trefor Higgins. I am the Director of Clinical Chemistry at DynaLIFEDx a large reference laboratory located in Edmonton, Alberta, Canada. I am also a Clinical professor at the University of Alberta in Edmonton. Welcome to this Pearl of Laboratory Medicine on "Thalassemias."

Slide 2:
Hemoglobin consists of 2 α and 2 non α globin chains forming a shell around a heme molecule. In the most common hemoglobin, Hb A, the globin chains are α and β. Hb A forms about 80-90% of the total hemoglobin. In Hb F, the hemoglobin found in the fetus, the globin chains are α and γ, and in Hb A2, a minor component of the total hemoglobin in adults, the globin chains are α and δ. There is a lot of similarity in the amino acid sequence of the β, δ, and γ chains.

Slide 3:
Thalassemias arise from changes in the production of globin chains and are observed as quantitative changes in the hematology, whereas hemoglobinopathies arise from qualitative changes in the amino acid sequence of the globin chains. Together, hemoglobinopathies and thalassemias form the most common genetic disease in the world. Hemoglobinopathies are discussed in a separate Pearl.

The name, thalassemia, derives from the Greek word for sea and was named because the first described cases of thalassemia were found in individuals from countries surrounding the Mediterranean Sea.

There are alpha, beta, delta, gamma, and delta beta thalassemias. Alpha thalassemia is the most common thalassemia worldwide and results from deletions within the alpha globin gene, whereas beta thalassemia is a result of modifications within the beta globin gene.

Slide 4:
This cartoon shows that 2 α chains are coded on each chromosome 16 and deletions in one or both α globin genes lead to α thalassemia. There is a wide range of phenotypic expression due to the variety of deletions and these deletions can be cis (on the same gene) or trans (on different genes).

Alpha thalassemia due to cis deletions tends to be more pronounced than those caused by trans deletions. The β, δ, and γ globin chains are coded on chromosome 11 and there is 1 gene per chromosome. Mutations in the β globin gene lead to decreased production of β globin chains resulting in β thalassemia. There may also be an increase in gamma chain production leading to delta beta thalassemia.

Slide 5:

By convention, the thalassemias are named after the gene in which there is decreased chain production. Therefore, α thalassemia results from a decreased production of α chains, and β thalassemia results from decreased production of β globin chains. Delta beta thalassemia results from decreased production of beta and delta chains and an increased production of gamma chains. The terms minor, major, and intermedia may be included in the description of the thalassemia and this describes the severity of the thalassemia.

Slide 6:

Clinical symptoms associated with thalassemia range from asymptotic through to a condition that is incompatible with life. A thalassemia investigation may be initiated by unexplained microcytosis in the complete blood count (CBC) or the discovery of thalassemia in a family member. In some parts of the world, testing for thalassemia is part of premarital screening. The presence of polyhydraminous at about week 26 of pregnancy may lead to an investigation to determine if Hb Barts is present. A child presenting at a checkup with failure to thrive and elongation of the long bones may initiate a family study for beta thalassemia.

Slide 7:

The presence of a thalassemia is noted in the complete blood count. The mean cell volume (MCV) is ≤ 74 fL indicating that the red blood cells are smaller than usual. However, the Red Blood Count (RBC) indicates a high number of cells and the Red Cell Distribution Width (RDW) indicates that there is uniformity of the size of red blood cells. One could say that thalassemia is characterized by the presence of many uniformly small red blood cells. In some of the more clinically significant thalassemias, this triad of results may not be seen. The presence of iron deficiency should be ruled out by measuring serum ferritin. Iron deficiency may be mistaken for thalassemia and the Hb A2, necessary to diagnose β thalassemia, is decreased in iron deficiency. The Metzner Index is a useful calculation to distinguish thalassemia from iron deficiency, although it has some shortcomings.

Slide 8:

Although the previous slide gave some common characteristics of thalassemias, these do not all apply to delta beta thalassemia and delta thalassemia. In delta beta thalassemia, the RDW is elevated with values between 20 and 25 indicating mixed distribution of cell size. Delta thalassemia may show no changes in the CBC and may only be noticed as a small Hb A2 peak. Since the quantity of Hb A2 is normally low, its absence makes little or no impact on the CBC.

Slide 9:
PCR analysis is the preferred way to establish a diagnosis of alpha thalassemia in the presence of thalassemic indices in the complete blood count and a Hb A2 concentration within the reference interval. Although HPLC, electrophoresis, and capillary electrophoresis are not used to detect the presence of 1 and 2 alpha gene deletions, they may all be used to detect the 3 gene deletion, Hb H. Quantitative measurements of Hb A2 and Hb F by HPLC or capillary electrophoresis are used to establish the diagnosis of other thalassemias.

Slide 10:
In this slide, the concentrations of Hb A2 and Hb F seen in the different thalassemias are summarized. In patients without a thalassemia and patients with alpha thalassemia, the quantities for Hb A2 and Hb F are within the reference intervals. The British Journal of Hematology recommends that an Hb A2 concentration greater than 4% of the total hemoglobin is diagnostic of beta thalassemia minor and no further testing need be performed. In delta beta thalassemia, the Hb F may be raised to 30% and may be differentiated from hereditary persistence of fetal hemoglobin by the presence of thalassemic indices in the CBC. In delta thalassemia, the only unusual result noted will be a low concentration of Hb A2.

Slide 11:
In this slide, 4 HPLC chromatograms of various thalassemias are shown. The x axis is the retention time and the y axis is the percentage of hemoglobin. In the top left hand corner, a chromatogram from a patient without thalassemia is shown but it is important to note that a chromatogram from a patient with alpha thalassemia will appear the same. In the top right hand corner, a chromatogram showing an elevated peak for Hb A2, characteristic of beta thalassemia minor, is noted. In the bottom left hand corner, a chromatogram from a patient with delta beta thalassemia is seen, and finally, in the bottom right corner, a chromatogram from a patient with delta thalassemia is seen.

Slide 12 :
As seen in this atlas, α thalassemia is widely distributed covering the area around the Mediterranean, much of North Africa, the Middle East, India, and South East Asia including China. There is a difference in the phenotype/genotype causing the α thalassemia. In Europe, Africa, and the Middle East, alpha thalassemia due to deletions in the alpha 1 gene is common. Alpha thalassemia due to deletions in the alpha 2 gene is more common in South East Asia.

Slide 13:
Alpha thalassemia arise from deletions in one or more of the α globin genes.

The deletion of 4 α globin genes results in Hb Barts, a condition which is incompatible with life. Pregnant females carrying a fetus with Hb Barts present at about the 26th week of pregnancy with polyhydramnios. The inability of the fetus to produce α globin chains due to the deletion of all 4 α genes results in the inability to make Hb F, the normal fetal hemoglobin, and fetal oxygen carrying capacity is markedly reduced.

Deletion of 3 α globin genes leads to Hb H disease in which there is a moderate anemia, reduced red cell volume (MCV), and unlike other thalassemias, an increased RDW.

Deletion of 2 α globin genes on the same chromosome leads to cis α thalassemia, whereas deletions on different α globin genes leads to Trans α thalassemia. In both cases, the MCV is decreased, the RBC number is increased, and the MCV is within or close to the reference interval. Iron studies show the patient is iron replete. The possibility of both parents having a cis α thalassemia greatly increases the risk of a baby with Hb Barts, and many parts of the world screen prospective parents for cis α thalassemia.

Slide 14:
Beta thalassemia is distributed in areas surrounding the Mediterranean, the Middle East, and South East Asia.

Slide 15:
When both of the β globin genes are deleted, the resulting condition is described as β thalassemia major, β^0 thalassemia, or Cooley's anemia. The latter name comes from the physician who first described the condition in Italian immigrant children in New York. The major hemoglobin found in these individuals is Hb F. There is elongation of the long bones, pallor, and frontal bossing. Treatment with hydroxyurea may be implemented and if that is unsuccessful, transfusion therapy may be required.

β thalassemia minor is characterized by an Hb A2 level greater than 4.0% and thalassemic indices in the CBC with replete iron status. When both β genes are not completely functional, a condition known as β thalassemia intermedia or β^+ thalassemia is noted. In this condition, there is an increased amount of Hb F and a decreased amount of Hb A with changes of varying severity in the CBC.

Slide 16:
The situation where there is a coexisting thalassemia and a hemoglobin variant is called a compound hemoglobin variant. When an α thalassemia and hemoglobin variant coexist, the amount of hemoglobin variant is less than that expected as there is preferred formation of Hb A. For example, Hb S normally forms about 40% of the total hemoglobin in the heterozygous state but in the presence of α thalassemia, the amount of Hb S falls to less than 30%. When both a β thalassemia and hemoglobin variant is present, Hb A may be absent or present in significantly lower amounts than the hemoglobin variant.

Slide 17:
There is a hemoglobin variant, Hb Lepore, that has thalassemic indices in the CBC with an increased Hb A2 and may be mistaken for beta thalassemia. Hb Lepore co-elutes with Hb A2 on HPLC, giving a total Hb A2 area greater than 10% of the total hemoglobin.

Slide 18:
In conclusion, there are several different thalassemias and thalassemias are widely distributed and arise from decreased globin chain production due to mutations or deletions in the globin gene. The laboratory is crucial to identify thalassemias.

Slide 19: References

Slide 20: Disclosures

Slide 21: Thank You from www.TraineeCouncil.org
Thank you for joining me on this Pearl of Laboratory Medicine on "Thalassemias." My name is Trefor Higgins.