TITLE: Hemoglobinopathies

PRESENTER: Trefor Higgins

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 "Hemoglobinopathies."

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, and 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 hemoglobin component 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:
The majority of hemoglobinopathies arise from changes in the amino acid sequence of the either the α or β globin chains or both. This change in amino acid sequence may be due to the removal, addition, or substitution of a different amino acid, or a combination of these. Thalassemias are due to quantitative changes in globin chain production and are discussed in another pearl.

Slide 4:
To date, over 1000 hemoglobinopathies have been found but fortunately, only a few have clinical significance.

Although there is a formal system for naming hemoglobinopathies, most hemoglobinopathies are known by a letter or identifier from the location where the hemoglobin variant was found or a combination of these. Hb Edmonton was initially found in an individual living in Edmonton, Alberta, where I live. Hb G Coushatta is named after a First Nations tribe in Louisiana. Hb Lepore is unique in that it is the only hemoglobinopathy named after a family.

Clinical effects of hemoglobinopathies include altered oxygen affinity, resulting in changes in the distribution of oxygen to the peripheral cells. Some hemoglobinopathies are associated with methemoglobin, in which the iron in the heme molecule is in the ferric state rather than the ferrous state, resulting in decreased oxygen carrying capability leading to cyanosis and brown blood. Some hemoglobinopthies are unstable leading to a decrease in red cell survival and a CBC pattern resembling anemia. Others produce a hemoglobinopathy in which sickling or changing of the shape of the red blood cell can happen under certain circumstances.

Slide 5:
There are 4 globin genes that are involved in the production of alpha chains. When one of these becomes mutated, a hemoglobinopathy is produced which usually forms 25% or less of the total hemoglobin. Hb G Philadelphia, found in blacks, is an example of an alpha chain variant. There are 2 beta globin genes and so a mutation in one of these produces a beta chain hemoglobin variant comprising of more than 25% but less than 50% of the total hemoglobin. Hb S is the typical beta chain variant. Due to a problem in messenger RNA transcription, the beta chain variant, Hb E, forms less than is usual for a beta chain variant. Sometimes, both beta chains are identically mutated, resulting in a homozygous hemoglobinopathy. Sickle cell disease, in which both beta chains are mutated to Hb S, is the most common. There is a small but significant hemoglobinopathy of the delta chain, called Hb A2', which is found almost exclusively in blacks.

Slide 6:
Complex hemoglobinopathies arise when there is a thalassemia alongside the hemoglobinopathy or there are changes in both the alpha and beta chains. In blacks, the combination of the alpha chain Hb G Philadelphia with the beta chain Hb S is not uncommon. A combination of alpha thalassemia and beta chain hemoglobinopathy usually leads to a decreased amount of beta chain variant found. For example, the combination of alpha thalassemia trait with Hb S results in the percentage of Hb S decreasing from around 40% to less than 30%. The combination of beta thalassemia and a beta chain variant usually leads to decreased formation of Hb A and an increase in production of Hb F.

Slide 7:
Hemoglobinopathies are found in the quantitation of Hb A1c by HPLC or capillary electrophoresis or as part of a clinical investigation as to the reason for sickle, boat, or target cells in the peripheral blood film. A hemoglobinopathy investigation may be initiated in the presence of unexplained microcytosis in an iron replete person. Family studies and genetic counselling for the investigation of hemoglobinopathies may be initiated by the finding of a hemoglobin variant in a family member. Neonatal screening for hemoglobinopathies is mandatory in many states in the United States and has recently been initiated in some European countries.

In Canada, it is required that immigrants from parts of the world with a high incidence of hemoglobinopathies be tested on arrival in Canada for hemoglobinopathies.

Hemoglobinopathy studies may be used in anthropological studies to evaluate migratory patterns.

Slide 8:
Methods for identifying hemoglobinopathies fall into 2 groups. The first group, presumptive methods, is used by many laboratories to identify the common hemoglobinopathies Hb S, C, D, and E. The British Hematology Society recommends that 2 presumptive methods, based on different analytical principles, should be used to establish a presumptive identification of a hemoglobinopathy. Definitive methods are used when presumptive methods fail to identify the hemoglobinopathy or a new hemoglobinopathy is found. LC-MS/MS has become the method of choice for identifying new hemoglobinopathies.

Slide 9:
In this table, the commonly used methods for the presumptive identification of hemoglobinopathies are summarized. Separation of hemoglobins is based on the differences in charge or column affinity of the different hemoglobin variants due to different amino acid sequences. Electrophoresis uses a lysed sample produced by treatment of the red blood cells with a hemolyzing agent or water HPLC and Capillary electrophoresis use whole blood samples. All have challenges in separating every hemoglobin variant, and electrophoresis has the additional disadvantage that Hb A2 and Hb F cannot be quantitated.

Slide 10:
Electrophoresis at alkaline pH (blue) and acid pH (purple) are the most commonly used methods to identify hemoglobins. In the top lane, a control containing Hb S, A, C,and F is seen. The lanes are labeled at the bottom of the slide with the common hemoglobin variant that is found at that migration position. Some hemoglobin variants like Hb E, shown in the second position from the top, migrate to a C position on alkaline pH but moves to the A position on acid electrophoresis. In lane 3, Hb D Punjab moves in the S position on alkaline electrophoresis but on acid electrophoresis it moves to the A position. This movement helps in the identification of a hemoglobin variant. Lane 6 (J trait) shows a fast moving hemoglobin I since it moves closer to the anode than A. Hemoglobins J and I are examples of fast moving hemoglobins. Heterozygous Hb S shows bands in the A and S positions on both alkaline and acid pH electrophoresis. Homozygous Hb S shows a single band in the S position in electrophoresis at both alaline and acid pH.

Slide 11:
Based on the amino acid sequence, hemoglobinopathies have different affinities for the column used in HPLC leading to the separation of the hemoglobins. This slide shows a combination of chromatograms of commonly found hemoglobin variants. The retention time is shown on the x axis and the percentage of hemoglobin is shown on the y axis. In the bottom right hand corner, a chromatogram is shown for a person without a hemoglobin variant. The dominant hemoglobin is Hb A, with Hb A2 and Hb F forming a small percentage of the total hemoglobin. In the top row of this slide, moving left to right, we have S trait, C trait, and SC disease. In the chromatogram for SC disease, there is no hemoglobin A. On the second row Hb E, Hb D Punjab are shown. One of the challenges with this method is the co-elution of Hb E with Hb A2.

Slide 12 :
Hemoglobin S (Hb S) is the most widely distributed hemoglobin variant in the world. It is widely distributed in central Africa, North and South America, the Middle East, and into Indonesia. There are many different sub-types of Hb S.

Slide 13:
On the left hand part of the slide, many classic S shaped sickle cells can be seen. A good example is located just above the label on the left hand side of the slide. The commonly used S solubility test is shown on the right hand side of this slide. The lack of transparency, as shown in the right hand tube, indicates the presence of Hb S. Other hemoglobinopathies can produce this cloudy effect, and the cloudy effect may not be seen when the hemoglobin concentration is low, despite the presence of Hb S.

Slide 14:
In this slide, an HPLC chromatogram and electrophoresis of a homozygous Hb S is shown. The HPLC shows a large band of Hb S with some Hb F and no Hb A. There is false slight increase in the Hb A2 due to co-elution of Hb S adducts with Hb A2. On the electrophoresis, the homozygous Hb S case is shown in the last lane of both electrophoresis gels and shows a single band in the S position.

Slide 15:
There are two treatment modalities for sickle cell disease. The first, shown in the left hand part of the slide, shows increased Hb F due to the action of the drug hydroxyurea. The right hand side shows a chromatogram from an individual receiving transfusion therapy.

Slide 16:
In conclusion, hemoglobinopathies (together with thalassemias) are the most commonly found genetic diseases in the world. There are a wide variety of hemoglobin variants but only a few have clinical significance.

Slide 17: References

Slide 18: Disclosures

Slide 19: Thank You from www.TraineeCouncil.org
Thank you for joining me on this Pearl of Laboratory Medicine on "Hemoglobinopathies." My name is Trefor Higgins.