Growth Differentiation Factor 15 Expression in Anemia of Chronic Disease and Iron Deficiency Anemia

Thesis

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بسم الله الرحمن الرحيم
وأنزل الله عليك
الكتاب والحكمة
وعلَّمَكَ مَا لَمْ تَكُن
تَعْلَمُ وكَانَ فَضْلُ
اللَّهِ عَلَيْكَ عَظِيماً
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I would like to dedicate this Thesis to my ***Family****,*
to them I will never find adequate words
to express my gratitude.

Also to my *Fiancé* ***for behaving tactfully***
and patiently during this work.

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List of Abbreviations

ACD : Anemia of chronic disease
AI : Anemia of inflammation
BM : Bone marrow
BMPs : Bone morphogenetic proteins
CBC : Complete blood count
CRP : C-reactive protein
DMT-1 : Divalent metal transporter-1
EGD : Esophagogastro-duodenoscopy
ELISA : Enzyme-linked immunosorbent assay
EPO : Erythropoietin
ESA : Erythropoiesis-stimulating agents
ESR : Erythrocyte sedimentation rate
FE : Serum iron
FEP : Free erythrocyte protoporphyrin
FISH : fluorescence in-situ hybridization (FISH)
Fpn : Ferroportin
GDFs : Growth and differentiation factors
HCP1 : Heme carrier protein 1
Hct : Hematocrit
HIV : Human immuno-deficiency virus
IBD : Inflammatory bowel disease
IDA : Iron deficiency anemia
IL : Interleukin
IRMA : Immunoradiometric assay
KDOQI : Kidney Disease Outcomes Quality Initiative

LR+ : Positive likelihood ratio
MCV : Mean corpuscular volume
MIC-1 : Macrophage inhibitory cytokine-1
NAG-1 : Non-steroidal anti-inflammatory drug activated gene
PB : Peripheral blood
PCV : Packed cell volume
PDF : Prostate-derived factor
PLAB : Placental bone morphogenetic protein
PTGFβ : Placental transforming growth factor-β
RDW : Red cell distribution width
rHuEPO : Recombinant human EPO
SD : Standard deviation
STAT-3 : Signal transducer and activator of transcription-3
TfR : Transferrin receptor
TGF-β : Transforming growth factor–β
TIBC : Total iron binding capacity
TNF-α : Tumor necrosis factor-alpha
UTR : Untranslated region

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Introduction

Growth differentiation factor 15 "GDF 15" is a divergent member of the transforming growth factor-B superfamily. It has been identified as a hypoxia-inducible gene product and as a molecule involved in hepcidin regulation ***(Lakhal et al., 2009).*** The "GDF 15" expression during late erythroid differentiation was discovered as part of an erythroblast transcriptome project ***(Tanno et al., 2010$_a$).*** Although "GDF 15" expression is associated with cellular stress or apoptosis, this hormone is a prerequisite for normal erythroid differentiation ***(Lakhal et al., 2009).***

The "GDF 15" is involved in ineffective erythropoiesis ***(Tanno et al., 2010$_a$)*** being strongly increased in B-thalassaemia and congenital dyserythropoietic anemia. The cytokine blocks hepcidin expression and increases iron absorption, thus, leading to iron loading in these anemias ***(Tamary et al., 2008).***In contrast, mildly increased "GDF 15" concentrations do not suppress hepcidin expression, as seen in-vitro and in patients with other types of iron-loading anemias (e.g., sickle cell anemia, myelodysplastic syndrome or pyruvate kinase deficiency) ***(Finkenstedt et al., 2009).***

Since intracellular iron availability affects "GDF 15" expression ***(Lakhal et al., 2009)***, recent studies are investigating the potential pathophysiological role of "GDF 15" in anemia of

chronic disease (ACD) and iron deficiency anemia (IDA). Moreover, detection of the association of "GDF 15" expression with serum iron parameters in patients suffering from IDA and ACD may be helpful to access the diagnostic and pathogenic impact of "GDF 15" in these anemias ***(Theurl et al., 2009).***

Anemia of chronic disease is a common clinical problem complicating diseases involving acute and chronic immune activation, such as infections, malignancies or autoimmune disorders ***(Weiss and Goodnough, 2005).*** The circulating concentrations of the "GDF 15" are significantly increased in ACD, most likely as a consequence of inflammation with yet not elucidated pathophysiological roles ***(Theurl et al., 2009).***

Iron deficiency anemia affects billions of persons worldwide. It develops when iron stores become insufficient to maintain normal erythropoiesis ***(Tanno et al., 2010$_b$).*** Iron depletion increases "GDF15" expression in cultured cells, and iron chelation causes a transient increase of "GDF 15" levels among human research subjects. Significant elevation of "GDF 15" was found among randomly chosen patients with ID, but other etiologies for increased "GDF 15" were not explored ***(Lakhal et al., 2009).*** Although "GDF 15" is probably linked to the degree of anemia and the need for erythropoiesis and iron homeostasis in IDA, the role of "GDF 15" in IDA is still controversial ***(Theurl et al., 2009).***

Human "GDF 15" protein level in cell supernatants and human sera is measured by Enzyme-linked immunosorbent assay (ELISA) or immunoradiometric assay. The ELISA is a sensitive, specific, quantitative and reliable technique, with relatively stable reagents. Moreover, it is quick, convenient, generally safe (no radioactive decay) ***(Harvey and Champe, 2008)*** and is used in a wide variety of laboratories.

Aim of the work

The aim of the present study is:

- Detection of GDF 15 expression in Egyptian patients with IDA and ACD.
- Comparison between GDF 15 level in IDA, ACD and healthy subjects.
- Correlation between GDF 15 level, iron profile and degree of anemia in both IDA and ACD.

Iron Metabolism

Body Iron:

Iron is critical to human life and important for growth. It plays the central role in the formation of hemoglobin, brain development and function, and muscle activity. Iron also functions in several key enzymes in energy production and metabolism including DNA synthesis ***(Wrighting and Andrews, 2008).***

Approximately 73% of body iron is normally incorporated into hemoglobin and 12% in the storage complexes "ferritin and hemosiderin". The remaining iron 15% is of great importance as it is incorporated into a variety of other iron-containing compounds of vital functions. Therefore, body iron can be considered as having two main components: functional iron and stoage iron ***(Anderson et al., 2009).***

The functional component of iron is found largely in the circulating hemoglobin and smaller quantity in body tissue, myoglobin and enzymes. A deficiency of iron in the functional component does not ordinarily occur until stores are completely exhausted. The storage component "ferritin and hemosiderin" in the liver and bone marrow, serves as source for functional component ***(Mun˜oz et al., 2009).***

Iron is required for the production of hemoglobin. One molecule consists of protein "globin" combined with four