INTRODUCTION

Thalassemia is an inherited blood disorder in which the body is unable to make adequate hemoglobin. This is due to an inborn error of metabolism that leads to absence or reduced synthesis of one or more types of globin polypeptide chains of the hemoglobin molecules ***(Asha Shah, 2004).***

While hypertransfusion and subcutaneous iron chelation therapy have increased longevity of patients with beta-thalassemia major, endocrinopathies have become more common and impair the quality of their lives ***(Wang et al., 2006).***

Growth retardation and failure of normal pubertal development are the most common endocrine complications. Short stature, delayed puberty, and hypogonadism have been recognized in thalassemia major patients for many years and continue to be common problems despite regular transfusion and chelation therapy ***(Karimi and Karamifar, 2004).***

Hypogonadotropic hypogonadism, which still remain the commonest endocrinopathy in patients with thalassemia major, has been proven to be the result of hemosiderosis of gonadotroph cells of the pituitary gland ***(Abdel Razek and Ghanem, 2007).***

Failure of pubertal development has been observed in 43% of patients over the age of 16 years. In some patients, this

was though to be primary gonadal in origin, whereas in others it was postulated to be secondary to pituitary insufficiency or to a combination of both primary and secondary hypogonadism ***(De Sanctis, 2006).***

The treatment of the pubertal disorders consists of hormone replacement therapy with sex steroid. Successful induction of spermatogenesis and ovulation has been reported after hormone stimulation with gonadotrophins ***(De Sanctis, 2006).***

AIM OF THE WORK

This study was aimed to evaluate puberty in a group of adolescent boys with beta-thalassemia major, to determine whether the cause of delayed puberty in those patients is hypothalamic-pituitary or gonadal or both and to determine the effect of chelation on spermatogenic function in pubertal beta-thalassemic males.

Chapter (1)

THALASSEMIA

Definition:

Thalassemia is a hereditary anemia resulting from defect in hemoglobin production ***(Rund and Rachmilewitz, 2005)***

Thalassemia is an inherited blood disorder in which the body is unable to make adequate hemoglobin. This is due to an inborn error of metabolism that leads to absence or reduced synthesis of one or more types of globin polypeptide chains of the hemoglobin molecules ***(Asha Shah, 2004).***

Historical back ground:

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 change ***(Cooley and Lee, 1925).*** Over the next decade, a milder form was described independently by several Italian investigators.

Because all early cases were reported in children of Mediteranean origin, the disease was later termed thalassemia, from the Greek word for sea, thalassa ***(Whipple and Bradford, 1936).***

Over the next 20 years, it became apparent that Cooley and Lee had described the homozygous or compound heterozygous state for a recessive mendelian disorder not

confined to the Mediteranean, but occurring widely throughout tropical countries. In the past 20 years, the two important forms of this disorder, alpha and beta thalassemia, resulting from the defective synthesis of the alpha and beta globin chains of hemoglobin, respectively, have become recognized as the most common monogenic disease in humans ***(Wheatherall et al., 1996).***

Prevalence and geographical distribution:

The thalassemias are wide-spread with about 5% of the world population affected by it. It is most prevalent around the Mediteranean Sea i.e. countries like Greece, Italy, Turkey, and North African countries. It is also seen in Saudi Arabia, Iran, Afghanistan, Pakistan and South East Asian countries like Thailand and Indonesia. The prevalence is high in Italy, Greece and Cyprus ***(Asha Shah, 2004).***

The high gene frequency of beta thalassemia in these regions is most likely related to the selective pressure from malaria, this distribution is quite similar to that of endemic Plasmodium falciparum malaria. However, because of population migration and, in a limited part, the slave trade, beta thalassemia is now common in Northen Europe, North and South America, the Caribbean, and Australia ***(Flint et al., 1998).***

β-thalassemia is endemic in all countries of the Arab world, probably due to the presence of malaria previously in

that region, and the frequency of carriers varies from 1% to 5% ***(Zahed, 2001).***

In Egypt, β-thalassemia represents a major public health problem, as it constitutes about 85% of the chronic haemolytic anaemias, with a gene frequency of 0.03% ***(El-Beshlawy et al., 1999).*** A carrier rate of 9-10.5% has been estimated in Egypt ***(El-Beshlawy et al., 1993).*** So, more than one thousand affected cases are expected to be born every year in the country ***(Omar et al., 2001).*** However a carrier rate of 2-4% was reported from Egypt ***(Hussein et al., 2000A).***

Structure of normal hemoglobin:

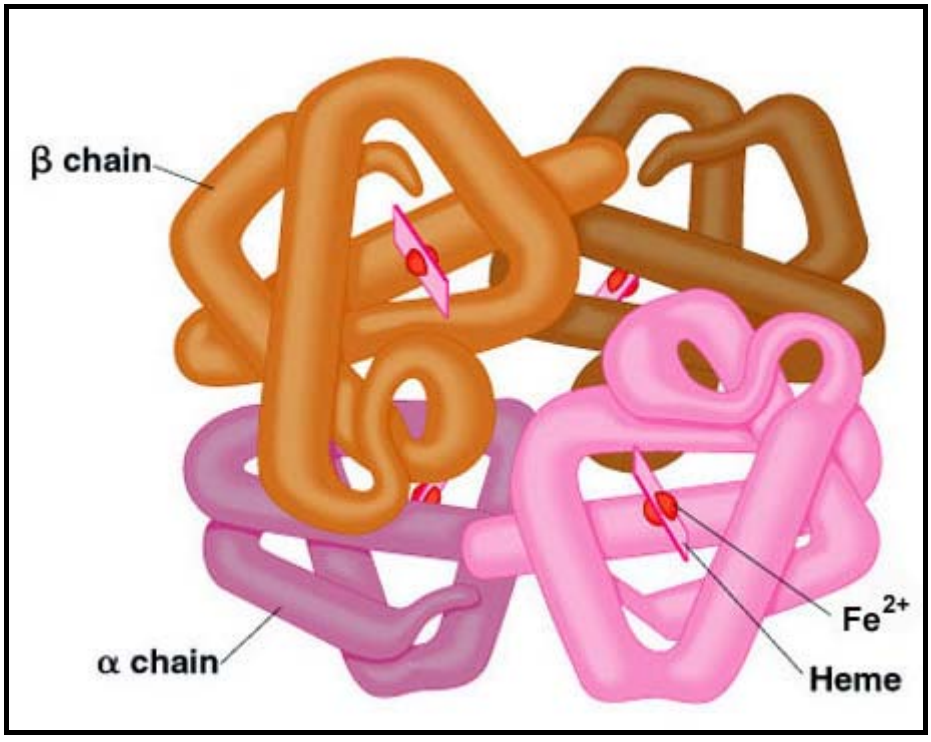

Fig. (1): Structure of hemoglobin ***(King, 2009).***

The hemoglobin molecule is made up of two parts - heme and globin. Heme is a porphyrin containing iron. Globin is made up of four polypeptide chains of two types - alpha and beta. Thus each globin molecule is made up of two alpha chains and two beta chains. This hemoglobin is called hemoglobin A because this forms the part of hemoglobin found in adults. In adults there is another small fraction of hemoglobin called HbA2, the globin portion of which is made up of two alpha chains and two delta chains. Normally the concentration of HbA2 is less than 3.5% of the total hemoglobin ***(Asha Shah, 2004).***

Within the RBCs of an embryo, fetus, child and adult, six different hemoglobins may normally be detected: the embryonic hemoglobin; Gower-1, Gower-2, and Portland, the fetal hemoglobin; Hb F and the adult hemoglobins; Hb A and Hb A2. The Gower-1 has the structure ζ2 ε2 and Gower-2, α2 ε2. Hb Portland has the structure ζ2 γ2. In embryos, the Gower hemoglobins predominate, but by the 3rd month they have disappeared. After the 8th gestational week, Hb F is the predominant hemoglobin. During the 3rd trimester, a gradual decline occures, so decrease rapidly postnatally, and by 6-12 months of age only a trace is present. Some Hb A (α2 β2) can be detected in even the smallest embryos. Accordingly, it is possible as early as 16-20 weeks gestation to make a prenatal diagnosis of major β-chain hemoglobinopathies, such as thalassemia major ***(Kliegman et al., 2008).***

The genetic control of gamma, alpha and beta chains is interrelated, so that after birth, the production of gamma chains slows down and beta chains increase correspondingly ***(Asha Shah, 2004).***

In chromosomally normal fetuses the percentage of erythroblasts expression the ζ chain was 25% to 10 weeks but this decreased exponentially with gestation to less than 1% by 17 weeks. Similarly, the percentage of cells expression the ε chain decreased from 97% at 10 weeks to less than 1% by 25 weeks. In contrast, expression of the γ chain increased from about 30% at 10 weeks to 90% by 16 weeks and decreased thereafter to 60% at 40 weeks ***(Al-Muftir et al., 2000).***

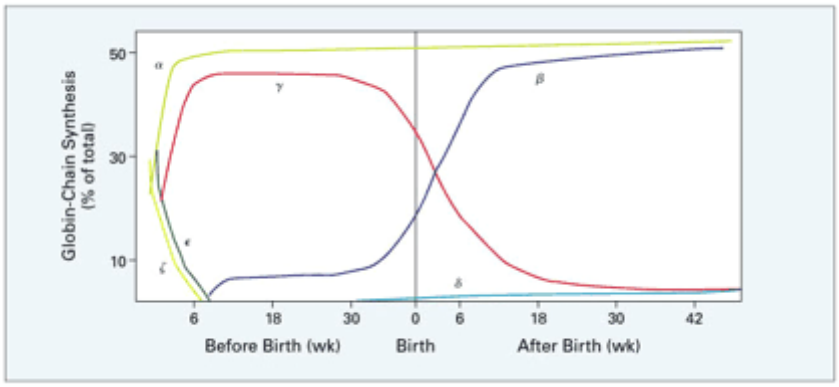

Fig. (2): Timing of the normal developmental switching of human hemoglobin ***(Olivieri, 1999).***

Classification of thalassemia:

A large number of thalassemic syndromes are currently known; each involves decreased production of one globin chain or more, which form the different Hbs normally found in RBCs.

The most important types in clinical practice are those that affect either alpha or beta chain synthesis ***(Yaish, 2007).***

Alpha thalassemia:

Several forms of alpha thalassemia are known in clinical practice. The most common forms are as follows:

Silent carrier alpha thalassemia:

This is a fairly common type of subclinical thalassemia usually found by chance among various ethnic populations, particularly African American, while the child is being evaluated for some other conditions. 2 alpha genes are located on each chromosome 16, giving alpha thalassemia the unique of gene duplication. This duplication is in contrast to only one beta globin gene on chromosome 11.

In the silent carrier state, one of the alpha genes is usually absent, leaving only 3 of 4 genes (aa, a0). Patients are hematologically healthy, except for occasional low RBC indices.

In this form, the diagnosis cannot be confirmed based on Hb electrophoresis results, which are usually normal in all alpha thalassemia traits. More sophisticated tests are necessary to confirm the diagnosis. One may look for hematologic abnormalities in family members (e.g. parents) to support the diagnosis. A CBC in one parent that demonstrate hypochromia and microcytosis in the absence of any

explanation is frequently adequate evidence for the presence of thalassemia ***(Yaish, 2007).***

Alpha thalassemia trait:

This trait is characterized by mild anemia and low RBC indices. This condition is typically caused by the deletion of two alpha (a) genes on the chromosome 16 (aa/00) or one from each chromosome (a0/a0). This condition is encountered mainly in Southeast Asia, The Indian subcontinent, and some parts of the Middle East. The a0/a0 form is much more common in black populations because of the doubly deleted (00) form of chromosome 16 is rare in this ethnic group.

Hb H disease:

This condition, which results from the deletion or inactivation of 3 alpha globin genes (00/a0), represents alpha thalassemia intermedia, with mildly to moderately severe anemia, splenomegaly, icterus, and abnormal RBC indices. When peripheral blood films stained with supravital stain or reticulocyte preparations are examined, unique inclusions in the RBCs are usually observed. These inclusions represent beta chain tetramers (Hb H), which are unstable and precipitate in the RBC, giving it the appearance of a golf ball. These inclusions are termed Heinz bodies.

Alpha thalassemia major:

This condition is the result of complete deletion of the alpha gene cluster on both copies of chromosome 16 (00,00), leading to the severe form of homozygous alpha thalassemia, which is usually incompatible with life and results in hydops fetalis unless intrauterine blood transfusion is given ***(Yaish, 2007).***

Beta thalassemia:

Similar to alpha thalassemia, several clinical forms of beta thalassemia are recognized; some of the more common forms are as follows:

Silent carrier beta thalassemia:

Similar to patients who silently carry alpha thalassemia, these patients have no symptoms, except for possible low RBC indices. The mutation that causes the thalassemia is very mild and represents a beta thalassemia.

Beta thalasemia trait:

Patients have mild anemia, abnormal RBC indices, and abnormal Hb electrophoresis results with elevated levels of HbA2, HbF, or both. Peripheral blood film examination usually reveals marked hypochromia and microcytosis (without the anisocytosis usually encountered in iron deficiency anemia), target cells, and faint basophilic stippling. The production of

beta chains from the abnormal allele varies from complete absence to variable degrees of deficiency ***(Yaish, 2007).***

Thalassemia intermedia:

This condition is usually due to a compound heterozygous state, resulting in anemia of intermediate severity, which typically does not require regular blood transfusions.

Beta thalassemia associated beta chain structural variants:

The most significant condition in this group of thalassemic syndromes is the HbE beta thalassemia, which may vary in its clinical severity from as mild as thalassemia intermedia to as severe as beta thalassemia major.

Thalassemia major (Cooley's anemia):

This condition is characterized by transfusion-dependent anemia, massive splenomegaly, bone deformities, growth retardation, and peculiar facies in untreated individuals, 80% of whom die within the first 5 years of life from complications of anemia. Examination of a peripheral blood preparation in such patients reveals severe hypochromia and microcytosis, marked anisocytosis, fragmented RBCs, hypochromic macrocytes, polychromatosia, nucleated RBCs, and, on occasion, immature leucocytes ***(Yaish, 2007).***

Inheritance of thalassemia:

Like all body characteristics and functions, hemoglobin formation is also controlled by a pair of genes, one inherited from each parent. Normal persons have inherited normal genes from both the parents and thus form normal hemoglobin.

Thalassemia carriers or traits have one normal and one abnormal gene. They are usually healthy because the normal gene masks the function of the abnormal gene. If a person inherits abnormal genes from both the parents, as occurs in thalassemia major, body cannot form enough hemoglobin and hence survival depends on regular transfusions ***(Asha Shah, 2004).***

The beta thalassemias are a consequence of mutations that impair the normal process of beta globin chain production. There are two beta globin genes, one located on each chromosome 11. Heterozygous beta thalassemia (beta thalassemia trait) is due to inheritance of one beta thalassemia gene, resulting in a lifelong mild hypochromic microcytic anemia (Hgb 8-11 g/dl) appearing at several months of age.

Homozygous beta thalassemia is due to the inheritance of two beta thalassemia genes, leading to a marked reduction or absence of beta globin chain production. Although deletion of the beta globin locus is an occasional cause of beta thalassemia, most cases are caused by point mutations that affect transcription, mRNA processing, or translation.

There are two general types of homozygous beta thalassemia, β° and β+. In β° thalassemia, no beta globin is reduced by thalassemic locus, whereas in β+ thalassemia, there is reduced but measurable output of beta globin.

The severity of homozygous beta thalassemia (or beta thalassemia major) is greatest when two β° thalassemia genes are inherited and is usually much milder when two β+ thalassemia genes are inherited. Severe beta thalassmia is associated with lifelong hemolytic anemia, dependence on regular RBCs transfusions for survival, and the gradual development of transfusion-associated hemosiderosis. The clinical abnormalities of beta thalassemia are not evident at birth but first present after three months of age, when beta globin normally becomes the dominant form of non-alpha globin that is synthesized ***(Pedro and Eric, 2005).***

Pathophysiology of thalassemia:

Despite discoveries concerning the molecular abnormalities that led to the thalassemic syndromes, it still not known how accumulation of excess unmatched alpha-globin in beta thalassemia and beta-globin in alpha thalassemia leads to red blood cell hemolysis in the peripheral blood, and in beta thalassemia particularly premature destruction of erythroid precursors in marrow (ineffective erythropoiesis). Oxidant injury may cause hemolysis, but there is no evidence that it causes ineffective erythropoiesis ***(Schrier, 2002).***

The central mechanism underlying the pathophysiology of the beta thalassemia can be related to the deleterious effects of imbalanced globin chain synthesis on erythroid maturation and survival. An imbalance of the alpha/non-alpha globin chains leads to an excess of unmatched alpha globin which precipitates out, damaging membrane structures leading to accelerated and premature destruction of the erythroid precursors in the bone marrow (ineffected erythropoiesis) ***(Thein, 2005).***

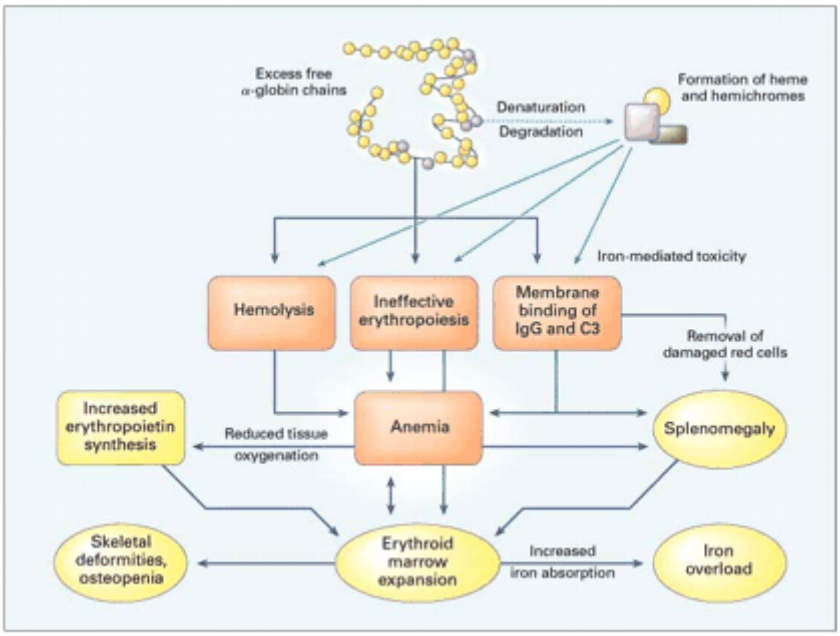

Fig. (3): Effects of Excess Production of Free α-Globin Chains ***(Olivieri, 1999).***

Today, more than 200 mutations, affecting different levels of beta-globin gene expression ***(Basak, 2007).***