A study of carrier detection rate of β thalassemia in normal population

Thesis

Submitted for partial fulfillment of

M.Sc. Degree in Pediatrics

By

Maha Zakariya Ramadan

(M.B.B.Ch.)

Under Supervision of

Prof. Galila Mohamed Mokhtar

Professor of Pediatrics

Faculty of Medicine- Ain Shams University

Dr/Nancy Samir Elbarbary

Lecturer of Pediatrics

Faculty of Medicine- Ain Shams University

Dr/Tarek Mostafa Kamal

Associate consultant for human genetics

Faculty of Medicine- Ain Shams University

2012

دراسة لاكتشاف معدل حاملي مرض انيميا البحر المتوسط في عدد من الاشخاص الطبيعيين

رسالة

توطئة للحصول على درجة الماجستير فى طب الأطفال

مقدمة من

مها زكريا رمضان محمد

بكالوريوس الطب و الجراحة

جامعة عين شمس (2008)

تحت إشراف

الأستاذة الدكتورة / جليلة محمد مختار

أستاذة طب الأطفال

كلية الطب- جامعة عين شمس

الدكتورة /نانسي سمير البربري

مدرس طب الأطفال

كلية الطب- جامعة عين شمس

الدكتور/ طارق مصطفى كمال

مساعد استشاري للوراثة البشرية

كلية الطب

جامعة عين شمس

2012

Introduction

The hemoglobin disorders are the most common clinically serious single gene disorders in the world. In Egypt, beta-thalassemia is the most common type with a carrier rate varying from 5.3 to ≥9% and a gene frequency of 0.03. So, it was estimated that 1,000/1.5 million per year live births will suffer from thalassemia disease in Egypt (total live births 1,936,205 in 2006)(***Elbashlawy et al., 2009).***

In contrast to a carrier frequency of approximately 0.1% in individuals of Northern European ancestry ***(Bernini et al., 2001).***

Beta-Thalassemia creates a social and financial burden for the patients' family .The high frequency of beta-thalassemia carriers with increasing rate of newly born cases is a pressing reason for the importance to develop prevention program for beta-thalassemia ***(Weatherall et al., 2001)***

Aim of the work

The objective of this study is to determine the prevalence of beta-thalassemia carrier status in the normal population . The major focus of population screening is the prevention of disease, since it allows the parents to take informed decisions about their reproductive health prior to having a child born with a severe genetic condition.

We also aimed to give predictive values of CBC parameters to predict thalassemia carriers.

Another aim was to detect gray zone individuals in which hemoglobin electrophoresis cannot be conclusive to diagnose them as thalassemia carriers.

Thalassemia

Production of red blood cells:

First, a transient cohort of embryonic red blood cells originate in the blood islands of the yolk sac. Definitive haemapoietic stem cells (HSCs), which persist throughout fetal and adult life, emerge from the ventral wall of the dorsal aorta. These cells migrate from the ventral wall to the fetal liver and, by about 60 days of gestation, the first fetal red blood cells are released into the circulation to replace embryonic red blood cells. During fetal development, HSCs migrate to the bone marrow, which is the site of erythropoiesis for the rest of normal adult life. In early postnatal life, adult red blood cells from the marrow replace the fetal cells ***(Palis, 2008).***

At all stages of development, senescent red blood cells are continually replaced with new blood cells. These new cells are derived from HSCs, which differentiate into mature red blood cells via erythroid progenitors and precursors (erythroblasts). For an adult to maintain a normal red blood cell

count, about 2 million to 3 million new cells must be produced every second. For severe forms of thalassaemia, in which many erythroblasts and mature red blood cells are damaged , erythropoiesis can be increased by 20–30 times ***(Weatherall & Clegg, 2001).***

Hemoglobin structure:

Hemoglobin is the oxygen-carrying moiety of erythrocytes. It is a polypeptide tetramer, globular in structure, and consisting of two pairs of unlike globin chains (i.e., α plus β, δ, or γ), which form a shell around a central cavity containing four oxygen-binding heme groups each covalently linked to α globin chain. In healthy adults, 95% of the Hb is Hb A ($\alpha_2\beta_2$) with small amounts (3.5%) of Hb A_2 ($\alpha_2\delta_2$) and Hb F ($\alpha_2\gamma_2$) present. During embryonic development, “pre alpha” ξ globin chains contribute to embryonic Hb. During fetal development, β-like globin chains ε and γ contribute to the Hb ***(Gwendolyn, 2000)***.

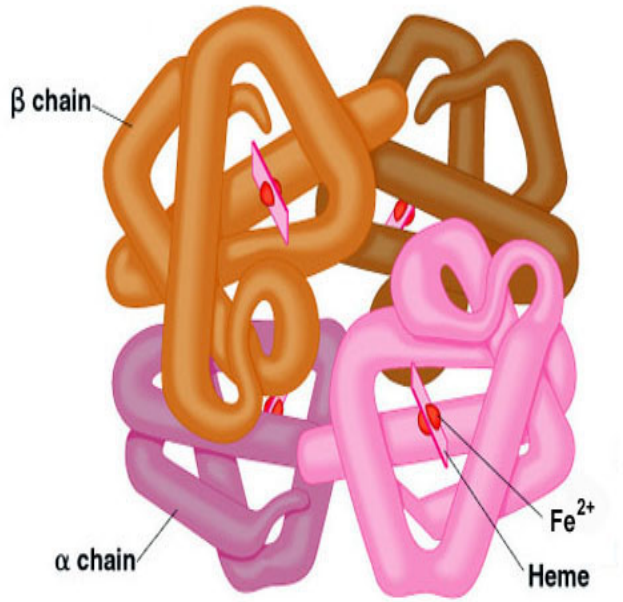


Figure (1) *Adult hemoglobin structure (2α chains and 2β chains).*

Quoted from (***themedicalbiochemistrypage.org)***

In the first trimester of intrauterine life, zeta, epsilon, alpha, and gamma chains attain significant levels and in various combinations form Hb Gower I ($\zeta_2\varepsilon_2$), Hb Gower II ($\alpha_2\varepsilon_2$), Hb Portland ($\zeta_2\gamma_2$), and fetal hemoglobin (HbF) ($\alpha_2\gamma_2$ 136-G and $\alpha_2\gamma_2$ 136-A) ***(Schroeder et al., 1968).***

Whereas Hb Gower and Hb Portland soon disappear, HbF persists and forms the predominant respiratory pigment during intrauterine life. Before birth, gamma-chain production begins to wane so that after the age of 6 months postpartum, only small amounts of HbF (<2%) can be detected in the blood ***(Marengo-Rowe et al., 1968).***

The α-globin chain is encoded in duplicate on chromosome 16, and the non-α chains (β, δ, γ) are encoded in a cluster on chromosome 11. A diploid cell therefore has four α-globin genes and two β-like genes. The α and β chains consist of 141 and 146 amino acid residues, respectively. There is some sequence homology between the two chains (64 individual amino acid residues in identical positions), and the β chain differs from the δ and γ chains by 39 and 10 residues, respectively ***(Lindeman, 1999).***

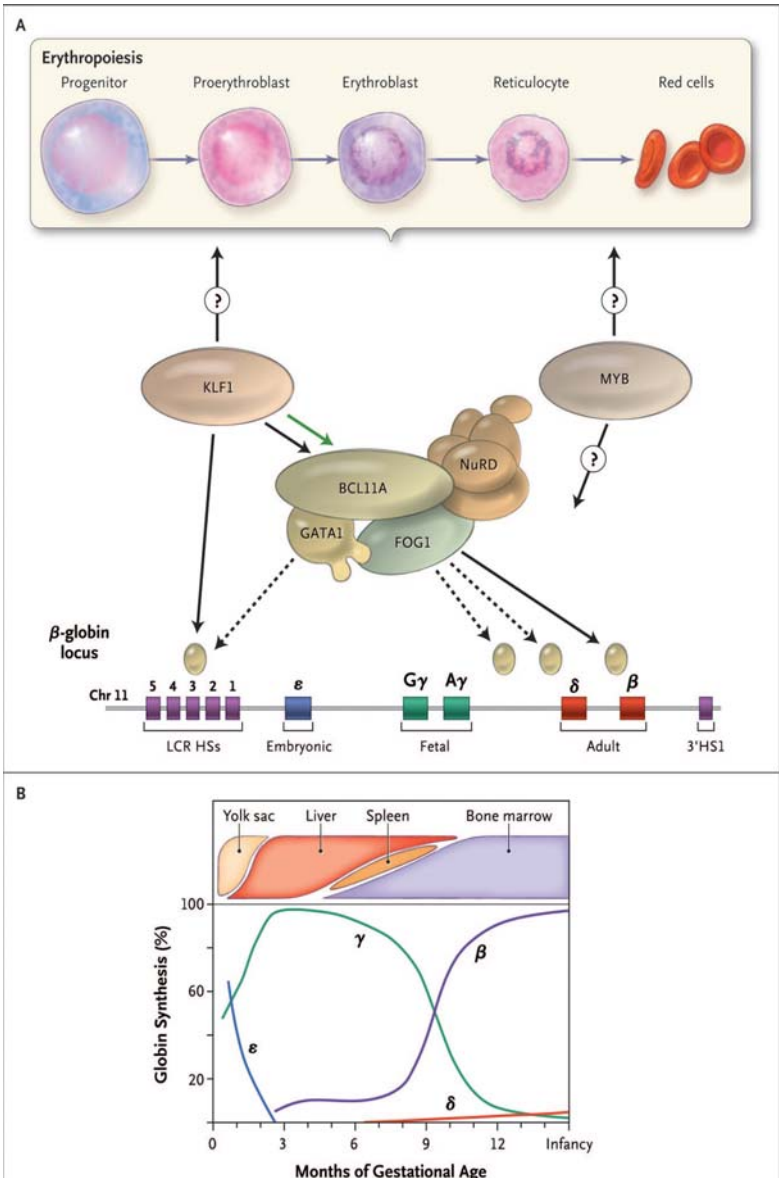


Figure (2). *Molecular regulation of the fetal-to-adult hemoglobin switch* ***(Sankaran, 2010).***

Definition and geographical distribution of β thalassemia syndrome:

Beta-thalassemias are heterogeneous autosomal recessive hereditary anemias characterized by reduced or absent β globin chain synthesis. The resulting relative excess of unbound α globin chains precipitate in erythroid precursors in the bone marrow, leading to their premature death and, hence, to ineffective erythropoiesis. β-thalassemia phenotypes are variable ranging from the severe transfusion dependent thalassemia major to the mild form of thalassemia intermedia. Patients with the major form of the disease have severe microcytic and hypochromic anemia, hepatosplenomegaly, and usually come to medical attention within the first two years of life. Without treatment, affected children have severely compromised growth and development and shortened life expectancy. Therapy, aimed at reducing transfusional iron overload, allows for normal growth and development and extends life expectancy into the third to fifth decade ***(Borgna-Pignatti et al., 2004)*.**

The extremely high frequency of the haemoglobin disorders compared with other monogenic diseases reflects several different processes. First, and foremost, is natural selection whereby carriers for these diseases have been protected during evolution from severe malaria, hence they have lived longer in malarious areas and produced more children until the numbers are balanced by the loss from the community of homozygotes. A second critical factor is the high frequency of any mendelian recessive disorder. The third factor is the epidemiological transition whereby, as neonatal and childhood mortality figures decline due to improved social conditions, babies who would formerly have died with the serious hemoglobin disorders before they came to diagnosis are now surviving to present for management. Additional factors that maintain the high gene frequency include founder effects and gene drift ***(Christianson, 2006).***

The thalassemias are found more commonly in certain ethnic groups, lending themselves to effective ethnicity-based population screening ***(Weatherall, 1997; Davies et al., 2000).***

The thalassemias are distributed across Africa, the Mediterranean region, the Middle East, the Indian subcontinent, and China and throughout southeast Asia in a line stretching from Southern China down the Malaysian peninsula to the Indonesian islands ***(Bernini, 2001)***.

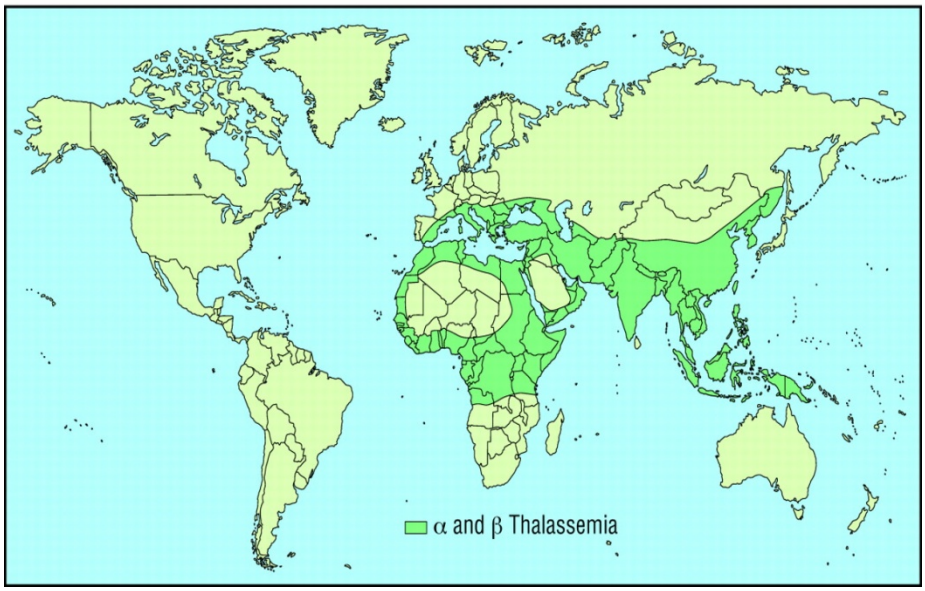


Figure (3): *Regions where thalassaemia is endemic.* Quoted from ***(Weatherall, 1997)***

In these populations, the carrier frequency is greater than 1%, in contrast to a carrier frequency of approximately 0.1% in individuals of Northern European ancestry ***(Weatherall & Clegg, 2001).***

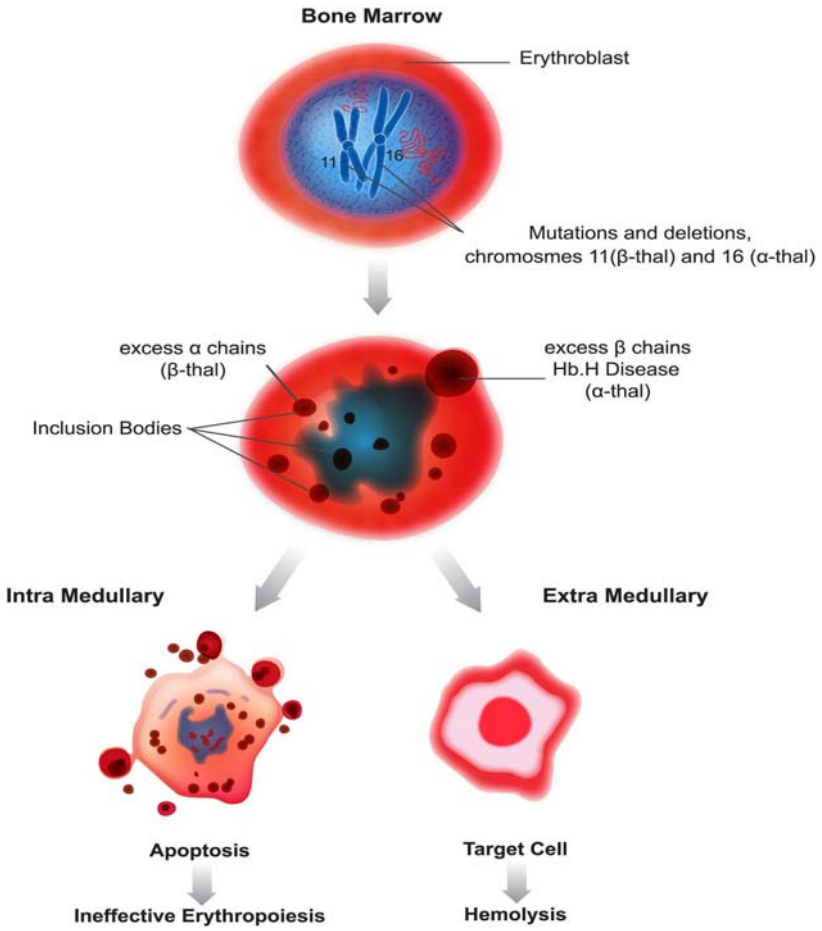
β-Thalassemia is the most frequent hemoglobinopathy in Egypt . The carrier rate of this disease varies between 5.3 and ≥ 9% and the gene frequency is 0.03, so it was estimated that 1,000/1.5 million per year live birth born with thalassemia disease (total live birth 1,936,205 in 2006) ***(El-Beshlawy et al., 2007).***

Pathology of thalassemia syndromes:

More than 200 disease-causing mutations have been described to date. The large majority of mutations are simple nucleotide substitutions or deletions or insertions of oligonucleotides leading to frameshift. Rarely the β-thalassemias are the result of gross gene deletions ***(Weatherall & Clegg, 2001).***

The major cellular pathogenic mechanisms are based primarily on the deleterious effects produced by the

accumulation of the excess α-globin chain in beta thalassemia. Indeed, studies on the synthesis of alpha and beta globin proved that the extent of mismatch determines the severity of the anemia in both alpha and beta thalassemia ***(Schrier, 2002)***.



Figure(4).*Mechanism of ineffective erythropoiesis and hemolysis in thalassemia* ***(Giardina, 2011b).***

Disease severity in patients with β-thalassemia varies greatly and patients are usually classified into Thalassemia Major (TM) or Intermedia (TI) according to clinical criteria. The requirement for at least 8 transfusions a year before the age of four years is often used to distinguish the 2 types of the disease ***(Modell et al., 2008; Thuret et al., 2010).***

The major determinant of disease severity is the degree of β-globin chain deficit (complete absence or variable reduction), resulting from the nature of the β-thalassemia alleles. Other genetic modifiers affecting the degree of α and non-α globin chain imbalance also impact the phenotypic severity ***(Thein, 2008)*.**

An associated α-thalassemia which minimizes the excess of α-globin chains tends to produce a less severe β-thalassemia condition ***(Kanavakis, 2004).***

An increased residual level of HbF in adult life which compensates the decreased β-globin chain is also a major determinant of less severe disease ***(Thein et al., 2009).***