Introduction

Thalassemias are a group of Hemoglobinopathy caused by genetic mutations of hemoglobin genes, resulting in reduced production or total absence of one or more globin chains ***(Yang et al., 2009).***

B-Thalassemia major is commonly caused by homozygous deletion of the β-globin chain gene. It is clinically characterized by lifelong severe hemolytic anemia that eventually affects many organs and is associated with high morbidity and mortality.

B-Thalassemia minor (or β-thalassemia trait) is the heterozygous form. Most patients are asymptomatic, and some patients have only mild anemia ***(Benz, 2008).***

B-thalassemia trait often shows microcytosis, a normal or an increased RBC count, and an elevated level of HbA2, which provides the basis for laboratory screening ***(Benz, 2008).***

HbA2 is a normal hemoglobin variant consisting of 2 α chains and 2 δ chains. δ and β chains have identical sequences in all but 10 of the 146 amino acids. HbA2 has a function similar to that of HbA. The percentage of HbA2 varies depending on the assay but generally is in the range of 1.5% to 3.5 % ***(Yang et al., 2009).***

The majority of patients with β-thalassemia trait show elevated HbA2 levels, and some authors have used an HbA2 level of more than 4% to diagnose β-thalassemia trait ***(Sirichotiyakul et al., 2005).***

Beta thalassemia trait is an important differential diagnosis of iron deficiency anemia (IDA), In addition, the mild anemia in β-thalassemia trait can be aggravated during pregnancy, and the incidence of intrauterine growth restriction and oligohydramnios has been reported to increase in patients with β-thalassemia trait ***(Sheiner et al., 2004).***

Although patients with β-thalassemia trait do not usually have increased morbidity and mortality, they have a significant risk of having a child with the devastating β-thalassemia disease ***(Yang et al., 2009).***

The frequency of the β-thalassemia gene is population-dependent.

B-Thalassemia is prevalent in a broad belt extending from the Mediterranean basin to Southeast Asia. It is estimated that 1.5% of the world's population carries β-thalassemia, i.e., at least 80 to 90 million people with an estimated 60,000 new carriers born each year ***(Rathod et al., 2007).***

Aim of the Work

The aim of this work is to evaluate the prevalence of B-thalassemia trait in healthy adult blood donors and differentiating it from iron deficiency anemia.

Chapter (I): B Thalassemia

Historical Considerations:

The thalassemias are inherited disorders of Hb synthesis their clinical severity widely varies, ranging from asymptomatic to severe or even fatal entities. The name Mediterranean anemia which Whipple introduced is misleading because the condition can be found in any part of the world ***(Galanello and Origa, 2010).***

In 1925, Thomas Cooley, a Detroit pediatrician, described a severe type of anemia in children of Italian origin. He noted abundant nucleated red blood cells (RBCs) in the peripheral blood, which he initially thought as erythroblastic anemia, an entity that Von Jaksh described earlier.

Although Cooley was aware of the genetic nature of the disorder, he failed to investigate the apparently healthy parents of the affected children. In Europe, Riette described Italian children with unexplained mild hypochromic and microcytic anemia ***(Kuypers, 2008).***

In addition, Wintrobe and Coworkers in the United States reported a mild anemia in both parents of a child with Cooley anemia, this anemia was similar to the one that Riette described in Italy. Only then was Coolys severe anemia recognized as the homozygous form of the mild hypochromic and microcytic

anemia that Riette and Wintrobe described. This severe form was then labeled as thalassemia major and the mild form as thalassemia minor **(Yaish, 2009).**

The first description in Egypt was by Professor El Diwany in 1944 ***(Habib and Book, 1982).***

Disease name and synonymous:

The term thalassemia is derived from the Greek, thalassa (sea) and haima (blood). B-thalassemia includes three main forms: thalassemia major, variably referred to as Cooley's anemia and Mediterranean anemia. Thalassemia intermedia and thalassemia minor also called β-thalassemia carrier, B-thalassemia trait, or heterozygous β-thalassemia.

Apart from the rare dominant forms, thalassemia major is homozygotes or compound heterozygotes for β0 or β^+ genes.

Subjects with thalassemia intermedia are mostly homozygotes or compound heterozygotes and subjects with thalassemia minor are mostly heterozygotes ***(Galanello and Origa, 2010).***

Classification:

The β-thalassemia can be classified at different levels.

Clinically

It is useful to divide them into three groups: the severe transfusion-dependent (major) varieties; the symptom less carrier states; and a group of intermediate severity that fall under the loose heading "thalassemia intermedia". This classification is retained because it has implications for both diagnosis and management.

Genetic level

Thalassemia can also be classified at the genetic level intoβ^0-thalassemia where no globin chain is synthesized at all, and hence they are called β^0-thalassemia, whereas in others some globin chain is produced but at a reduced rate; these are designated β^+thalassemia.

Because the β thalassemia occurs in populations in which structural hemoglobin variants are also common, it is not unusual to inherit a β thalassemia gene from one parent and a gene for a structural variant from the other. Furthermore, since both α and β-thalassemia occur commonly in some countries, individuals may receive gene for both types. All these different interactions produce an extremely complex and clinically diverse family of genetic disorders, which range in severity from death in utero to extremely mild, symptomless, hypochromic anemias ***(Higgs and Weatherall, 2009)***

Epidemiology:

Prevalent in Mediterranean countries, The Middle East, Central Asia, India, Southern China, and The Far East as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%), and Southeast Asia ***(Wetherall and clegg, 2001).***

Population migration and intermarriage between different ethnic groups has introduced thalassemia in almost every country of the world, including Northern Europe where thalassemia was previously absent. It has been estimated that about 1.5% of the global population (80 to 90 million people) are carriers for β thalassemia, with about 60,000 symptomatic individuals born annually. The total annual incidence of symptomatic individuals is estimated at 1 in 100,000 throughout the world and 1 in 10,000 people in the European Union. However, accurate data on carrier rates in many populations eg: Egypt is lacking ***(Vichinsky, 2005).***

Genetic aspect of β-thalassemia:

The beta-thalassemias are inherited in an autosomal recessive manner. The parents of an affected child are obligate heterozygotes and carry a single copy of a disease-causing beta globin gene mutation.

At conception, each child of heterozygotes parents has 25% chance of being affected, 50% chance of being an

asymptomatic carrier, and 25% chance of being unaffected and not carrier ***(Cao and Galanello, 2010).***

Pathophysiology of β thalassemia:

1) Anemia

Normal adult Hb formed of 2α and 2 β globin chains and heme. In β-thalassemia the underlying genetic defect is responsible for the inability of the erythroid cells to synthesize adequate amounts of β globin chains. This causes excessive accumulation of free α chain since there is no complementary β chain to form a tetramer.

The unbound α chains precipitate within erythroblasts and red cells. These α chain inclusions damage the cell membrane leading to lysis of erythroblasts and red cells in the bone marrow (ineffective erythropoiesis).

The red cells containing α chain aggregates have reduced flexibility and are trapped in the spleen. Removal of the inclusions by the splenic macrophages damages the red cell membranes; such red cells are ultimately destroyed by the macrophages in the spleen and liver. Thus, in addition to intramedullary destruction, red cells are also destroyed peripherally in the spleen (extra medullary hemolysis) ***(Kazazian, 2002).***

Reduced synthesis of hemoglobin due to lack of β globin production leads to the formation of microcytic hypochromic cells. Excessive peripheral destruction of peripheral red cells invariably leads to splenomegaly. Pooling of considerable proportion of red cells within large spleen further aggravates the anemia.

HbF is the predominant Hb in β-thalassemia major and is due to increased proliferation of cells capable of synthesizing γ chains. HbF does not release oxygen as readily to the tissues as HbA since it poorly binds to 2, 3 DPG and thus exacerbates tissue hypoxia ***(Kazazian, 2002).***

2) Skeletal changes:

Severe anemia and tissue hypoxia stimulate erythroboietic drive and cause extreme bone marrow hyperplasia. Expansion of hyperactive bone marrow causes weakening and deformities of skull and facial bones. Thinning of cortex lead to pathological fractures ***(Dhaliwal et al., 2004).***

3) Iron overload:

Iron absorption from the intestine is increased in thalassemia major due to ineffective erythropoiesis. Chronic regular blood transfusion therapy markedly increases the iron accumulation and causes iron overload ***(Angelucci et al., 2008).***

Mechanism of iron overload:

At the end of their life span, transfused red cells are phagocytosed by reticuloendothelial macrophages in the liver, bone marrow, and spleen.

Their hemoglobin is digested, and the iron is freed from heme and released into the cytosol. Early in the course of long-term transfusion, most of this additional iron can be stored within reticuloendothelial macrophages. Gradually, limits on the capacity of macrophages to retain iron result in the release of excess iron into plasma.

Transferrin binds the released iron, with an increase in the plasma iron concentration and transferrin saturation. As the transferrin saturation increases, hepatocytes are recruited to serve as storage sites for the excess iron.

With continued transfusion, macrophages and hepatocytes can no longer retain all the surplus iron. Iron then enters plasma in amounts that exceed the transport capacity of circulating transferrin. As a consequence, non-transferrin-bound iron appears in the plasma as a heterogeneous assortment of iron complexes that appear to be the major mediators of extrahepatic tissue damage in transfusional iron overload.

Non–transferrin-bound plasma iron enters specific cells, particularly hepatocytes, cardiomyocytes, anterior pituitary

cells, and pancreatic beta-cells. In these cells, iron accumulation leads to the generation of reactive oxygen species, resulting in damage to lipids, proteins, DNA, and subcellular organelles, including lysosomes and mitochondria. This injury may result in cellular dysfunction, apoptosis, and necrosis.

Therapy with chelating agents that form a complex with iron and promote its excretion can clear plasma non–transferrin-bound iron, remove excess iron from cells, and maintain or return body iron to safe levels ***(Gary and Brittenham 2011).***

Investigations of β-thalassemia:

A) Beta thalassemia major (BTM)

1) CBC:

- Profound hypochromic, microcytic anemia accompanied by bizarre red cell morphology is a hallmark of BTM ***(Forget 2000).***

- The hemoglobin level may be as low as 3 to 4 g/dL. Red cell morphology is dramatically abnormal in most patients, with extreme hypochromia and poikilocytosis, a predominance of microcytes, tear drop and target cells and the visibility of clumped inclusion bodies representing precipitates of alpha globin within the red cell. These precipitates (Heinz bodies) can be more readily

appreciated by staining with methyl violet or other supravital stains.

- The white blood cell (WBC) count is often strikingly high. The high white count may be misleading, since these patients release many nucleated red blood cells (NRBC) into the peripheral blood. Depending on the counting method used, NRBC can be miscounted as leukocytes. However, even when corrected for this phenomenon, a true neutrophilia is often encountered.

- The reticulocyte count surprisingly low reflecting the severe degree of ineffective erythropoiesis underlying the disorder, resulting in many fewer than the expected number of reticulocytes being released from the bone marrow.

- The platelet count is usually normal. However, hypersplenism can lower both white cell and platelet counts. Splenectomy usually produces exaggerated rises in circulating NRBC, WBC, and platelets in the peripheral blood.

- High RDW reflecting severe degree of anisicytosis ***(Martin and Thompson 2013).***

2) **Qualitative and quantitative Hb analysis** (by cellulose acetate electrophoresis or HPLC) identifies the amount and type of Hb present.

The Hb pattern in beta-thalassemia varies according to beta-thalassemia type. **In beta^0 thalassemia**, homozygotes HbA is absent and HbF constitutes the 92-95% of the total Hb**. In beta+ thalassemia homozygotes and beta+/beta^0 genetic compounds** HbA levels are between 10 and 30% and HbF between 70-90%. HbA2 is variable in beta thalassemia homozygotes and it is enhanced in beta thalassemia minor.

Hb electrophoresis and HPLC also detect other hemoglobinopathies (S, C, E) that may interact with beta-thalassemia ***(Galanello and Origa, 2010).***

3) **Iron studies**

- Because of the high rate of erythroid cell turnover, the serum iron level is usually elevated.

- The transferrin saturation, expressed as the ratio of serum iron to total iron binding capacity (or transferrin), is very high ***(Forget, 2000).***

- Serum ferritin levels in those with thalassemia major may be quite elevated, reflecting the presence of iron overload primarily from multiple blood transfusions, but to a lesser extent from increased absorption of dietary iron from the gastrointestinal tract ***(Danjou et al., 2012).***

4) Other laboratory studies:

The serum is often icteric; increased concentrations of indirect (unconjugated) bilirubin and lactate dehydrogenase, and low levels of haptoglobin, findings typical of hemolytic disease, are usually present ***(Danjou et al., 2012).***

5) Vitamin and mineral levels:

Vitamin and mineral levels relevant to bone marrow homeostasis, such as folate, vitamin B12, and pyridoxine, are usually normal.

- Folate deficiency can develop in these patients, due to the high rate of cellular turnover.
- Serum zinc levels tend to be particularly low in these patients, likely due to increased requirements for this essential element and/or increased excretion subsequent to the use of iron chelating agents (***Erdoğan 2013).***

6) Bone marrow examination

Bone marrow examination reveals profound erythroid hyperplasia that is unusual for the degree of immaturity and bizarre morphology of the erythroid progenitors. Early erythroblasts are abundant, and often appear megaloblastic, likely reflecting limited supplies of folate and other nutrients. Later erythroid progenitors are less abundant than expected, due

to their intramedullary destruction (ie, ineffective erythropoiesis), Alpha globin inclusions are readily apparent, particularly if supravital dyes are used ***(Dragean et al., 2011).***

7) Molecular Genetic Analysis

Commonly occurring mutations of the beta globin gene are detected by PCR-based procedures. The most commonly used methods are reverse dot blot analysis or primer-specific amplification, with a set of probes or primers complementary to the most common mutations in the population from which the affected individual originated.

If targeted mutation analysis fails to detect the mutation, beta globin gene sequence analysis can be used to detect mutations in the beta globin gene ***(Vrettou et al., 2003).***

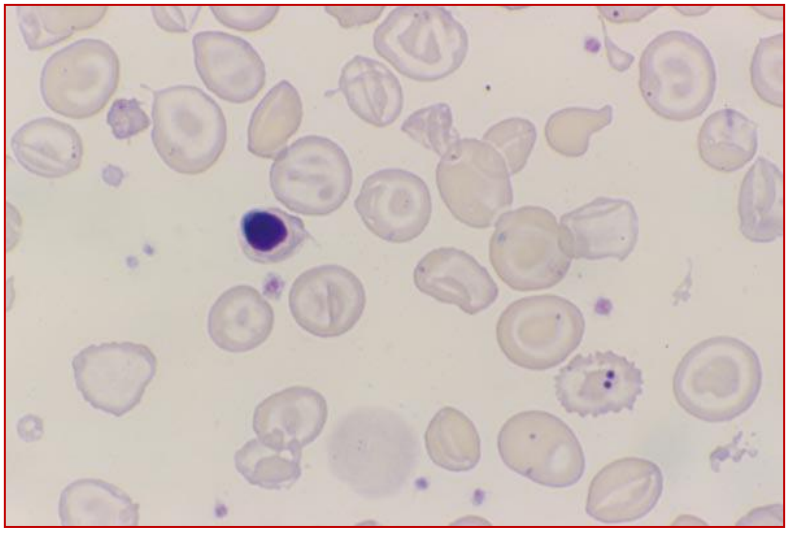

Fig. (1): Thalassemia major (Cooley's anemia). Note the bizarre erythrocyte morphologyand the nucleated erythrocyte ***(Drew provan 2007)***.