Evaluation Of Serum Levels Of Erythroferrone And Hepcidin In Egyptian Beta Thalassemia Intermedia Patients And Its Correlation To Iron Overload

Thesis

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CONTENTS

List of Abbreviations

ALT	:	Alanine transaminase
ANC	:	Absolute neutrophil count
ARMS	:	Amplification refractory mutation system
AST	:	Aspartate transaminase
BMD	:	Bone mineral density
BMP	:	Bone morphogenetic protein
B-TI	:	Beta thalassemia intermedia
B-TM	:	Beta thalassemia major
CDAII	:	Congenital dyserythropoeitic anemia type II
CRDB	:	Covalent reverse dot blot hybridization
CT	:	Computed tomography
CVS	:	Chorionic Villus Sampling
DE-52	:	Diethylaminoethyl cellulose
DEXA	:	Dual Energy X-ray absorptiometry
DFO	:	Deferoxamine
DFP	:	Deferiprone
DFX	:	Deferasirox
DMT1	:	Divalent metal transporter-1
DNA	:	Deoxyribonucleic acid
ECG	:	Electrocardiogram
ELISA	:	Enzyme Linked Immunosorbent Assay
EPO	:	Erythropoietin
ERFE	:	Erythroferrone
FBS	:	Fetal blood sampling
Fe^{2+}	:	Ferrous state
Fe^{3+}	:	Ferric state
GDF-15	:	Growth differentiation factor 15
Hb	:	Hemoglobin
HbA_1	:	Hemoglobin A1
HbA_2	:	Hemoglobin A2
HbE	:	Hemoglobin E
HbF	:	Fetal hemoglobin

✍List of Abbreviations

HbH	:	Hemoglobin H
HBV	:	Hepatitis B virus
HCC	:	Hepatocellular carcinoma
HCP1	:	Heme carrier protein 1
HCV	:	Hepatitis C virus
HPLC	:	High-performance liquid chromatography
HU	:	Hounsfield units
JAK2	:	Janus Kinase 2
LCI	:	Labile cellular iron
LIC	:	Liver iron concentration
LVEDD	:	Left ventricular end diastolic dimesnsion
LVEF	:	Left ventricular ejection fraction
LVESD	:	Left ventricular end systolic dimension
MCH	:	Mean corpuscular hemoglobin
MCHC	:	Mean corpuscular hemoglobin concentration
MCV	:	Mean corpuscular volume
MRI	:	Magnetic Resonance Imaging
NIPD	:	Non-invasive prenatal detection
NTBI	:	Non-transferrin bound iron
NTDT	:	Non-transfusion-dependent thalassaemia
PAH	:	Pulmonary artery hypertension
PO4	:	Phosphorus
PTH	:	Parathyroid hormone
RBC	:	Red blood cells
RDW	:	Red cell distribution width
RVSP	:	Right ventricullar systolic pressure
SF	:	Serum ferritin
SQUID	:	Superconducting quantum interference devices
T3	:	Triiodothyronine
T4	:	Thyroxine
TDT	:	Transfusion-dependent thalassaemia
TIBC	:	Total iron-binding capacity

TIF	:	Thalassemia International Federation
TLC	:	Total leucocytic count
TMPRSS-6	:	Transmembrane protease serine-6
TSH	:	Thyroid stimulating hormone
TWSG1	:	Twisted gastrulation
WBC	:	White blood cells count
WHO	:	World Health Organization

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ABSTRACT

Background and aims: Iron overload is an important feature of beta thalassemia, as it mediates much of the morbidity and mortality of the disease. Currently, iron overload is assessed with laboratory markers such as serum ferritin and imaging studies such as magnetic resonance imaging (MRI) T2* on the liver and the heart. However, they are not without their drawbacks, such as serum ferritin is not a specific marker, rises in many acute inflammatory conditions and MRI is not widely available and is a relatively expensive investigation. This study aimed at investigating the accuracy of evaluation of serum levels of erythroferrone and hepcidin in predicting iron overload and its complications. Methods: We performed this cross-sectional case-control study with a total number of 56 participants; 40 cases with beta thalassemia intermedia and 16 healthy age and sex matched controls. Patients were recruited from the outpatient clinic of the Clinical Hematology and Oncology Unit, Internal Medicine Department, Ain Shams University, Cairo, Egypt during the period between January 2018 and July 2018. We collected blood samples and measured the levels of serum erythroferrone and hepcidin. We compared the ability of these levels to predict iron overload. Results: cases had lower mean level of hepcidin in relation to controls being 170.250 ± 108.663 ng/ml versus 278.125 ± 72.316 ng/ml respectively. As for erythroferrone levels; the marker of the study; cases had a mean value which is nearly triple that of controls. Cases had a mean of 638.500 ± 407.567 ng/l; however, controls had a mean of 252.500 ± 83.944 ng/l. Upon correlating erythroferrone to ferritin and hepcidin, it was not correlated to either of them with P-values of 0.145 and 0.122. Also, no significant correlation between it and iron overload complicatons. Conclusions: hepcidin and erythroferrone cannot be used as markers of iron overload.

Keywords: hepcidin, errythroferrone`

INTRODUCTION

β-Thalassemia is a hereditary anemia characterized by ineffective erythropoiesis and hemolysis ***(Rund and Rachmilewitz, 2005).*** The underlying mechanism is defective production of hemoglobin β-chains, resulting in excess of α-chains, which are unstable and precipitate to form intracellular inclusion bodies. This excessive intracellular deposition of α-chain material is responsible for accelerated apoptosis of the erythroid precursors and for peripheral hemolysis of the erythrocytes ***(Pootrakul et al., 2000).***

Distinction between the various phenotypes of β-thalassemia relies primarily on the clinical severity of the disease, which should be assessed both at initial presentation and over a period of close follow-up ***(Rund and Rachmilewitz 2005).*** Beta thalassemia intermedia (β-TI) is a disease of intermediate severity where affected patients usually present with a later onset of microcytic anemia and milder clinical symptoms compared to beta thalassemia major (β-TM) ***(Taher et al., 2006).*** In contrast to patients with TM, in whom iron loading occurs mainly as a result of transfusion therapy, patients with TI accumulate iron primarily due to increased intestinal iron absorption ***(Origa et al., 2007).*** Excess iron is extremely toxic to all cells of the body and can cause serious and irreversible organic damage, such as cirrhosis, diabetes, heart disease, and hypogonadism ***(Melchiori et al., 2010).***

For the last decade, investigators have focused on understanding the mechanisms underlying iron overload in ineffective erythropoiesis, hypothesizing that correction of ineffective erythropoiesis would significantly reduce iron overload and improve anemia, ultimately increasing overall survival of patients with β-thalassemia. Hepcidin, a small peptide mainly produced by the liver, is absolutely required for the maintenance of systemic iron homeostasis in basal conditions ***(Nicolas et al., 2003).*** Hepcidin acts by binding to ferroportin, the sole known cellular iron exporter, leading to ubiquitination, internalization, and degradation of ferroportin in lysosomes. Decreased hepcidin production leads to the stabilization of ferroportin at the cellular membrane and promotes the absorption of dietary iron in the duodenum ***(Qiao et al., 2012).***

The erythroid regulator erythroferrone (ERFE) is produced by erythroid precursors in the marrow and the spleen and acts directly on the liver to decrease hepcidin production, and thereby increase iron availability for new red blood cell synthesis. ERFE also contributes to the recovery from anemia of inflammation by suppressing hepcidin and increasing iron availability. In contrast to its adaptive role in the physiological recovery from anemia, ERFE may also act pathologically to suppress hepcidin production and mediate iron overload and severe clinical complications in inherited anemias with ineffective erythropoiesis such as β-thalassemia ***(Kautz et al., 2014)***.

Aim of the work

The aim of the present study is to first measure levels of erythroferrone and hepcidin in the serum of adult patients with beta thalassemia intermedia. Next, to correlate their serum levels to each other. Finally, both will be evaluated for their relationships to degree of iron overload and its associated complications.

INTRODUCTION TO BETA THALASSEMIA

Anemia; definition

Anemia is a global public health problem affecting both developing and developed countries at all ages. According to the World Health Organization (WHO), anemia is defined as hemoglobin (Hb) levels <12.0 g/dL in women and <13.0 g/dL in men. However, normal Hb distribution varies not only with sex but also with ethnicity and physiological status. New lower limits of normal Hb values have been proposed, according to ethnicity, gender, and age. Anemia is often multifactorial and is not an parameters, the underlying pathological mechanism and patient history should be taken into account. ***(Cappellini and Motta, 2015)***

Symptoms and signs of anemia

In general, the signs and symptoms of anemia are unreliable in predicting the degree of anemia. Several factors determine the symptomatology of anemia, with time of onset and overall baseline health of the patient being the most important. Patients who gradually develop anemia over a period of months can tolerate lower hemoglobin owing to the use of compensatory mechanisms. Because blood delivers oxygen, many of the signs are related to lack of oxygen delivery, chiefly, fatigue and shortness of breath. On physical examination, anemia is manifested by paleness