SERUM HEPCIDIN LEVEL IN β-THALASSEMIA MINOR

Thesis

Submitted for Partial Fulfillment of M.Sc. Degree
In Clinical and Chemical Pathology

By

Eman Nagy Mohamed Aly

M.B., B.Ch. (٢٠٠٦)

Ain-Shams University

Supervised by

Professor Dr. / Hala Mahmoud Hamdi Abaza

Professor of Clinical Hematology
Faculty of Medicine – Ain-Shams University

Professor Dr./ Soha Raouf Youssef

Professor of Clinical and Chemical Pathology
Faculty of Medicine – Ain-Shams University

Assistant Professor/ Dina Samir Mohamed Eissa

Assistant professor of Clinical and Chemical Pathology
Faculty of Medicine – Ain-Shams University

Faculty of Medicine
Ain-Shams University
2014

Contents

Contents (Cont…)

List of Tables

List of Figures

List of Abbreviation

α	Alpha.
β	Beta.
δ	Delta.
ε	Epsilon.
γ	Gamma.
κ	Kappa.
Ψ	Psi.
Z	Zeta.
μg	Microgram.
μL	Microliter.
ACD	Anemia of chronic disease.
AG	Adenine and guanine.
BM	Bone marrow.
BMP	Bone morphogenetic protein.
CBC	Complete blood count.
CLD	Chronic liver diseases.
Cm	Centimeter.
Dl	Deciliter.
DNA	Deoxyribonucleic acid.
DFO	Desferrioxamine.
EMH	Extramedullary hematopoiesis.
Epo	Erythropoietin.
ELISA	Enzyme-Linked immunosorbent assay.
FPN	Ferroportin.
fl	Femtoliter.
G	Gram.
GDF-15	Growth differentiation factor-15.
GT	Guanine and thymine.
HAMP	Hepcidin antimicrobial peptide.
Hb	Hemoglobin.
Hct	Hematocrit.

List of Abbreviation (Cont…)

HE	Hemoglobin electrophoresis.
Hfe	Hemochromatosis gene.
HPLC	High performance liquid chromatography.
HS	Hypersensitive site.
HJV	Hemojuveline.
IQR	Inter quartile range.
IU/L	International unit/Liter.
IVS	Intervening sequence.
LAR	Locus activating region.
LC	Liquid chromatography.
LCR	Locus control region.
LDH	Lactate dehydrogenase.
LPS	Lipopolysacharide.
MCH	Mean corpuscular hemoglobin.
MCHC	Mean corpuscular hemoglobin concentration.
MCV	Mean corpuscular volume.
MS	Mass spectrometry.
NMR	Nuclear Magnetic Resonance.
Mg	Milligram.
ML	Milliliter.
mRNA	Messenger ribonucleic acid.
ng	Nanogram.
nm	Nanometer.
NMD	Nonsense–mediated decay.
PB	Peripheral blood.
PCR	Polymerase chain reaction.
Pg	Pictogram.
PPV	Positive predictive value.
RBC	Red blood cell.
R	Spearman's rank correlation coefficient.
RDW	Red cell distribution width.

List of Abbreviation (Cont…)

RT-PCR	Reverse transcription-polymerase chain reaction.
RE	Reticuloendothelial system.
RGM	Repulsive guidance molecule.
RIA	Radio-immuno assay.
Rpm	Revolution per minute.
SD	Standard deviation.
STAT3	Signal transducer and activator of transcription.
Tfr-1	Transferrin receptor 1.
Tfr-2	Transferrin receptor 2.
TIBC	Total iron binding capacity.
TMPRSS6	(Matreptase-2) type 2 member of the trans-membrane serine protease family.
TLC	Total leucocytic count.
TOF	Time of flight.
TWSG-1	Twisted gastrulation protein-1.
UTR	Untranslated region.

Introduction

Beta-thalassemia is a genetic disorder caused by mutations in the β-globin gene. It is characterized by chronic anemia caused by ineffective erythropoiesis. It is accompanied by a variety of serious secondary complications such as extramedullary hematopoiesis, splenomegaly and iron overload ***(Gardenghi et al., ٢٠١٠).*** β-thalassemia minor is a common, usually asymptomatic abnormality, discovered on routine blood test. It is characterized by hypochromic microcytic blood picture (MCV, MCH are very low, normal RDW), but high red cell count (>٥.٥×١٠^{١٢}/L) and mild anemia (haemoglobin ١٠-١٢g/dl). A raised Hb $A_٢$ (α_2 δ_2) (>٣.٥%) confirms the diagnosis ***(Provan et al., 2009)***.

Iron overload is the principal cause of morbidity and mortality in β-thalassemia with or without transfusion dependence. Iron homeostasis is regulated by the hepatic peptide hormone "hepcidin" which is a small peptide hormone secreted by hepatocytes. Hepcidin controls dietary iron absorption, plasma iron concentrations and tissue iron distribution. Dysregulation of hepcidin production underlies many iron disorders ***(Nemeth, ٢٠١٠).***

In thalassemia major patients, iron absorption contributes less to the total iron load than transfusions. However, in non-transfused thalassemic patients, low hepcidin and the

consequent hyper-absorption of dietary iron is the major cause of systemic iron overload. Hepcidin measurement has only recently become available with the development of assays for bioactive mature hepcidin in serum and urine ***(Nemeth , ٢٠١٠).*** Hepcidin, ferroprotin and their regulators represent potential targets for the diagnosis and treatment of iron disorders and anemias ***(Ganz et al., ٢٠١٢).***

Aim of the Work

The aim of this work is to evaluate the hepcidin levels and iron status in Egyptians having β-Thalassemia minor.

Thalassemias

Definition

Thalassemia syndromes are a heterogeneous group of inherited anemias, characterized by defects in the synthesis of one or more of the globin chain subunites of the hemoglobin tetramer. The result is imbalanced globin chain production, ineffective erythropoiesis, and hemolytic anemia ***(Giardina and Rivella, 2013).***

Currently, repeated blood transfusions and red cell hemolysis are the major causes of secondary iron overload and oxidative stress in thalassemia ***(Pognatti and Galanello, 2009).***

Geographical distribution and epidemiology

Thalassemias represent the most common mono-genetic disorder worldwide. Because thalassemia heterozygosity confers some immunity against malaria, there is a particularly high incidence of thalassemia (2.5%-25%) in the Mediterranean basin, the Middle East, the tropical and sub-tropical regions of Africa, the Asian subcontinent, and Southeast Asia, where milder forms of the disease are most commonly seen ***(Rachmilewitz et al., 2011)***. Around 3% of the world population carries genes for β-thalassemia (Figure 1) ***(Omar et al., 2005).***

It has been estimated that about 1.5% of the global population (80 to 90 million people) are carriers of β-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. According to Thalassemia International Federation, only about 200,000 patients with thalassemia major are alive and registered as receiving regular treatment around the world ***(Galanello and Origa, 2010).***

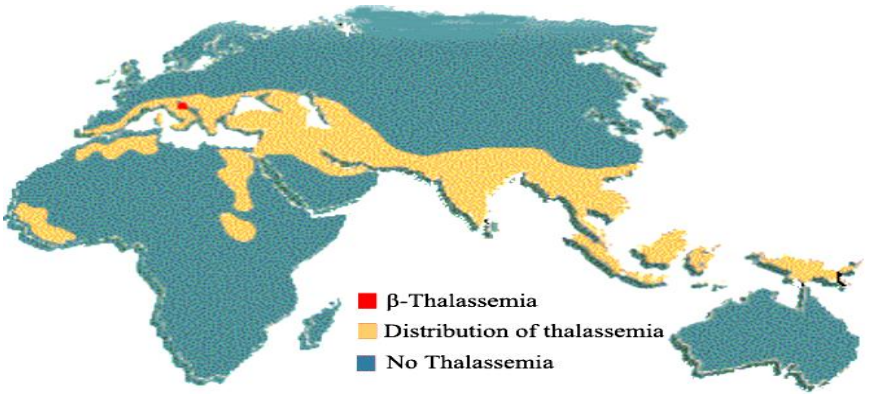

Fig.(1): Geographic distribution of β-thalassemia ***(Weatherall and Clegg, 2001).***

In Egypt, thalassemia is considered the most common hemoglobinopathy and is one of its major health problems ***(El Danasoury et al., 2011).*** It is considered the most common genetically determined, chronic haemolytic anemia ***(Madani et al., 2011)***, where >1000 of the annual 1.5 million newborns are expected to be affected with this disorder ***(Tantawy et al., 2009; Mansour et al., 2012)***, with an estimated carrier rate of 9%-

10.5% ***(Madani et al., 2011),*** and a gene frequency of 0.03 ***(Mansour et al., 2012).***

Classification

According to which globin chain is produced at a reduced rate thalassemia is classified into $\alpha, \beta, \delta\beta, \gamma\delta\beta, \delta, \gamma, \varepsilon\gamma\delta\beta$ thalassemias (Table 1). Functionally, some thalassemia mutations cause a complete absence of globin chain synthesis, and these are called α^0 or β^0 thalassemias; in others, the globin chain is produced at a reduced rate and these are called α^+ or β^+thalassemia ***(Thien and Rees, 2011).***

Table (1): The thalassemias and related disorders.

α-Thalassemia	α^0 α^+ Deletion ($-\alpha$) 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^+
$\varepsilon\gamma\delta\beta$ –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

(Thien and Rees, 2011)