Serum Adeponectin In Beta-Thalassemia Patients

Thesis

Submitted for Partial Fulfillment of M. Sc. Degree

in Clinical and Chemical Pathology

By

Nagwa Kamal Ragheb Poles

M.B., B.Ch.

Faculty of Medicine, Assuit University

Supervised by

Professor / Mohamed Amen Mekawey

Professor of Clinical and Chemical Pathology

Faculty of Medicine- Ain Shams University

Professor / Soha Raouf Youssef

Professor of Clinical and Chemical Pathology

Faculty of Medicine- Ain Shams University

Doctor/ Mahera Ismail Almogy

Assistant Professor of Clinical and Chemical Pathology

Faculty of Medicine- Ain Shams University

Faculty of Medicine – Ain Shams University

2012

INTRODUCTION

Adipose tissue plays a critical role in energy homeostasis, not only by storing triglycerides, but also by responding to nutrient, neural and hormonal signals andsecreting adipocytokines that control feeding, thermogenesis, neuroendocrine function, immunity and inflammation ***(Ronti et al., 2006)***. Among these cytokines, much attention has been paid to adiponectin, which has significant effects on the inflammatory process ***(Chaliasos et al., 2010)***. Adiponectin is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism, and also has anti-inflammatory, anti-atherogenic and anti-diabetic properties ***(Sun et al., 2009)***. According to the current hypothesis, chronic inflammation inhibits production of adiponectin, probably through increased levels of pro-inflammatory cytokines. Low adiponectin levels promote inflammation, thus generating a self sustaining loop ***(Ouchi et al., 2007).***

However, adiponectin exerts not only anti-inflammatory but also pro-inflammatory actions in a number of cell types since increase in adiponectin levels was seen during inflammatory conditions that are unrelated to adiposity ***(Popa et al., 2009; Fayad et al., 2007; Tsatsanis et al., 2005; Chung et al., 2009).***

It is suggested that adipose tissue dysfunction can play a role in the neuroendocrine and haematopoietic dysfunctions affecting thalassaemic patients ***(Chaliasos et al., 2010)***.Mounting evidence supports the role of inflammation in the pathogenesis of vascular complications in beta-thalassaemia. Although beta-thalassaemia is a hereditary haemoglo-binopathy, a chronic inflammatory state is present in these patients, with increased levels of pro-inflammatory cytokines ***(Kanavaki et al., 2009)***. Adiponectin was originally detected exclusively in adipocytes, but later studies revealed that it could be expressed also by other mesenchymal cells such as osteoblasts ***(Berner et al., 2004),*** synovial fibroblasts ***(Ehling et al., 2006)*** and skeletal muscle cells ***(Delaigle et al., 2004)*** or even endothelial cells ***(Chaliasos et al., 2010)***. Endothelial dysfunction is also believed to play an important role in the pathophysiology of beta-thalassaemia major, through haemolysis, increased erythrocyte adherence to endothelium and thrombosis ***(Aggeli et al., 2005)***. Findings also support the hypothesis that a serious degree of endothelial activation and damage along with a state of chronic inflammationunderlie the pathophysiology of beta-thalassemia intermedia ***(Kanavaki et al., 2009).***

The role of adipocytokines in the inflammation process in beta-thalassaemia has not yet been fully investigated. It is not known how adiponectin is produced or acts in thalassaemia patients, but further research in this field is clearly necessary.

AIM OF THE WORK

The aim of our study is to analyze the serum levels of adiponectinin Egyptian beta thalassemia patients and determine any possible correlations with disease severity or complications.

Chapter 1

THALASSEMIA

Introduction

The thalassemias are a heterogeneous group of inherited disorders of globin synthesis, all of which result from a reduced rate of production of one or more of the globin chains of hemoglobin.The result is imbalanced globin chain production, ineffective erythropoiesis, hemolysis and variable degree of anemia ***(Omar et al., 2005).***

Hemoglobin Structure

Hemoglobin consists of four polypeptide chains (i.e. a tetramer) of two different types; the α-like chains (e.g. α chain) and the β-like chains (e.g. β, γ or δ). The different hemoglobin tetramers are composed of two α-like chains and two β-like chains. Conjugated to the globin moiety is a heme subunit, each having a ferrous (Fe^{++}) iron ***(Thomas, 2004).*** The heme groups are located in the cervices near the exterior of the molecule, one in each subunit.

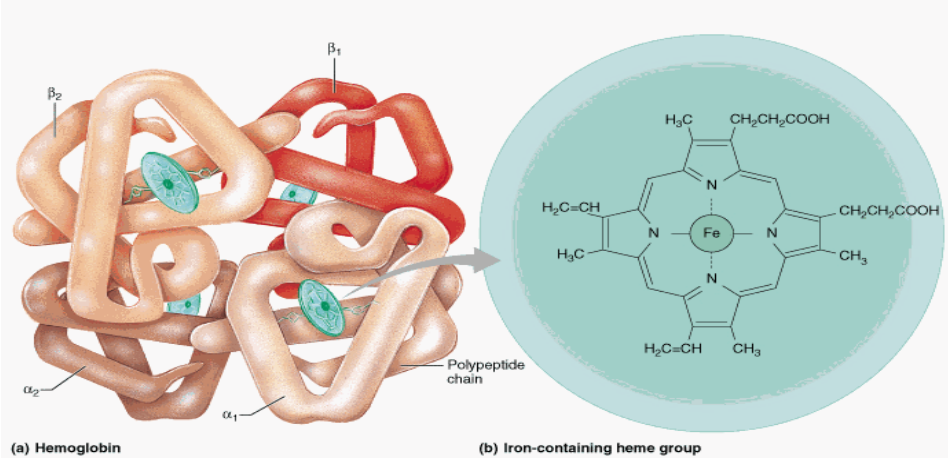

Figure (1): Model of hemoglobin ***(Stryer, 1995).***

Hemoglobin types

Globin is a protein with 4 polypeptide chains joined together by non covalent bonds. Several different kinds of hemoglobin are normally found in humans. They vary in the primary structure of the peptide chains of globin. These include transient embryonic hemoglobins, Hb F, Hb A, Hb A_2 ***(Ahmed et al., 2002)***.

Table (1): Composition of hemoglobin in the human embryo, fetus and neonate ***(Weatherall and Clegg, 2001 b).***

Hemoglobin	Globin chains α-Globin gene cluster	β-Globin gene cluster	Gestation
Embryonic			
Hb Gower1	ζ_2	ε_2	From 3 weeks
Hb Gower2	α_2	ε_2	
Hb Portland	ζ_2	γ_2	From 4 weeks
Fetal			
HbF	α_2	γ_2	From 4 weeks
Adult			
Hb A	α_2	β_2	From 6-8 weeks
Hb A_2	α_2	δ_2	From 30 weeks

Transient embryonic hemoglobins includes Hb Portland, Hb Gower 1 and Hb Gower 2 ***(Weatherall and Clegg, 2001 b)***.

Fetal hemoglobin is the predominant hemoglobin of fetal life and comprises the major proportion of hemoglobin found at birth. It accounts for 1% of adult human hemoglobin ***(Manca and Masala, 2008)***.

Hemoglobin A is the major hemoglobin in adult (97%). Its globin comprises two α-chains (141 aminoacids) and two β-chains (146 aminoacids) ($\alpha_2\beta_2$). Each polypeptide chain has a helical structure and folded into 8 stretches labeled A to H creating a pocket inside it for heme binding ***(Manca and Masala, 2008)***.

Hemoglobin A_2accounts for about 2% of adult human hemoglobin ***(Nagel and Steinberg, 2001).***

Hemoglobin genes

The α chain synthesis is directed by 2 α genes (α_1 and α_2) located on short arm of chromosome 16, while β and δ chain synthesis are directed by a single β and δ genes on short arm of chromosome 11. γ chain synthesis is directed by two genes, $^{G}\gamma$ and $^{A}\gamma$ also on chromosome 11 ***(Wild and Bain, 2001).***

The structure of the human globin genes is similar to that of all mammalian genes. They consist of long strips of nucleotides which are divided into coding regions, or exons, and non-coding inserts called intervening sequences (IVS), or introns. The β–like globin genes contain two introns between codon 30 and 31 and between codon 104 and 105, respectively ***(Weatherall, 2005b).*** And three exons first exon encodes amino acids 1 to 29 together with the first two bases for codon 30, exon 2 encodes part of residue 30 together with amino acids 31 to 104, and exon 3, amino acids 105 to 146 ***(Forget, 2001).***

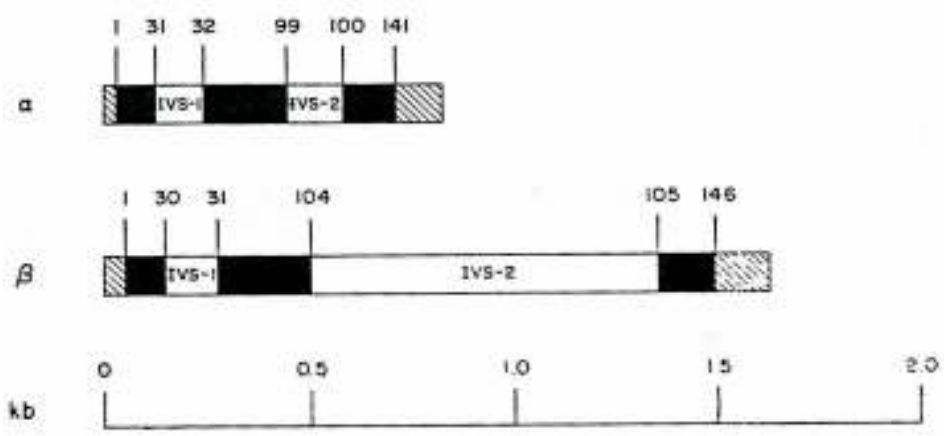

Figure (2): Fine structure of α and β globin genes ***(Forget, 2001).***

Regulation of Globin Gene Expression

i- Promoters

The promoter of a gene is the DNA sequences required for accurate and efficient initiation in transcription as they are responsible for binding RNA polymerase enzyme that catalyzes mRNA synthesis. Promoters are composed of several functional elements or "motifs", ranging from 6 to 20 nucleotides in length. These elements are thought to interact with specific DNA-binding proteins that facilitate and regulate transcription ***(Dynan and Tjian, 1985).***

Globin gene promoter regions are highly conserved during evolution and contain three elements:TATA box (position -28 to -31) a CCAAT box (position -72 to -76), and duplicated CACCC motifs (proximal at positions -86 to -90, and distal at position -101 to -105) ***(Marini et al., 2004)***.

ii- Enhancers

These are segments of DNA that increase effectiveness of a promoter. They may lie 5' or 3' to the gene or within the gene itself. The identification of sequences that enhance expression of globin genes has been of particular importance in designing vectors for gene transfer to remedy the hemoglobinopathies ***(Beutler, 2006).***

iii- Locus Control Region (LCR)

Upstream of the entire β-globin complex is the locus control region (LCR), which is essential for the expression of all the genes in the complex. This region consists of five DNAase hypersensitive (HS) sites (designated HS1-5). The two extreme HS sites flanking the β-complex have been suggested to mark the boundaries of the β-globin gene domain ***(Thein, 2008)***.

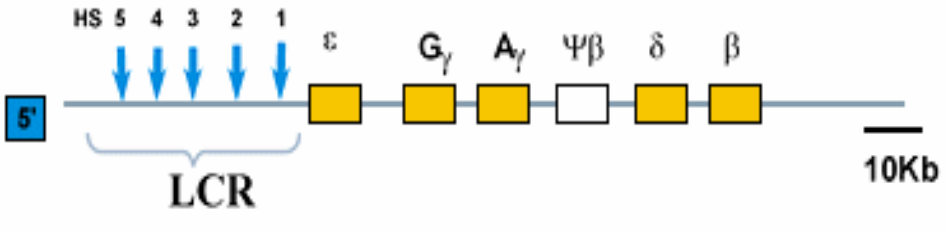

Figure (3): Organization of globin genes ***(Thein, 2005a).***

Expression of globin genes:

Transcription and processing

When a globin gene is transcribed, messenger RNA (mRNA) is synthesized from one of its strand by the action of RNA polymerase II. The primary transcript is a large mRNA precursor that contains both introns and exons, while in the nucleus, it undergoes a number of modifications first, the introns are removed and the exons are spliced together ***(Weatherall, 2001a)***.

The exon –intron junctions always have the sequence GT at their 5' end and AG at their 3' end, if there is a mutation at these sites, normal splicing cannot occur. The mRNA is modified at its 5' end by the addition of A cap structure, and at its 3' end by the addition of adenylic residues (poly A). The processed mRNA now moves into the cytoplasm to act as a template for globin chain production ***(Steinberg et al., 2001).***

Translation:

Amino acids are transported to the mRNA template on carriers called transfer RNAs, there are specific transfer RNAs for each aminoacid. The order of amino acids in a globin chain is determined by a triplet code i.e. three bases (codons) code for a particular amino acid ***(Forget et al., 2001).***

The transfer RNAs also contain three bases, anticodons, which are complementary to mRNA codons for particular amino acids. The transfer RNAs carry amino acids to the template, where they find the right position by codon-anticodon base pairing ***(Higgs et al., 2001)***.

The mRNA is translated from the 5' end to the 3' end. The transfer RNAs are held in an appropriate steric conformation with the mRNA by the two subunits that make up the ribosomes ***(Steinberg et al., 2001).***

There are specific initiation (AUG) and termination (UAA, UAG, UGA) codons. When the ribosomes reach the termination codon, translation ceases, the completed globin chain is released, and the ribosomal subunits fall apart and are recycled ***(Steinberg et al., 2001).***

Individual globin chains combine with heme, which is synthesized through a separate pathway, and with themselves to form definitive hemoglobin ***(Weatherall, 2005b)***.

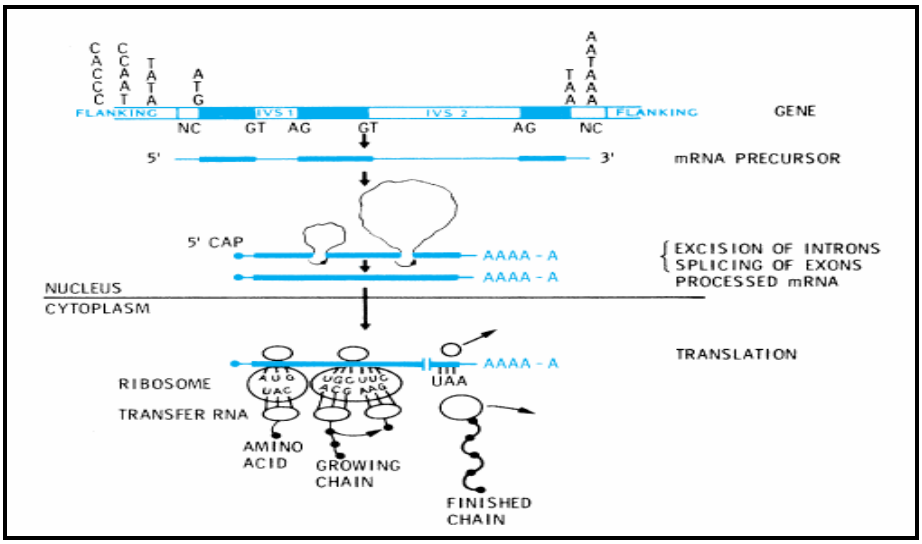

Figure (4): The mechanism of globin gene transcription and translation ***(Weatherall, 2001a).***

Classification of the disorders of haemoglobin

Mutations in the globin genes can cause either a quantitative reduction in output from that gene or alter the amino acid sequence of the protein produced. Quantitativedefects cause thalassaemia whereas qualitative changes, referredto as haemoglobin variants, cause a wide range of problems including sickle cell disease, unstable haemoglobins, decreased oxygen affinity, increased oxygen affinity and methaemoglobinaemia ***(Thein and Rees, 2011).***

Thalassaemia

History

A form of severe anemia occurring early in life and associated with splenomegaly and bone changes was first described by Cooley and Lee in 1925 ***(Cooley and Lee, 1925).***

Definition

Beta-thalassaemias are a group of hereditary human diseases caused by more than 200 mutations of the human β-globin gene, leading to low or absent production of adult β-globin and an excess of α-globin, causing ineffective erythropoiesis and low or absent production of HbA (adult haemoglobin). Currently, repeated blood transfusions and red cell hemolysis are the major causes of secondary iron overload and oxidative stress in thalassemia ***(Pignatti and Galanello, 2009).***

Classification of Thalassemia

Thalassemia is classified according to which globin chain is produced at a reduced rate. Theoretically, there are as many types of thalassemias as there are types of globin chains. **Table (3)** sets the main varieties of thalassemia that have been defined with certainty ***(Weatherall, 2005b).*** In some thalassemias, no globin chain is synthesized at all and these are called α^0 or β^0 thalassemia; in others, designated α^+ or β^+thalassemia, the globin chain is produced at a reduced rate.

Table (3): The thalassemias and related disorders ***(Weatherall, 2005b).***

α Thalassemia	α^0 α^+ Deletion (−α) Non deletion (α^T)
β-Thalassemia	β^0 β^+ Normal HbA_2 Silent
δ-β Thalassemia	$(\delta\beta)^0$ $(^A\gamma\delta\beta)^0$ $(\delta\beta)^+$
γ-Thalassemia	
δ-Thalassemia	δ^0 δ^+
εγδβ –Thalassemia	
Hereditary persistence of fetal hemoglobin	Deletion $(\delta\beta)^0$, $(^A\gamma\delta\beta)^0$ Non deletion Linked to β-globin genes $^G\gamma\beta^+$, $^A\gamma\beta^+$ Unlinked to β-globin genes

β- Thalassemia

Geographic distribution and epidemiology:

Thalassemia is the most common genetic disorder allover the world. Around 3% of the world population carries genes for β-thalassemia ***(Omar et al., 2005)***.