Assessment of Adrenal Function In β-Thalassemia Patients by Estimation of Δ4- Androstenedione Before and After ACTH Stimulation by Synacthen

Thesis
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Introduction

Endocrine disorders are common in patients with B- thalassemia major. The prevalence varies depending on the age, country of origin of the patient, the total number of transfusions, the intensity of chelation therapy, the family history and health status of the patient **(Brittenham et al., 1994).**

Multi-transfused thalassemia major patients may develop severe endocrine complications due to iron overload. The anterior pituitary is particularly sensitive to iron overload which disrupts hormone secretion **(Skordios and Toumba, 2007).**

Growth, sexual development, fertility, bone mineral density, diabetes mellitus, hypothyroidism, hypoparathyroidism, and hypoadrenalism are the main issues to be addressed in the long-term follow-up of patients with thalassemia **(Tiosano and Hochberg, 2001).**

Since several endocrine glands may be affected in patients with thalassaemia major, and their life expectancy is now much longer, it is important for physicians to be aware of the endocrine abnormalities that may develop. Therefore, periodic evaluation of these problems should be carried out in thalassaemic patients with iron overload and poor compliance to chelation therapy **(De Sanctis et al., 2004).**

Aim of the work:

The current study aims at evaluating the adrenal function by estimation of Δ4 androstenedione before and after ACTH stimulation in adolescent patients with β- thalassemia major.

Thalassemia

Thalassemias are genetic disorders in globin chain production. In individuals with β-thalassemia, there is either a complete absence of β-globin production (β°-thalassemia) or a partial reduction (β+-thalassemia) **(Debaun and Vichinisky, 2007).**

The thalassemias are heterogenous group of heritable hypochromic anemias of various degrees of severity. Underlying genetic defects include total or partial deletions of globin chain genes and nucleotide substitutions, deletions or insertions. The consequence of these various changes is a decrease or absence of mRNA for one or more of the globin chains or the formation of functionally defective mRNA. The result is a decrease or total suppression of hemoglobin polypeptide chain synthesis **(Honig, 2000).**

Thalassemia major is also known as Cooleys anemia and it is a clinically severe disorder due to presence of 2 identical β-thalassemia mutations one on each chromosome no. 11 **(Roth et al., 1997).**

Prevalence and geographic distribution:

Thalassemia is considered the most common genetic disorder worldwide, about 3% of the world population (150 million people) carry β-thalassemia genes and in Southeast Asia 5-10% of the population carry genes for α-thalassemia **(Honig, 2004).**

The thalassemias are common in the Mediterranean basin such as Italy, Greece, Turkey, Lebanon and Egypt. The malaria hypothesis suggests that geographic overlap of malaria and thalassemia is due to a heterozygous selective advantage that

carriers exhibit against malaria caused by plasmodium falciparum **(Mange and Mange, 1994).**

In Egypt, it represents the commonest cause of hemolytic anemia, the carrier rate in different studies ranged from 9-10.2 % **(El-Beshlawy et al., 1999).**

Khalifa et al 1997, considered an annual incidence of 1000 thalassemic patients per 1.5 million live births.

Classification of thalassemias:

Thalassemias can be classified at the genetic level into the α-, β-, δβ- or εγδβ- thalassemias, according to which globin chain is produced in reduced amounts (table 1). In some thalassemias, no globin chain is synthesized, and hence these are called α^o- or β^o- thalassemias, where in others, some globin chains are produced but at a reduced rate; these are designated α+- or β+- thalassemias. The δβ thalssemias, in which there is defective δ and β chain synthesis, can be subdivided in the same way i.e into (δβ+) and ($\delta\beta^o$) **(Olivieri et al., 1999).**

Table (1): Classification of the common thalassemias and related disorders

- β-Thalassemia
  - β+
  - βº

- δβ-Thalassemia
  - (δβ) + Hb Lepore thalassemia
  - (δβ) º
  - (Aγδβ) º
- εγδβ-Thalassemia
  - (εγδβ) º

- δ-Thalassemia
- B or δβ-thalassemia associated with β-chain variants.
  - Hb S. β-thalassemia
  - Hb E. β-thalassemia
  - Many others

- α- Thalassemia
  - α+ (deletion)
  - α+ (non deletion)
  - α º

- Hereditary persistence of Hb F
  - Deletion (δβ) º
  - Non deletion Aγβ+ G γβ+
  - Unlinked to β-globin gene cluster

(Olivieri and Weatherall, 2006)

Types of β-thalassemias:

Absence of beta chain causes beta zero-thalassemia (βº). Reduced amounts of detectable beta globin causes beta plus-thalassemia (β +).

For clinical purposes, beta thalassemia is divided into thalassemia major (transfusion dependent), thalassemia intermedia (of intermediate severity), and thalassemia minor (asymptomatic) **(Weatherall et al., 1995).**

Beta thalassemia minor (heterozygous) (β +):

This is the most common type of thalassemia. Beta chain production is less than normal due to failure of one of the genes coding for beta chains. Alpha chain production continues at near normal rate. The alpha chains combine with the available beta chains resulting in decreased level of hemoglobin A. There still remain excess alpha chains and this stimulates the increased production of delta chains. The alpha and delta chains continue to form increased amounts of hemoglobin A2. If there is still an excess of alpha chains the normal mechanism which switches off gamma chain production does not function correctly and the rate of gamma chain production is greater than a normal adult. This results in the formation of increased amounts of hemoglobin F **(Bunn and Forget, 1989).**

Beta thalassemia major (homozygous) (β^o):

In beta thalassemia major there is a complete failure of beta chain production. Hence there is very little, if any hemoglobin A present. Raised delta and gamma chains produced high levels of hemoglobin A2 and hemoglobin F **(Bunn and Forget, 1989).**

Beta thalassemia intermedia:

The term beta thalassemia intermedia is used to describe a wide spectrum of conditions, ranging from those that are almost as severe as β thalassemia, with marked anemia and growth

retardation, to those which are almost as β-thalassemia trait and which may only be discovered on routine hematological examination **(Weatherall, 2001).**

Signs and symptoms of thalassemia intermedia are comparable to those of thalassemia major but are of lesser magnitude. Although chronically anemic, individuals with thalassemia intermedia do not require transfusions, except in association with intercurrent illness. Growth and development during childhood are relatively uncompromised, pubescence takes place normally, and fertility is preserved. Neverthless, pallor, intermittent icterus, splenomegaly, and facial bony changes similar to those of thalassemia major are observed regularly. Survival into adulthood is the rule and patients typically enjoy a full life span. Complications in adult life include pathologic fractures, cholelithiasis and thoracic masses composed of hematopoietic tissue. The primary cause of premature death is myocardial hemosiderosis **(Lukens, 1993).**

Beta thalassemia minor:

This syndrome is almost always discovered accidentally during examination for unrelated symptoms or as a consequence of a study designed to characterize better the nature of symptomatic anemia in a family member. No symptoms are present, and physical findings are the exception rather than the rule. Affected women are more anemic than are non thalassemic women during pregnancy, but blood transfusion is not required **(Schuman, 1973).** Because hematologic alterations frequently are mistaken for iron deficiency, therapeutic iron use often is prescribed for extended periods. Medical iron intake, compounded by greater than normal iron absorption, has been implicated in the pathogenesis of hemosiderosis noted in some adults with thalassemia minor. Most iron overload, however, results from coincidental inheritance of the hemochromatosis gene **(Edwards, 1981).**

Molecular pathology of homozygous beta-thalassemia:

Thalassemias are uniquely characterized at molecular level because much is known about the structure of the globin genes and behaviour in the developing cells **(Chao and Wang, 1996).**

The genes encoding for the α and β chains occur in clusters, the α globin cluster is found on the tip of chromosome 16 while the β globin gene spanks 7kb on the short arm of chromosome 11. The β globin gene cluster is under the control of β locus control region (BLCR) a major regulatory element located approximately 5 to 20 kb up stream of the gene **(Olivieri, 1999).**

The β thalassemia is inherited as pathologic alleles of one or more globin genes located on chromosome 11. These lesions range from total deletion to point mutations that affect every step in pathway of globin gene expression, transcription, processing of mRNA precursor, translation of mRNA and post translation integrity of β polypeptide chain **(Forget, 2001).**

Over 200 mutations have been described in the thalassemia phenotype and they have been identified and classified as β + or βº depending on whether they reduce or abolish the production of β globin chains **(Honig, 2004).**

About 200 mutations account for the vast majority of affected patients with the remainder responsible for the disorder in only relatively few patients. It has been determined that five to six mutations usually account for more than 90% of cases of β thalassemia which are: IVS 1nt 6, IVS 1nt1, IVS 1nt745, IVS 2nt 1, and codon 39 **(Kazazian, 1990).** It has been found that IVS 1nt110 is the most common mutation followed by IVS 1nt1 followed by IVS 1nt6 **(Hafez et al., 1990).** Patients having these mutations receive more frequent blood transfusions than

the rest of the patients particularly those having mutation IVS 1nt110. This last group also manifests significant reduction in weight. Patients having the mutations IVS 1nt6 and / or IVS 1 receive the least frequent blood transfusions and none of them had weight below the fifth percentile **(Khalifa et al., 1995).**

Pathophysiology

The thalassemia syndromes were among the first genetic diseases to be understood at the molecular level. More than 200 β-globin and 30 α-globin mutation have been identified; these mutations result in decreased or absent productions of one globin chain (α or β) and relative excess of the other. The resulting imbalance leads to unpaired globin chains which precipitate and cause premature death (apoptosis) of red cell precursors within the marrow, termed ineffective erythropoiesis. Of the damaged but viable red blood cells that are released from the bone marrow, many are removed by the spleen or hemolyzed directly in the circulation due to the hemoglobin precipitatants. Red blood cell destruction in the marrow, spleen and periphery causes anemia and, ultimately, an escalating cycle of pathology resulting in the clinical syndrome of severe thalassemia **(Kearney et al., 2007).**

Damaged erythrocytes enter the spleen and are trapped in this low pH and low oxygen environment; subsequent splenomegaly exacerbates the trapping of cells and worsens the anemia. Anemia and poor tissue oxygenation stimulate increased kidney erythropoietin production that further drives marrow erythropoiesis, resulting in increased ineffective marrow activity and the classic bony deformities associated with poorly managed thalassemia major and severe thalassemia intermedia. Anemia in severe thalassemia phenotypes necessitates multiple red blood cell transfusions and, over time, without proper chelation, results in transfusion-associated iron absorption and result in iron overload, even in untransfused

patients who have thalassemia intermedia **(Kearney et al., 2007).**

It has been recognized that the severity of ineffective erythropoiesis affects the degree of iron overloading, but until the recent discovery of hepcidin and understanding its role in iron metabolism the link was not understood. Hepcidin; an antimicrobial hormone, is recognized as playing a major role in iron deficiency and iron overload. Hepcidin initially was discovered due to its role in the etiology of anemia of chronic inflammation or chronic disease **(Weinstein et al., 2002).**

Elevated levels, associated with increased inflammatory markers, maintain low levels of circulating bioavailable iron in two important ways: by preventing iron absorption and transport from the gut and by preventing release and recycling of iron from macrophages and the reticuloendothelial system **(Ganz, 2003).** Conversely, inadequate hepcidin allows increased gastrointestinal absorption of iron and ultimately may lead to excess iron sufficient to result in oxygen toxicity. Iron not bound to transferrin, also referred to as nontransferrin-bound iron, damages the endocrine organs, liver, and heart. Nontransferrin-bound iron can result in myocyte damage leading to arrhythmia and heart failure; the primary cause of death in patients who have thalassemia **(Borgna-Pigantti et al., 2004).**

Clinical features of beta thalassemia major:

The clinical picture of beta thalassemia major includes features that are due to the disease itself, as well as others that represent the consequences of therapy and are, in sense, iatrogenic **(Caterina and Renzo, 2004).**

1- Anemia:

Hypochromic microcytic anemia becomes apparent 3-6 months after birth when switch from gamma to beta chain production should take place **(Honig, 2000).**

2- Iron overload:

The primary long-term complication of chronic red blood cell transfusion for thalassemia is iron loading and the resultant parenchymal toxicity. As iron levels build up in the body, transferrin becomes saturated. Iron begins to accumulate in the tissues, bound to protein storage molecules ferritin and hemosiderin. In iron overload, the capacity to bind iron is exceeded both within the cells and in the plasma compartment. This is highly reactive and alternates between the ferrous (Fe^{2}) and ferric (Fe^{3}) forms. This results in gain and loss of electrons which can generate harmful free radicals **(Borgna-Pignatti, 2005).**

3- Skeletal changes:

Bone disease in beta thalassemia is related to erythroid expansion **(Rojita et al., 1990).**

The most striking skeletal changes are seen in the skull and facial bones. There is bossing of the skull and overgrowth of maxillary region, the whole face gradually assumes a mongoloid appearance **(Lukens, 1993).** The skull X-ray shows classic hair on end appearance **(Honig, 2000).**

In the long bones, widening of the medullary portions and thinning of the compact bone of the cortex occur, predisposing to pathological fractures **(Modell and Berdoukas, 1984).**

Low bone mineral density is often present in patients with thalassemia, although recognized late. Early diagnosis should be done during childhood, in order to improve the quality of life in adulthood **(Beningo et al., 2003).**

4- Liver and gall bladder:

Hepatomegaly is prominent in severly affected patients. This is a consequence of extramedullary hematopoiesis initially, so that early hepatomegally can be reduced by hypertransfusion **(Ehlers et al., 1991).**

Later in the course of the disease, hepatomegaly is associated with extensive cirrhosis. Iron deposition, first in the Kupffer cells, ultimately engorges the parenchymal cells, resulting in an appearance that is indistinguishable from that of hemochromatosis. The hepatocellular injury of iron overload may be due to the liberation of hydrolases resulting from initiation by the ferrous form of peroxidative damage of lysosomal membrane lipids **(Mak et al., 1985).**

Many of these patients have also had viral hepatitis which may augment liver damage. A high frequency of chronic active hepatitis was observed and it was postulated that liver iron overload facilitates persistence of virus-induced liver diseases **(Pearson et al., 1996).**

Liver biopsy revealed four types of lesions in various combinations namely, foci of hematopoiesis, siderosis, hepatitis and collagen deposition **(Khalifa et al., 1991).**

El Alfy et al., 2004 stated that during the last years liver disease has emerged as a major cause of morbidity in patients with beta thalassemia major. In spite of its clinical relevance, beta thalassemia major associated liver damage has been insufficiently characterized. The liver is one of the major

storage sites of iron; measurement of the liver iron content has been described as the gold standard for the monitoring of body iron load and for assessing the risk of iron toxicity. Modern transfusion practices in the last decade and compliance to chelation therapy are the mainstay for prevention of liver cirrhosis.

Beta thalassemia patients suffer from a high incidence of gallstones as well as systemic complications of iron overload. Laparoscopic cholecystectomy should be advised in carefully selected thalassemia patients **(Katz et al., 2003).**

5- Splenomegaly and hypersplenism:

Progressive hepatosplenomegaly is a constant finding in homozygous beta thalassemia. There is some evidence that children maintained on a high transfusion regimen develop less marked splenomegaly. However, when children are maintained on inadequate transfusion, progressive enlargement of the spleen is the rule.

There may be physical discomfort due to the size of the spleen. The formed elements of the blood may be trapped in the spleen producing anemia, thrombocytopenia and some degree of neutropenia. Trapped red cells in the splenic pool may account for 9 to 40% of the total red cell mass. Certainly in any patient with beta thalassemia in whom there is splenomegaly with either increasing transfusion requirement or pancytopenia, the diagnosis of hypersplenism should be considered **(Olivieri, 1997).**

6- Infection:

Bacterial infection in patients with transfusion-dependent beta thalassemia was studied by Peng et al., (2000). The micro-organisms, outcome of infections and the risk factors were

evaluated in these patients. Bacteremia accounted for 72.7% of all infections. Few patients developed meningitis, some patients had liver abscesses, and few patients had soft tissue infections. One patient had a urinary tract infection and one patient had lobar pneumonia, interestingly, a large portion of the patients were infected by gram negative bacteria. Patients who were implanted with intravascular catheters were most susceptible to bacterial infection. The frequency of bacterial infections in patients with splenectomies was also significantly higher than that of the average patients. In conclusion, three major risk factors of bacterial infection were identified in this group of patients: intravascular catheterization, high seum ferritin levels and splenectomy. The infection rate of these patients is about 20-folds higher than that of general pediatric patients.

Infection is a major complication and the leading cause of death in thalassemia, especially E- beta thalassemia. The spectrum of infections in E-beta thalassemia include mild and severe infections, therapy related infections such as Yersinia enterocolitica infection associated with desferrioxamine therapy, and transfusion-transmitted diseases, as well as unique infections **(Wanachiwananwin, 2000).**

The level of antibodies to Yersinia enterocolitica was higher in patients receiving desferrioxamine **(Khalifa et al., 1989).** Desferrioxamine enhances the growth and virulence of Yersinia enterocolitica infection among patients who underwent splenectomy versus those with intact spleen.

Regular blood transfusions for patients with thalassemia have improved their overall survival although these transfusions carry a definite risk of the transmission of certain viruses. Infection with hepatitis B (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV) and human immunedeficiency virus (HIV) leads to complications which contribute to the morbidity and mortality of patients with thalassemia. **Jamal et al., (1998)** found that the seroprevelance rates for HBV, HCV and CMV