

# **Growth Differentiation Factor ١٥ in Beta Thalassemia**

*Thesis*

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*By*

**Heidy Maher Fouad**

*M.B.B.Ch*

*Ain Shams University*

*Supervised by*

**Professor/ Azza Al Sayed Hashem**

*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/ Deena Samir Mohamed**

*Lecturer of Clinical and Chemical Pathology  
Faculty of Medicine -Ain Shams University*

**Faculty of Medicine  
Ain Shams University**

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## List of Abbreviations

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<b>5'UTR</b>	: 5' untranslated region
<b>ALT</b>	: Alanine transaminase
<b>AST</b>	: Aspartate transaminase
<b>ATO</b>	: Arsenic trioxide
<b>BM</b>	: Bone marrow
<b>BMI</b>	: Body mass index
<b>BMPRs</b>	: Bone morphogenic protein receptors
<b>BMPs</b>	: Bone morphogenic proteins
<b>C/EBP<math>\alpha</math></b>	: CCAAT/enhancer-binding protein
<b>CE- HPLC</b>	: cation exchange high performance liquid chromatography
<b>CHO</b>	: Carbohydrate
<b>CHOP</b>	: CCAAT/enhancer-binding homologous protein (negative regulator of C/EBP $\alpha$ )
<b>CREBH</b>	: cAMP-responsive element binding protein H
<b>DFO</b>	: Desferoaxamine
<b>DFP</b>	: Deferiprone
<b>DNA</b>	: Deoxyribonucleic acids
<b>EDTA</b>	: Ethylene diamine tetra-acetic acid
<b>EMH</b>	: Extramedullary hematopoiesis
<b>EPO</b>	: Erythropoietin
<b>EPOR</b>	: EPO receptor
<b>ESR</b>	: Erythrocyte sedimentation rate
<b>GDF<math>\beta</math></b>	: Growth differentiation factor $\beta$
<b>Hb</b>	: Hemoglobin
<b>HFE</b>	: HFE gene product

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## List of Abbreviations

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<b>HJV</b>	: Haemojuvelin
<b>HPFH</b>	: Hereditary persistence of fetal hemoglobin
<b>HPLC</b>	: High-performance Liquid chromatography
<b>HS</b>	: Hypersensitive sites
<b>HSM</b>	: Hepatosplenomegaly
<b>IL-<math>\gamma</math></b>	: interleukin - $\gamma$
<b>IL-<math>\gamma</math>R</b>	: IL- $\gamma$ receptor
<b>IQR</b>	: Interquartile range
<b>IVS</b>	: Intervening sequence
<b>LAR</b>	: Locus activating region
<b>LCR</b>	: Locus control region
<b>MCH</b>	: Mean corpuscular hemoglobin
<b>MCHC</b>	: Mean corpuscular hemoglobin concentration
<b>MCV</b>	: Mean corpuscular volume
<b>MDD</b>	: Minimum detectable dose
<b>MIC-<math>\gamma</math></b>	: Macrophage inhibitory cytokine – $\gamma$
<b>MMP</b>	: Mitochondrial membrane potential
<b>Mr</b>	: Monomer molecular mass
<b>mRNA</b>	: Messenger ribonucleic acid
<b>NAG-<math>\gamma</math></b>	: Nonsteroidal anti-inflammatory drug activated gene
<b>PB</b>	: Peripheral blood
<b>PCR</b>	: Polymerase chain reaction
<b>PDF</b>	: Prostate derived factor
<b>PLAB</b>	: Placental bone morphogenic protein
<b>PLT</b>	: Platelets

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## List of Abbreviations

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<b>PTGFb</b>	: Placental transforming growth factor-b
<b>RDW</b>	: Red cell distribution width
<b>ROC</b>	: Receiver operator curve
<b>SCF</b>	: Stem cell factor
<b>sHJV</b>	: Soluble form of Haemojuevelin
<b>Smad</b>	: Small molecules against decapentaplegic proteins
<b>STAT</b>	: signal transducer and activator of transcription
<b>TF</b>	: Transcription factor
<b>Tf</b>	: Transferrin
<b>TGF-β</b>	: Transforming growth factor-β
<b>TIBC</b>	: Total iron binding capacity
<b>TLC</b>	: Total leucocytic count
<b>TM</b>	: Thalassemia major
<b>TNFα</b>	: tumour necrosis factor α
<b>TT</b>	: Thalassemia trait
<b>TWSG<sup>1</sup></b>	: Twisted gastrulation
<b>Tx</b>	: Thromboxne
<b>UPR</b>	: Unfolded protein response
<b>XBP-<sup>1</sup></b>	: X-box binding protein <sup>1</sup> (endoplasmic reticulum stress-activated transcription factor)
<b>γGT</b>	: Gamma glutamyl transferase

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## Introduction

The thalassemia syndromes (alpha and beta thalassemia) represent the most common causes of ineffective erythropoiesis (*Tanno et al., 2007*). The increased but ineffective erythropoiesis resulting in tissue iron overload (*Casanovas et al., 2007*) induces numerous endocrine diseases, hepatic cirrhosis, cardiac failure and even death (*Weatherall and Clegg, 2007*).

Hepcidin regulated intestinal absorption represents a principal mechanism for iron homeostasis in humans (*Donovan et al., 2007*). It is commonly believed that ineffective erythropoiesis inhibits expression of hepcidin, a hepatic peptide hormone secreted from liver that regulates the release of iron into the blood stream from duodenal enterocytes, hepatocytes and macrophages (*Ramey et al., 2007*). It was shown that hepcidin levels are decreased in individuals with beta thalassemia syndromes (*Kattamis et al., 2007*).

It's hypothesized that the erythroid expansion could influence the regulation of hepcidin expression through systemic release of transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily members that are secreted from erythroblasts during human erythropoiesis (*Tanno et al., 2007*).

The TGF- $\beta$  superfamily consists of numerous molecules that regulate cellular processes such as growth differentiation and oncogenesis. Members of the TGF- $\beta$  superfamily include

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**Introduction**

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several TGF- $\beta$  proteins, bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs) and other proteins (*De Caestecker and M, 2007*).

Growth differentiation factor 15 (GDF15), a member of the transforming growth factor  $\beta$  superfamily acts by inhibiting hepcidin expression, thereby contributing to iron overload in thalassemia syndromes (*Tanno et al., 2014*).

GDF15 measurements may be helpful for predicting ineffective or apoptotic erythropoiesis (*Tanno et al., 2014*); further evaluation of its levels in thalassemia patients may have useful clinical applications.

## **Aim of the Work**

In this study we aim to analyze GDF<sup>15</sup> levels in  $\beta$ -thalassemia patients in conjunction with their iron status and clinical presentation.

## Thalassemia

The thalassemys are a heterogeneous group of inherited anemias characterized by defects in the synthesis of one or more of the globin chains of hemoglobin. The result is imbalanced globin chain production, ineffective erythropoiesis, hemolysis and variable degrees of anemia (*Weatherall, 1997*).

### Geographic distribution and epidemiology

Thalassemia is the most common genetic disorder all over the world. Around 3% of the world population carries genes for  $\beta$ -thalassemia (*Omar et al., 1999*).

$\beta$ -thalassemia is distributed widely in Mediterranean populations, Middle East, parts of India and Pakistan, and throughout Southeast Asia. The disease is common in parts of the southern Soviet republics and in the People's Republic of China.  $\beta$ -thalassemia is rare in Africa, except for isolated pockets in West Africa, notably Liberia, and in parts of North Africa (*Weatherall and Clegg, 1991a*).

In Egypt  $\beta$ -thalassaemia is the most common genetically determined, chronic haemolytic anaemia with an estimated carrier rate of 9%–10,0% (*Madani et al., 1997*) and a gene frequency of 0,03. So, it was estimated that 1000/1,0 million per year live births suffer from thalassemia disease in Egypt (*Ahmed et al., 1997*).

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## Thalassemia

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The most common forms of thalassemia are those that are prevalent in the malarial tropical and sub-tropical regions where a few mutations have reached high gene frequencies because of the protection they provide against malaria. The epidemiology of the disease; however is changing due to a fall in total birth rate, prevention programs and recent population movements (*Swee, 1998*).



Figure (1): Geographic distribution of  $\beta$ -thalassemia (*Weatherall and Clegg, 2001*).

## Classification of Thalassemias

They are divided into two main classes,  $\alpha$  and  $\beta$  according to which globin chain is produced in reduced amounts (*Cornelis and Douglas, 1998*).

### $\beta$ -Thalassemias:

$\beta$ -thalassemias, which are caused by a decrease in the production of  $\beta$ -globin chains, are the most important types (*Cunningham et al., 1998*).  $\beta$ -thalassemia is divided into two main varieties: