Serum Hepcidin Level As An Indicator Of Iron Status And Disease Activity In Patients With Rheumatoid Arthritis

Thesis
Submitted for Partial Fulfillment of Master degree in Clinical Pathology

By

Rawda Ahmed Alaa El-Din
M.B.B.Ch
Ain Shams University

Supervised by

Professor/Dahlia Ahmed El-Sewefy
Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Professor/ Dalia Fayez Mohamed
Professor of Internal Medicine and Rheumatology
Faculty of Medicine - Ain Shams University

Doctor/ Heba Mostafa El-Maraghy
Lecturer of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Faculty of Medicine
Ain Shams University
2016

First thanks to **ALLAH** *to whom I relate any success in achieving any work in my life.*

All praise to Allah and all thanks, he guided and enabled me by his mercy to fulfill this thesis, which I hope to be beneficial for people.

I would like to express my deep appreciation and gratitude to ***Prof. Dahlia Ahmed El-Sewefy,*** *for her enormous effort, excellent guidance, supervision, advice and help during the entire course of this research.*

I am deeply grateful to ***Prof. Dalia Fayez Mohamed,*** *for her valuable help and supervision.*

I wish to express my sincere thanks and gratitude to ***Dr. Heba Mostafa El-Maraghy,*** *for her continuous help, valuable remarks, advice and supervision with continuous guidance through out this research.*

I am also grateful to my ***Dad*** *for his excellent guidance and powerful support.*

Dedicated to my family; my Parents and my husband. Thank you all for your continous help, cooperation, support, encouragement and understanding

Rawda Ahmed Alaa El-Din

List of Contents

Title Page No.

List of Tables

List of Figures

List of Abbreviations

Abb.	Full term
aa	Amino acid
ACD	Anemia of chronic disease
AI	Anemia of chronic inflammation
ALK2&3	Activin-like kinase or anaplastic lymphoma kinase 2&3
ANA	Antinuclear antibody
ANOVA	Analysis of variance
anti-CCP	Anti cyclic citrullinated peptide
BFU-e	Burst-forming unit-erythrocyte
BMP	Bone morphogenetic protein
BMPR	Bone morphogenetic protein receptor
CFU-e	Colony-forming unit-erythrocyte
CFU-G/M	Colony-forming unit-granulocyte/macrophage
CKD	Chronic kidney disease
CRP	C-reactive protein
DAS	Disease activity score
DMARDs	Disease-modifying antirheumatic drugs
E. Coli	Escherichia Coli
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ESAs	Erythropoiesis-stimulating agents
ESR	Erythrocyte sedimentation rate
ESRD	End stage renal disease
ETS	E26-transformation-specific
Fe-Tf	Holo-transferrin
Fig	Figure
GCs	Glucocorticoids
GFR	Glomerular filtration rate
HAMP	Hepcidin antimicrobial peptide
Hb	Hemoglobin
HCC	Hepatocellular carcinoma
HCT	Hematocrit
HD	Hemodialysis

List of Abbreviations **cont...**

Abb.	Full term
HFE	Product of a high iron gene
HH	Hereditary hemochromatosis
hr	Hour
HRP	Horseradish Peroxidase
IDA	Iron deficiency anemia
IF-γ	Interferon-gamma
IL-1	Interleukin-1
IL-6	Interleukin-6
IQR	Interquartile range
IRF-1	Interferon regulatory factor-1
IV	Intravenous
Jn	Junior
LEAP1	Liver expressed antimicrobial peptide
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MRI	Magnetic resonance imaging
mRNA	messenger ribonucleic acid
MTX	Methotrexate
OD	Optical density
P	Probability
r	Correlation
RA	Rheumatoid arthritis
RBCs	Red blood cells
RCC	Renal cell carcinoma
RDW	Relative distribution width
RF	Rheumatoid factor
rhEPO	Recombinant human erythropoietin
rPM	Repeat per minute
s. irom	Serum iron
SLE	Systemic lupus erythematosus
SMAD	Sma and Mad, Mothers against decapentaplegic
SSZ	Sulfasalazine

List of Abbreviations **cont...**

Abb.	Full term
STAT3	Signal transducer and activator of transcription
TfR1	Transferrin receptor-1
TIBC	Total iron binding capacity
TMP	3,3',5,5'-Tetramethylbenzidine
TNFα	Tumor necrosis factor alpha
UK	United Kingdom

Abstract

Pro-inflammatory stimuli result in the development of anemia of chronic disease through direct inhibition of erythropoiesis and indirect reduction of iron supplied for heme synthesis. This is associated with increased levels of hepcidin..

Hepcidin is an acute phase reactant protein synthesized in liver. It is iron homeostasis key regulator. Hepcidin limits intestinal iron absorption as well as iron release from hepatocytes and macrophages. Because inflammation increases hepcidin production and increased hepcidin reduces iron available for erythropoiesis, hepcidin likely has an important role in anemia of inflammation.

Key words: Amino acid **-** Anemia of chronic disease **–** Antinuclear antibody- Analysis of variance- Chronic kidney disease- Disease activity score

INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic systemic inflammatory disease causing tissue inflammation that is mostly localized at the level of synovial joints. Anemia' is a common morbidity' in patients with RA' ***(Sabau et al., 2013)***.

Anemia in RA is a process associated with chronic Inflammatory disease. It also may occur as iron deficiency mostly due to drug-induced gastrointestinal bleeding and disorders as well as iron redistribution into inflamed joint structure ***(Victor et al., 2014)***.

Chronic or acute inflammation induces hepcidin expression ***(Hentze et al., 2010)***. The inflammatory cytokine interleukin-6 (IL-6) directly regulates hepcidin expression ***(Wrighting et al., 2006).*** Hepcidin is an iron regulatory protein mainly produced in the liver ***(Zhang et al., 2013)***.

Elevated serum hepcidin decreases intestinal iron absorption due to the occurrence of changes in the molecule of the cell iron exporter – ferroportin, which leads to iron retention in macrophages and iron sequestration in the reticulo-endothelial system ***(Goodnough et al., 2010).*** Consequently, the total content of iron in the body is normal, but less is supplied for erythropoiesis ***(Victor et al., 2014).***

Hepcidin is type II acute phase protein. ***Sabau et al. (2013)***, suggest that hepcidin is a key mediator of persistent anemia of chronic inflammation.

Bone marrow biopsy is considered the best method for diagnosing iron deficiency anemia in the presence of inflammation, but has the disadvantage of being an invasive and expensive diagnostic tool. Ferritin is used as a marker for iron deficiency but even increased or normal values do not exclude with certainty the presence of iron deficiency, ferritin being an acute phase reactant which increases during inflammatory process ***(Patiu et al., 2007).***

Aim of the Work

The aim of this study is to evaluate serum hepcidin level in Rheumatoid Arthritis patients and to correlate it with iron status and disease activity.

Chapter one

HEPCIDIN

Hepcidin is a regulatory protein of serum iron produced mainly in the liver ***(Sabau et al., 2013). Park et al. (2001)*** isolated two cysteine- rich small peptides from human urine, these two peptides were encoded by hepatic messenger ribonucleic acid (mRNA). The 20 and the 25 amino acid peptides were named hepcidin because of their hepatic origin and because of their activity against bacteria and fungi. The liver is the major source of hepcidin expression. However, expression of hepcidin has also been documented in human, rat and mouse kidney ***(Kulaksiz et al., 2004)***.

Hepcidin is the central regulatory hormone of body iron metabolism. Its binding to the cellular iron exporting channel ferroportin causes its internalization and degradation, and thus decreasing iron efflux from iron exporting macrophages and enterocytes into plasma ***(Kemna et al., 2008)***.

Hepcidin synthesis is induced by inflammation and increased iron stores, whereas its suppression is induced by anemia, hypoxia and ineffective erythropoiesis in the bone marrow. Furthermore, hepcidin deficiency has a central role in the iron loading in thalassemias and hereditary hemochromatosis. It is induced during infection decreasing the available iron that is essential for pathogens ***(Bergmans et al., 2007)***.

Most of the evidence on regulation of hepcidin and its mode of action comes from mice and in vitro studies that use mRNA of hepcidin as a read out. Until recently, human studies were impeded as suitable assays were not available. Thus, although discoveries on the mode of action and regulation of hepcidin have had wide effects throughout the field, a lot of work remains to define the hepcidin role in healthy and diseased states ***(Ganz, 2008)***.

Current understanding of the iron metabolism regulation is based on a number of proteins, including ferroportin, hemojuvelin, iron regulatory proteins, transferrin, transferrin receptors, ferritin, divalent metal transporter 1, and hepcidin **(Andrew, 2005)**. Among these proteins, plasma transferrin and ferritin are generally measured in the laboratory as the total iron-binding capacity and an indicator of overall iron storage, respectively. The peptide hepcidin controls plasma iron levels through regulating the absorption of iron from the intestine and the release of iron from the intestine and from macrophages. Hepcidin is an acute-phase reactant which is induced by inflammation and has antimicrobial activity in vitro ***(Nicolas et al., 2002$_a$)***.

Structure:

Structurally, the hepcidin is a peptide resembling a hairpin connected together by four disulfide bonds. Hepcidin binding to its receptor requires one of the disulfide bonds ***(Nemeth et al., 2006)***.