Assessment of Serum Visfatin Level in Beta Thalassemia Patients

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By

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Amr Hany Mohamed Amin

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List of Abbreviations

Abbreviation	Meaning
AHSP.....	Alpha Hemoglobin Stabilizing Protein
AP-1, 2.....	Activator Protein-1, 2
AT.....	Adipose Tissue
AUC.....	Area Under Curve
BCL11a	B-Cell Lymphoma/Leukemia 11A
BMT	Bone Marrow Transplantation
CAD.....	Coronary Artery Disease
CBC.....	Complete Blood Count
CRP	C-Reactive Protein
CVD.....	Cardiovascular Disease
DFO.....	Desferrioxamine
DFP.....	Deferiprone
DNA	Deoxynucleic acid
DPP-4	Dipeptidyl Peptidase-4
dw	Dry Weight
ELISA.....	Enzyme-Linked Immunosorbent Assay
FN……..False Negative	
FP……...False Positive	
GH	Growth Hormone
Hb	Hemoglobin
HBV.....	Hepatitis B virus
HCC.....	Hepatocellular Carcinoma
hCG	Human Chorionic Gonadotrophin
Hct	Hematocrit
HCV.....	Hepatitis C Virus
HIV-I/II	Human Immunodeficiency Virus-I/II
hMG.....	Human Menopausal Gonadotrophin
HPLC.....	High Performance Liquid Chromatography
HPSC.....	Hematopoietic Stem Cells
HRP	Horseradish Peroxidase
HSM	Hepatosplenomegaly

List of Abbreviations (Cont.)

Abbreviation	Meaning
HSP70	Heat Shock Protein 70
HSPCs	Hematopoietic Stem and Progenitor Cells
ICAM-1	Intra Cellular Adhesion Molecule-1
IF	Immunofluorescence
IgG	Immunoglobulin G
ILs	Interleukins
iPSCs	Induced Pluripotent Stem Cells
IRA	Infarct-Related Artery
k2-EDTA	Potassium ethylene Diamine Tetra-Acetic Acid
MCH	Mean Corpuscular Hb
MCP-1	Monocyte Chemoattractant Protein-1
MCV	Mean Corpuscular Volume
MYB	Myeloblastosis
NAMPT	Nicotinamide Phosphoribosyltransferase
NAD	Nicotinamide Adenine Dinucleotide
NAT	Nucleic Acid Amplification Technology
NF-1	Nuclear Factor -1
NF-κB	Nuclear Factor-Kappa B
NGS	Next Generation Sequencing
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NIPD	Non-Invasive Prenatal Diagnosis
NMN	Nicotinamide Mononucleotide
NTBI	Non-Transferrin Bound Iron
O.D	Optical Density
PAI-1	Plasminogen Activator Inhibitor-1
PBEF	Pre-B Cell Colony-Enhancing Factor
PCR	Polymerase Chain Reaction
PH	Pulmonary Hypertension
RA	Rheumatoid Arthritis
RBC	Red Blood Corpuscles/Cells
Redox	Reduction-Oxidation

List of Abbreviations (Cont.)

Abbreviation	Meaning
ROC.....	Receiver Operating Characteristic Curve
ROS	Reactive Oxygen Species
SCA	Sickle Cell Anemia
SIR.....	Silent Information Regulator
STEMI	ST-Segment Elevation Myocardial Infarction
TMB	Tetramethylbenzidine
TN……..True Negative	
TP……...True Positive	
TNFα	Tumor Necrosis Factor α
TRL	Triglyceride Rich Lipoprotein
TSH	Thyroid Stimulating Hormone
TTIs	Transfusion Transmitted Infections
VOC.....	Vaso-Occlusive Crisis
α-thalassemia	Alpha Thalassemia
β-thalassemia	Beta Thalassemia
δ-thalassemia	Delta Thalassemia
β-TM.....	β-Thalassemia Major
β-TI……	β-Thalassemia Intermedia
β-TT.....	β-Thalassemia Trait
γ-globin.....	Gamma Globin

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Abstract

Background: Thalassemias are monogenetic hematologic disorders which are caused by faulty synthesis of one or more of the Hb chains which leads to imbalance in globin chains resulting in hemolysis and impaired erythropoiesis and chronic inflammatory condition. Visfatin is a pro-inflammatory adipocytokine which is mostly expressed in visceral adipose tissue and its testing may help in assessment of severity of the disease. **Aim of the work:** This study aims at measuring serum visfatin level in Beta thalassemia patients and its possible correlation to disease severity (Major and Intermedia). **Patients and methods:** Forty-one patients diagnosed as β-thalassemia (major, intermedia) and twenty age and sex-matched healthy individuals as the control group were tested for serum visfatin levels by enzyme-linked immunosorbent assay. **Results**: Serum visfatin was higher in the ß-thalassemia major (ß-TM) group than in the control group (P<0.001). Serum visfatin and serum ferritin were higher in ß-TM group than in ß-thalassemia intermedia group (ß-TI) (p< 0.001).Serum visfatin was found positively correlated with platelet count (P< 0.001) in the ß-TM group. As per ROC curve analysis, measured serum visfatin level was found to discriminate ß-TM group from β-TI group and control group. **Conclusion:** There is an association between serum visfatin and the degree of severity of β thalassemia disease. **Recommendations:** Testing of the relationship between serum visfatin level and other vascular inflammatory markers in β-thalassemia disease (as adiponectin, resistin, intracellular adhesion molecule (ICAM)) to clarify the exact mechanism of the inflammatory process in the disease and its complications to alter the course of the disease. Also to study serum visfatin in complicated β-thalassemia patients to establish its possible role in cardiac, vascular, endocrine or any other complications and comparing it with non-complicated patients.

Keywords: Beta thalassemia major, visfatin, inflammation.

Introduction

Thalassemia is the most common form of all inherited diseases of the red blood corpuscles (RBCs) ***(Higgs et al., 2012).*** It represents a major public health problem in Egypt. The carrier rate varies between 5.5% to ≥9% and it is estimated that there are 1000/1.5 million per year live births born with beta thalassemia (β-thalassemia) ***(El-Beshlawy et al., 2007)***.

Thalassemias are monogenetic hematologic disorders which are caused by faulty synthesis of one or more of the hemoglobin (Hb) chains. Decrease or complete absence in alpha or beta globin chains synthesis respectively leads to alpha (α) and beta (β) thalassemia. Imbalances in globin chains lead to hemolysis and impair erythropoiesis ***(Santosh, 2017).***

β-thalassemias are a group of hereditary hematological disorders caused by more than 300 changes of the adult β-globin gene of which the most common subgroup is Hb E β-thalassemia ***(Kountouris et al., 2014).***

Globally, β-thalassemia changes introducing gene deletions, splicing, or premature stop codons have the most prominent affect in terms of worldwide illness burden and clinical severity ***(Ip and So, 2013; Traeger-Synodinos and Harteveld, 2014).*** β-thalassemia causes varying degrees of anemia, which can range from severe to life-threatening. People of Mediterranean, Middle East, Africa, and Southeast

Asia descent are at higher risk of carrying the genes for thalassemia ***(Weatherall, 1997).***

A much rarer dominantly inherited β-thalassemia disease occurs in heterozygous individuals because of a highly unstable β-globin variant formation ***(Thein, 1999)***.

β-thalassemia includes three main forms: β-thalassemia major (β-TM) which is also known as "Mediterranean Anemia" and "Cooley's Anemia", β-thalassemia intermedia (β-TI) and β-thalassemia minor referred to as "beta-thalassemia carrier", "beta-thalassemia trait" (β-TT) or "heterozygous beta-thalassemia". In contrast to the uncommon dominant forms, individuals who have β-TM disease are homozygotes or compound heterozygotes for β0 or β+ genes, individuals who have β-TI disease are mostly homozygotes or compound heterozygotes and individuals with β-thalassemia minor are mostly heterozygotes. Because thalassemias are inherited disorders, they can result in significant issues, so newborn screening and prenatal diagnosis are important in treating patients ***(Chernoff, 1959).***

Silent carriers of alpha thalassemia (α-thalassemia) and people with α or β-TT show no symptoms of the disease and don't need treatment. Hemolytic anemia could be caused by α-TI disease (also known as Hb H disease). α-TM with Hb Bart's causes fatal hydrops fetalis ***(Santosh, 2017).***

β-TM results in hemolytic anemia, poor growth, and skeletal abnormalities during the first year of life. Affected children need blood transfusions for life. β-TI disease is less

severe when compared with β-TM disease and may require less often blood transfusions. By the age of 30, cardiac complications of iron overload often lead to death of β-TM individuals ***(Santosh, 2017).***

The survival of subjects with β-TM is dependent on the blood transfusion for life which is complicated by iron overload and its toxicity effects on various organs including the endocrine glands ***(Perera et al., 2010).***

Progress in the research of disease modifiers, ***(Thein, 2013)*** chemical modulation of gene expression, ***(Pourfarzad et al., 2013; Suzuki et al., 2014)*** and tools and approaches for deoxynucleic acid (DNA)-based therapies ***(Breda et al., 2013; Gaj et al., 2013)*** have opened new fields toward novel and more personalized strategies to manage or cure β-thalassemia ***(Gambari, 2012; Finotti and Gambari, 2014).***

Chronic inflammatory state with increased level of pro-inflammatory cytokines is believed to be present in patients with β-TM ***(Kanavaki et al., 2009)***. The most common studied adipocytokines are adiponectin and leptin and visfatin ***(Fukuhara et al., 2005)***.

Visfatin is a pro-inflammatory adipocytokine which is mostly expressed in visceral adipose tissue ***(Karachaliou et al., 2006; Moshtaghi-Kashanian et al., 2009)***. It appears that if we understand its possible role in the pathogenesis of β-TM and its relationship with markers of endothelial function may help in finding more effective therapeutic approaches for treatment of patients with β-TM and its related complications ***(Dehkordi et al., 2014)***.

Aim of the Work

This study aims at measuring serum visfatin level in β-thalassemia patients and its possible correlation to disease severity (β-TM, β-TI or β-thalassemia minor).