INTRODUCTION

Thalassemia syndromes are group of disorders, each resulting from an inherited defect in rate of synthesis of one or more of globins chains *(**Weatherall, 2001**)*. This disease remains a big threat to a substantial number of patients, most of them remain untreated, resulting in early death of them due to complications of the disease *(**Olivieri and Birttenham**, **1997**)*.

Thalassemia patients are prone to hepatitis C virus (HCV) infection caused by multiple transfusions ***(Ansar and Kooloobandi, 2002**)*.

Antiphospholipid antibodies (aPLAbs) are auto antibodies directed against anionic phospholipids or protein-phospholipid complexes which includes anticardiolipin antibodies (aCLAbs), and lupus anticoagulant (LA) ***(Kashef et al.,2008),*** known to have strong association with thrombosis, recurrent fetal loss, and thrombocytopenia, have been found in HCV infections with an increased risk of thromboembolic event ***(Levine, et al., 2002).***

An increased incidence of LA and IgG ACA as apart of a hypercoagulable state *(**Naithani et al.,***

2006), is found in polytransfused patients with beta-thalassemia major , leading to thromboembolic events when compared to the normal healthy population, but their clinical significance is yet not clearly understood ***(Sharma et al., 2006).***

AIM OF THE WORK

To investigate, the association of anticardiolipin antibodies (aCLAbs), lupus anticoagulant (LA) and their relation with HCV infection in Egyptian thalassemic patients.

THALASSEMIA

In 1925, Thomas Cooley and Pearl Lee described a form of severe anemia, occurring in children of Italian origin and associated with splenomegaly and characteristic bone changes. Over the next decade, a milder form was described independently by several Italian investigators. Because all early cases were reported in children of Mediterranean origin, the disease was later termed thalassemia, from the Greek word thalassa for sea and haima for blood ***(Whipple and Bradford, 1936).***

Definition

Thalassemia syndromes are a group of hereditary blood disorders characterized by reduced synthesis of one of the two types of polypeptide chains(alpha or beta) that form the normal adult human haemoglobin molecule(HbA,α2β2) resulting in reduced Hb in red blood cells (RBC), decreased RBC production and anemia. Depending on which of the genes the defect occurs and the corresponding effecton the production of globin chains, alpha thalassemia or beta thalassemia results. Most thalassemias are inherited as recessive traits ***(Thein, 2005).***

*Beta-thalassemia

Disease name and synonyms:

Beta-thalassemia includes three main forms: Thalassemia Major variably referred to as "Cooley's Anemia" and "Mediterranean Anemia", Thalassemia Intermedia, and Thalassemia Minor also called "beta-thalassemia carrier", "beta-thalassemia trait" or "heterozygous beta-thalassemia". Apart from the rare dominant forms, subjects with thalassemia major are homozygotes or compound heterozygotes for beta0 or beta+ genes, subjects with thalassemia intermedia are mostly homozygotes or compound heterozygotes and subjects with thalassemia minor are mostly heterozygotes ***(Renzo and Raffaella, 2010).***

Beta-thalassemias can be classified into:

- Beta-thalassemia
    - Thalassemia major
    - Thalassemia intermedia
    - Thalassemia minor
- Beta-thalassemia with associated Hb anomalies
    - HbC/Beta-thalassemia
    - HbE/Beta-thalassemia
    - HbS/Beta-thalassemia (clinical condition more similar to sickle cell disease than to thalassemia major or intermedia)

- Hereditary persistence of fetal Hb and beta-thalassemia
- Autosomal dominant forms
- Beta-thalassemia associated with other manifestations
  - Beta-thalassemia-tricothiodystrophy
  - X-linked thrombocytopenia with thalassemia

Mode of Inheritance:

The β-thalassemias are inherited in an autosomal recessive manner

Epidemiology

Beta-thalassemia is 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 ***(Flint et., al 1998)***. The high gene frequency of beta-thalassemia in these regions is most likely related to the selective pressure from Plasmodium falciparum malaria. 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 of beta-thalassemia, with about 60,000 symptomatic individuals born annually, the great majority in the developing world. 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 are lacking, particularly in areas of the world known or expected to be heavily affected ***(Vichinsky, 2005).*** According to Thalassemia International Federation, only about 200,000 patients with thalassemia major are alive and registered as receiving regular treatment around the world ***(Thalassemia International Federation, 2008).*** The most common combination of beta-thalassemia with abnormal Hb or structural Hb variant with thalassemic properties is HbE/beta-thalassemia which is most prevalent in Southeast Asia where the carrier frequency is around 50% ***(Renzo and Raffaella, 2010).***

Pathophysiology

The reduced amount (beta+) or absence (beta0) of beta globin chains result in a relative excess of unbound alpha globin chains that precipitate in erythroid precursors in the bone marrow, leading to

their premature death and hence to ineffective erythropoiesis. The degree of globin chain reduction is determined by the nature of the mutation at the beta globin gene located on chromosome 11.

Peripheral hemolysis contributing to anemia is less prominent in thalassemia major than in thalassemia intermedia, and occurs when insoluble alpha globin chains(forming inclusion bodies) induce membrane damage to the peripheral erythrocytes. These chain inclusions can be demonstrated by both light and electron microscopy in the erythroid precursors in the bone marrow as well as in the peripheral red cells following splenectomy. Anemia stimulates the production of erythropoietin with consequent intensive but ineffective expansion of the bone marrow (up 25 to 30 times normal), which in turn causes the typical described bone deformities. Prolonged and severe anemia and increased erythropoietic drive also result in hepatosplenomegaly and extramedullary erythropoiesis ***(Thein, 2005).***

Clinical description:

Beta-thalassemia major

Clinical presentation of thalassemia major occurs between 6 and 24 months. Affected infants fail to thrive and become progressively pale. Feeding

problems, diarrhea, irritability, recurrent bouts of fever, and progressive enlargement of the abdomen caused by spleen and liver enlargement may occur. In some developing countries, where due to the lack of resources patients are untreated or poorly transfused, the clinical picture of thalassemia major is characterized by growth retardation, pallor, jaundice, poor musculature, genu valgum, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoiesis, and skeletal changes resulting from expansion of the bone marrow. Skeletal changes include deformities in the long bones of the legs and typical craniofacial changes (bossing of the skull, prominent malar eminence, depression of the bridge of the nose, tendency to a mongoloid slant of the eye, and hypertrophy of the maxillae, which tends to expose the upper teeth).

If a regular transfusion program that maintains a minimum Hb concentration of 9.5 to 10.5 g/dL is initiated, growth and development tends to be normal up to 10 to 12 years ***(Thalassemia International Federation, 2008).*** Transfused patients may develop complications related to iron overload. Complications of iron overload in children include growth retardation and failure or delay of sexual maturation. Later iron overload-related compli-cations include involvement of the heart

(dilated cardiomyopathy or rarely arrythmias), liver (fibrosis and cirrhosis), and endocrine glands (diabetes mellitus, hypogonadism and insufficiency of the parathyroid, thyroid, pituitary, and, less commonly, adrenal glands) ***(Borgna-Pignatti and Galanello, 2004).*** Other complications are hypersplenism, chronic hepatitis (resulting from infection with viruses that cause hepatitis B and/or C), HIV infection, venous thrombosis, and osteoporosis. The risk for hepatocellular carcinoma is increased in patients with liver viral infection and iron overload ***(Borgna-Pignatti et al., 2004).*** Compliance with iron chelation therapy mainly influences frequency and severity of the iron overload-related complications. Individuals who have not been regularly transfused usually die before the second-third decade. Survival of individuals who have been regularly transfused and treated with appropriate chelation extends beyond age of 40 years. Cardiac disease caused by myocardial siderosis is the most important life-limiting complication of iron overload in beta-thalassemia. In fact, cardiac complications are the cause of the deaths in 71% of the patients with beta-thalassemia major ***(Borgna-Pignatti et al., 2005).***

Beta-thalassemia intermedia:

Individuals with thalassemia intermedia present later than thalassemia major, have milder

anemia and by definition do not require or only occasionally require transfusion. At the severe end of the clinical spectrum, patients present between the ages of 2 and 6 years and although they are capable of surviving without regular blood transfusion, growth and development are retarded. At the other end of the spectrum are patients who are completely asymptomatic until adult life with only mild anemia. Hypertrophy of erythroid marrow with the possibility of extramedullary erythropoiesis, a compensatory mechanism of bone marrow to overcome chronic anemia, is common. Its consequences are characteristic deformities of the bone and face, osteoporosis with pathologic fractures of long bones and formation of erythropoietic masses that primarily affect the spleen, liver, lymph nodes, chest and spine. Enlargement of the spleen is also a consequence of its major role in clearing damaged red cells from the bloodstream. Extramedullary erythropoiesis may cause neurological problems such as spinal cord compression with paraplegia and intrathoracic masses. As a result of ineffective erythropoiesis and peripheral hemolysis, thalassemia intermedia patients may develop gallstones, which occur more commonly than in thalassemia major ***(Galanello et al., 2001).*** Patients with thalassemia intermedia frequently develop leg ulcers and have an increased predisposition to thrombosis as compared to

thalassemia major, especially if splenectomised. Such events include deep vein thrombosis, portal vein thrombosis, stroke and pulmonary embolism ***(Taher et al., 2008).***

Although individuals with thalassemia intermedia are at risk of iron overload secondary to increased intestinal iron absorption, hypogonadism, hypo-thyroidism and diabetes are not common ***(De Sanctis et al., 1998).*** Women may have successful spontaneous pregnancies. However, if blood transfusions are necessary during pregnancy, those never or minimally transfused are at risk of developing hemolytic alloantibodies and erythrocyte autoantibodies. Intrauterine growth retardation, despite a regular transfusion regimen, has been reported ***(Nassar et al.,2008).*** Cardiac involvement in thalassemia intermedia results mainly from a high-output state and pulmonary hypertension, while systolic left ventricle function is usually preserved ***(Aessopos et al., 2005)***. Pseudoxantoma elasticum, a diffuse connective tissue disorder with vascular manifestation caused by degeration of the elastic lamina of the arterial wall and calcium deposition, has been described in such patients ***(Aessopos et al., 2002).***

Beta-thalassemia minor

Carriers of thalassemia minor are usually clinically asymptomatic but sometimes have a mild

anemia. When both parents are carriers there is a 25% risk at each pregnancy of having children with homozygous thalassemia ***(Renzo and Raffaella, 2010).***

Dominant beta-thalassemia:

In contrast with the classical recessive forms of beta-thalassemia, which lead to a reduced production of normal beta globin chains, some rare mutations result in the synthesis of extremely unstable beta globin variants which precipitate in erythroid precursors causing ineffective erythro-poiesis. These mutations are associated with a clinically detectable thalassemia phenotype in the heterozygote and are therefore referred to as dominant beta-thalassemias. The presence of hyper-unstable Hb should be suspected in any individual with thalassemia intermedia when both parents are hematologically normal, or in families with a pattern of autosomal dominant transmission of the thalassemia intermedia phenotype. Beta globin gene sequencing establishes the diagnosis ***(Luo et al., 2005).***

β-thalassemia associated with other Hb anomalies

The interaction of HbE and beta-thalassemia results in thalassemia phenotypes ranging from a condition indistinguishable from thalassemia major

to a mild form of thalassemia intermedia. Depending on the severity of symptoms three categories may be identified:

- Mild HbE/beta-thalassemia: It is observed in about 15% of all cases in Southeast Asia. This group of patients maintains Hb levels between 9 and 12 g/dl and usually does not develop clinically significant problems. No treatment is required.
- Moderately severe HbE/beta-thalassemia: The majority of HbE/beta-thalassemia cases fall into this category. The Hb levels remain at 6-7 g/dl and the clinical symptoms are similar to thalassemia intermedia. Transfusions are not required unless infections precipitate further anemia. Iron overload may occur.
- Severe HbE/beta-thalassemia: The Hb level can be as low as 4-5 g/dl. Patients in this group manifest symptoms similar to thalassemia major and are treated as thalassemia major patients

(Pathrapol et al., 2010)

Patients with HbC/beta-thalassemia may live free of symptoms and be diagnosed during routine tests. When present, clinical manifestations are anemia and enlargement of the spleen. Blood transfusions are seldom required. Microcytosis and hypochromia are found in every case. The blood film

shows distinctive Hb C crystals with straight parallel edges, target cells, and irregularly contracted cells with features of thalassemia such as microcytosis.

The association of hereditary persistence of fetal Hb (HPFH) with beta-thalassemia mitigates the clinical manifestations which vary from normal to thalassemia intermedia. Individuals with HbS/beta-thalassemia have a clinical course similar to that of Hb SS. Beta-thalassemia associated with other features. In rare instances the beta-thalassemia defect does not lie in the beta globin gene cluster. In cases in which the beta-thalassemia trait is associated with other features, the molecular lesion has been found either in the gene encoding the transcription factor TFIIH (beta-thalassemia trait associated with tricothiodystrophy) or in the X-linked transcription factor GATA-1 (X-linked thrombocytopenia with thalassemia ***(Freson et al., 2002).***

Etiology

More than 200 mutations have been so far reported; the large majority are point mutations in functionally important regions of the beta globin gene . Deletions of the beta globin gene are uncommon. The beta globin gene mutations cause a