

# Study of serum hepcidin in Hereditary hemolytic anemias

## *Thesis*

Submitted for partial fulfillment  
Of The MD Degree  
Of Pediatrics

## *Submitted by*

Hossam El-Din Maged Abdel Rahman Mohamed ***M.B.B.Ch,***  
***Misr University for Science and Technology (MUST) University***  
***Msc. Ain Shams University***

Under supervision of

Prof. Dr. Amal El Beshlawy

**Professor of Pediatrics**  
**Faculty of Medicine**  
**Cairo University**

Prof. Dr. Ibrahim El Araby

**Professor of Pediatrics**  
**Faculty of Medicine**  
**MUST University**

Dr. Mohamed Salah  
El-Din Mohamed  
**Lecturer of Pediatrics**  
**Faculty of Medicine**  
**(MUST) University**

Dr. Dina Hesham  
Ahmed  
**Lecturer of Clinical Pathology**  
**Faculty of Medicine**  
**Cairo University**

**2012**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا سبحانك لا علم لنا إلا  
ما علمتنا إنك أنت العليم  
الحكيم

صدق الله العظيم

(البقرة ٣٢)

Dedicated To:

**My Father & My Mother**

Maged Abdel-Rahman Mohamed

Sanaa Mohamed Ali

**My Wife & My Sons**

Dr. Nermeen Salah

Omar & Ali

**And my brother**

Dr. Mohamed Maged

# **ACKNOWLEDGMENT**

## **Heart to heart talk**

Glory to ALLAH , most high full of grace and mercy. To him I owe sincere prayers of thanks. Truly without his grace and guidance this work couldn't have been possible.

It gives me great pleasure to express deep and everlasting gratitude to Prof. **Dr. Amal El Beshlawy**, Professor of Pediatrics Cairo University, for choosing the subject and her continuous encouragement during supervision this work.

Also I wish to express my deepest gratitude to Prof. **Dr. Ibrahim El Araby**, Professor of Pediatrics, Misr University for Science and Technology (MUST), whose guide and help were a real drive to complete this work.

My deepest thanks to **Dr. Mohamed Salah El-Din Mohamed**, Lecturer of Pediatrics, Misr University for Science and Technology (MUST) for his kind help, indispensable advice, and valuable comments during the course of the study.

I am also indebted to **Dr. Dina Hisham Ahmed**, Lecturer of Clinical and Chemical Pathology, Cairo University, for her effort in the laboratory work and her meticulous help.

My hearty thanks and indebtedness to each and every member of my family is beyond measure for their understanding and support during the preparation of the whole work.

Last but not least, I submit my deep thanks for the great favour done to me and all colleagues of MUST giving all the chance and pave the way for better university education, I honestly express my great thanks to the founder of MUST being the first MUST graduate to have achieved M.D. degree.

# **Abstract**

## **Study of serum hepcidin in hereditary hemolytic anemias**

**(Key words): Hereditary haemolytic anemias, Thalassemia, hepcidin, iron overload disorders.**

---

Thalassemia, the most common genetic disorder in Egypt, is a major health problem with an estimated carrier rate of 5.3%-9%. Registered cases in large centers in Egypt in September 2007 were 9912 cases, and in Cairo University hematology clinic were 2597 cases. Heparidin, a 25 amino-acid peptide hormone synthesized in the liver is the key regulator of iron homeostasis. We measured the level of hepcidin in congenital chronic hemolytic anemias including sickle cell anemia, hereditary spherocytosis, thalassemia syndromes. This study revealed the decrease of hepcidin level in all congenital chronic hemolytic anemias in comparison to control. The use of hepcidin as an adjuvant therapy with oral iron chelators is important as it has a vital role in combating hemosidrosis.

## **LIST OF ABBREVIATIONS**

<b>ACD</b>	: Anemia of chronic disease
<b>AML</b>	: Acute myleogenous leukemia
<b>ATCUN</b>	: Amino terminal Cu and Ni binding
<b>BMD</b>	: Bone mineral density
<b>BMP</b>	: Bone morphogenetic protein
<b>CFU-E</b>	: Colony-forming unit-erythroid
<b>CHr</b>	: Reticulocyte hemoglobin content
<b>CKD</b>	: Chronic kidney disease
<b>CV</b>	: Coefficient of variance
<b>CVAD</b>	: Central vascular access devices
<b>DFO</b>	: Desferrioxamine
<b>DFP</b>	: Deferiprone
<b>DMT1</b>	: Divalent metal transporter 1
<b>DNA</b>	: Deoxy ribonucleic acid
<b>EPO</b>	: Erythropoietin
<b>ESAs</b>	: Erythropoiesis-stimulating agents
<b>FE (2+)</b>	: Ferrous
<b>FE (3+)</b>	: Ferric
<b>FID</b>	: Functional iron deficiency
<b>FPN1</b>	: Ferroportin-1
<b>HAMP</b>	: Hepcidin antimicrobial peptide
<b>HAART</b>	: Highly Active Anti-Retroviral Therapy
<b>HB</b>	: Hemoglobin
<b>HCP</b>	: Heme-carrier protein

<b>HCV</b>	: Hepatitis C virus
<b>HFE</b>	: Haemochromatosis gene
<b>HH</b>	: Hereditary Haemochromatosis
<b>HIF</b>	: Hypoxia-inducible transcription factor
<b>HII</b>	: Hepatic iron index
<b>HJV</b>	: Haemojuvelin
<b>HO</b>	: Heme oxygenase
<b>HS</b>	: Hereditary spherocytosis
<b>HSCT</b>	: Hematopoietic stem cell transplantation
<b>ID</b>	: Iron deficiency
<b>IDA</b>	: Iron deficiency anemia
<b>IFN</b>	: Interferon
<b>IFU</b>	: Instructions for use
<b>IL</b>	: Interleukin
<b>IRE/IRP System</b>	: Iron responsive element/Iron regulatory protein
<b>IRIDA</b>	: Iron refractory iron-deficiency anemia
<b>LEAP</b>	: Liver-expressed antimicrobial peptide
<b>LIC</b>	: Liver iron concentration
<b>LPS</b>	: Lipopolysaccharide
<b>MCV</b>	: Mean corpuscular volume
<b>MCHC</b>	: Mean corpuscular hemoglobin concentration
<b>MDS</b>	: Myelodysplastic syndromes
<b>MS</b>	: Mass spectrometry
<b>OD</b>	: Optical density
<b>OPG</b>	: Osteoprotegerin
<b>OsM</b>	: Oncostatin M

<b>RA</b>	: Renal anemia
<b>RA</b>	: Rheumatoid arthritis
<b>RBC</b>	: Red blood cell
<b>RES</b>	: Reticuloendothelial system
<b>RGM</b>	: Repulsive Guidance Molecule
<b>rHuEPO</b>	: Recombinant human erythropoietin
<b>RIA</b>	: Radioimmunoassay
<b>SELDI-TOF MS</b>	: Surface-enhanced laserdesorption/ ionization time- of- flight mass spectrometry
<b>SCD</b>	: Sickle cell disease
<b>SQUID</b>	: Superconducting quantum-interference device
<b>TI</b>	: Thalassemia intermedia
<b>TM</b>	: Thalassemia major
<b>TMPRSS6</b>	: Transmembrane serine protease- 6
<b>TNF</b>	: Tumour necrosis factor
<b>TR</b>	: Transferrin receptor
<b>TS</b>	: Transferrin saturation
<b>VHL</b>	: Von Hippel–Lindau



## **LIST OF TABLES**

Table	Title	Page
1.	Iron absorption	15
2.	Positive and negative regulators of hepcidin production	30
3.	Proteins involved with hepcidin regulation of iron transport	42
4.	Major categories of liver iron overload	58
5.	Major categories of hereditary hemochromatosis	59
6.	Improvements in Supportive Care of $\beta$ –Thalassemia	82
7.	Chronic hemolytic anemia types in the study group	89
8.	Comparison between thalassemia major and intermedia as regard general data	91
9.	Comparison between cases and controls as regard general data	92
10.	Comparison between cases and controls as regard laboratory data	93
11.	Comparison between thalassemia major versus intermedia as regard laboratory data	97
12.	Distribution of cases as regard number of blood transfusion	102
13.	Comparison between thalassemia major versus intermedia as regard number of blood transfusion	102
14.	Clinical data (associated findings) concerning cases group	104
15.	Comparison between TM patients and TI patients as regard associated findings	105
16.	Correlation between hepcidin versus general data among cases	106
17.	Correlation between hepcidin( pretransfusion) versus	107

laboratory data among cases	
18. Correlation between hepcidin (pretransfusion) versus number of blood transfusion among cases	111
19. Comparison between hepcidin (pretransfusion) versus type of chelating agent	112
20. Comparison between males and females as regard hepcidin among both groups	112
21. Comparison between hepcidin pre and post transfusion	113
22. Comparison between negative and positive CRP patients as regard hepcidin pretransfusion	113
23. Validity of hepcidin (pretransfusion) in case of iron overload	115

## **LIST OF FIGURES**

Figure	Title	Page
1.	Iron uptake and recycling	8
2.	Main pathways of iron absorption by enterocytes in mammals	9
3.	Regulation of intestinal iron absorption	11
4.	Iron homeostasis	12
5.	Incorporation of iron from plasma transferrin into haemoglobin in developing red cells	14
6.	Main pathways of iron storage and exportation by hepatocytes in mammals	18
7.	Effects of inflammation on erythropoiesis and iron homeostasis in mammals	19
8.	Molecular structure of human synthetic hepcidin-25	26
9.	The physiological equilibrium of hepcidin	37
10.	Hepcidin expression	41
11.	Model of pathways involved in hepcidin regulation	43
12.	Morbidities related to iron overload in relation to age	68
13.	Management of Thalassemia and Treatment-Related Complications	81
14.	Chronic hemolytic anemia types in the control group	89
15.	Diagram showing sex distribution in cases of control group	90
16.	Diagram showing sex distribution in cases of study group	90
17.	Plots of mean and 95% confidence interval serum hepcidin in cases and control group	94
18.	Mean values of laboratory data of the control group	95
19.	Mean values of laboratory data of the study group	95

20. Comparative analysis of Serum Hepcidin, Serum iron and Serum Ferritin between control and study groups	96
21. Comparative analysis of the laboratory parameters between control and study groups	96
22. Comparative analysis of Serum Hepcidin, Serum iron and Serum Ferritin between Thalassemia major and Thalassemia intermedia groups	98
23. Comparative analysis of the laboratory parameters between Thalassemia major and Thalassemia intermedia groups	98
24. Plots of mean and 95% confidence interval serum hepcidin in different types of chronic hemolytic anemia	99
25. Plots of mean and 95% confidence interval of serum iron in different types of chronic hemolytic anemia	99
26. Plots of mean and 95% confidence interval of serum ferritin in different types of chronic hemolytic anemia	100
27. Mean values of laboratory data of the Thalassemia major group	100
28. Mean values of laboratory data of the Thalassemia intermedia group	101
29. Plots of mean and 95% confidence interval of number of blood transfusion in different types of chronic hemolytic anemia	103
30. Comparison between TM patients and TI patients as regard clinical features	105
31. Scatter curve represent a significant positive correlation between age and hepcidin among cases	106
32. Scatter curve represent a significant positive correlation between AST and hepcidin among cases	108

33. Scatter curve represent a significant positive correlation between ALT and hepcidin among cases 108
34. Scatter curve represent a significant positive correlation between platelets and hepcidin among cases 109
35. Scatter curve represent a significant positive correlation between serum iron and hepcidin among cases 110
36. Scatter curve represent a significant positive correlation between serum ferritin and hepcidin among cases 110
37. Scatter curve represent a significant positive correlation between number of blood transfusion and hepcidin among cases 111
38. Scatter curve represent a significant positive correlation between CRP and hepcidin among cases 114

## **CONTENTS**

	Page
Introduction and Aim of the Work	<b>1</b>
Review of Literature	<b>4</b>
Iron physiology	<b>4</b>
Hepcidin hormone	<b>23</b>
Iron overload disorders	<b>56</b>
Management of iron overload disorders	<b>73</b>
Subjects and Methods	<b>83</b>
Results	<b>89</b>
Discussion	<b>116</b>
Summary and Conclusion	<b>133</b>
Recommendations	<b>138</b>
References	<b>139</b>
Arabic Summary	

## INTRODUCTION AND AIM OF THE WORK

---

Thalassemia is the most common genetic disorder in Egypt; it composes a major health problem with an estimated carrier rate of 5.3-9%. Registered cases in large centers in Egypt September 2007 were 9912 cases, and in Cairo University hematology clinic were alone 2597 cases (**El-Beshlawy et al., 2007**). Patients with thalassemia major requiring regular blood transfusions accumulate iron that is toxic to the heart, liver, and endocrine systems (**El-Beshlawy et al., 2008**).

Complications secondary to iron overload should essentially be prevented as the treatment is difficult and often lifelong. Endocrinopathies secondary to iron overload include hypogonadism, hypothyroidism, diabetes mellitus and hypoparathyroidism. Most of these occur towards the end of second decade of life and often require lifelong replacement therapy. Iron related cardiac disorders vary from rhythm disturbances to chronic heart failure. The latter being the chief cause of death in young adults with thalassemia major. They need inotropic and anti-arrhythmic medications (**Agarwal, 2009**).

**Park et al., (2001)** discovered Hepcidin, a 25 amino-acid peptide hormone synthesized in the liver, and described it as a key regulator of iron homeostasis. **Pigeon et al., (2001)** found that the expression of hepcidin mRNA in the mouse liver was increased in setting of iron overload. In **2005**, **Ganz** explained that downstream effect of hepcidin is to inhibit intestinal iron absorption, recycling of iron from the reticuloendothelial system, and mobilization of iron from hepatic stores. On the molecular level, this is achieved by hepcidin binding to, and inducing internalization of the cellular iron exporter ferroportin (**Nemeth**