Courses

Course-01

Hemoglobınopathies

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Hereditary hemoglobin disorders with thalassemia and sickle-cell anemia are the most common monogenic diseases in the world. It is estimated that about 1-5% of the global population is the carriers of a genetic thalassemia mutation. Hemoglobinopathies are among the most common hereditary blood diseases also in Turkey and are an important health problem especially in the southern and western part of Turkey. The beta thalassemia carrier frequency in Turkey is 2.1% and there are about 1.3 million carriers and around 4.500 patients. When the frequency of carriers is considered, 300-400 sick children are estimated to be born each year, which causes financial and emotional harm to the families and society. Even though Cooley and Lee described the pathophysiology of thalassemia syndromes 90 years ago, the management of these diseases is still complex and requires a gradual process. The high frequency of hereditary hemoglobin variants in some regions may reflect the heterozygous resistance to malaria and with further studies, the resistance was determined to be in alpha, beta thalassemia and hemoglobin E diseases.

Patients with β-thalassemia were typically categorized as minor, major or intermedia based on α-globin or β-globin chain disorders, severity of anemia, and the clinical presentation. Over 400 mutations in the β-globin gene causing the disease ranged from the silent (silent β) ones to the mild mutations leading to a relative reduction in β-globin chain production (β +) and severe mutations. The complete absence of β-globin chain synthesis (β 0), deletion of the gene is rare. β-Thalassemia minor (trait or carrier) represents the heterozygous inheritance of a β-thalassemia mutation. Patients usually have (asymptomatic) microcytic anemia clinically, but it can be stated that the others (silent carriers) have the unidentified hematological abnormalities. Whereas the patients with β-thalassemia major usually present with severe anemia in infancy and can be dependent on transfusion for life, the patients with β-thalassemia intermediate may develop with mild-moderate anemia later and require variable transfusion. Both β-thalassemia major and the intermedia may result from the homozygous or compound-heterozygous inheritance of the mutations in the β-globin gene.

There are two main forms of α-Thalassemia: α + -thalassaemia and α0-thalassemia. Their classifications depend on, by mutation, the deletion of one or both of the α-globin genes and whether its activity diminishes or not. Two common α + -thalassemia forms are called –α 3 · 7 and –α 4 · 2 to define the lengths of the underlying deletions. α + - Thalassemia has various forms caused by point mutations. The most common one is α- CS α which is caused by the chain-termination mutation hemoglobin Constant Spring.

Abnormal hemoglobin variants are also an important health problem for Turkey. Sickle cell anemia (Hemoglobin S) is caused by the replacement of glutamic acid at the 6th position in the beta globin chain with valine (β6 Glu→Val). While the frequency was between 0.37% and 0.6% across Turkey, this frequency was determined to be between 3% and 44% in some regions especially in Cukurova. The term "sickle cell anemia" is used for the patients who carry the sickle cell hemoglobin in genetically homozygous state. The clinical presentation comprised of the findings seen in the people who carry the sickle cell hemoglobin in homozygous or heterozygous state or who carry it with other hemoglobins is called "sickle cell syndromes." Inheritance of HbS from one parent and another hemoglobinopathy (for example beta thalassemia or HbC) from the other parent can result in many sickle cell syndromes. The most common of these syndromes are HbSC disease, HbS/Beta thalassemia, HbS/DPunjab, HbS/OArab, and HbS/E. The clinical findings vary based on the accompanying mutation.

Now, the programs for screening and prevention of thalassemia are widespread, their acceptance depends on the regional distribution and cultural factors. Various screening programs and premarital screening are carried out nationwide. These screening programs are carried out in line with the recommended algorithms using the complete blood count and electrophoresis or HPLC methods. The DNA analysis is required to confirm the diagnosis of thalassemia and hemoglobin variant. In Turkey, in our central laboratories where the prenatal diagnosis is performed, the final diagnosis can be established using the advanced molecular methods such as ARMS, RFLP, Gene Sequencing (classical/new generation).

References

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Course-02

What is LC-MS/MS ? What not?

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Standard techniques of analyte detection in clinical chemistry rely on indirect characteristics of an analyte, e.g. its absorption of light, chemical reactivity or physical interaction with macro-molecules. In mass spectrometric methods, in contrast, analytes are detected directly from molecular characteristics as molecular mass and molecular disintegration patterns.

Beginning in the 1970s, gas chromatography-mass spectrometry (GC-MS) instrumentation has evolved to an essential tool for both biomedical research and some important routine clinical chemistry applications.

This technology seems to be of similar novelty for clinical chemistry as immunoassays in the 1970s and PCR in the 1980. The use of HPLC-MS/MS systems in bio-analytical research started already in the late 1980s. Tandem mass spectrometry found its way into clinical laboratories in the early 1990s when first flow injection analysis (FIA)-MS/MS methods were introduced for neonatal screening assays. First implementations in clinical routine laboratories started about a decade ago with realizing the first therapeutic drug monitoring (TDM) assay.

HPLC-MS/MS systems are expensive analytical tools. Investments ranging from 300.000 € to 500.000 € have to be expected to purchase systems suitable for above mentioned routine applications in clinical laboratories. This roughly corresponds to twenty ultrasounds instruments, ten intensive care respirators, of half a modern CT scanner and exceeds the costs for immunoanalyzer systems wide spread in routine laboratories.HPLC-MS/MS systems require substantial hands on time of skilled technicians-typically one instrument occupies one person fully.

It is optimistic but not unrealistic to expect that the typical lifetime of a HPLC-MS/MS is about 10 years. Given a degree of utilization which is