Pentraxin-3 level as a vascular endothelial marker in newly diagnosed and multi-transfused patients with Beta-thalassemia major: relation to oxidative stress

Thesis

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List of Abbreviations

Abb.	Full term
ACS	*Acute coronary syndrome*
AHSP	*α-Hb-Stabilizing Protein*
ALT	*Alanine aminotransferase*
AST	*Aspartate aminotransferase*
BP	*Blood pressure*
BP	*Blood pressure*
C4BP	*C4b-binding protein*
CBC	*Complete blood count*
CIMT	*Carotid intima media thickness*
CKD	*Chronic kidney disease*
CRP	*C reactive protein*
CTD	*Connective tissue disease*
cTnI	*Cardiac troponin I*
DFO	*Deferoxamine*
DFP	*Deferiprone*
DFX	*Deferasirox.*
ECM	*Extracellular matrix*
ECs	*Endothelial cells*
ESRD	*End stage renal disease*
GVHD	*Graft-versus-host disease*
Hb	*Hemoglobin*
HPLC	*High performance liquid chromatography*
HSC	*Hemopoietic stem cell*
HSPGs	*Heparin sulphate proteoglycans*
IL-6	*Interleukin-6*
IMT	*Intima-media thickness*
IRD	*Inflammatory rheumatic disease*
LDH	*Lactate dehydrogenase*
LIC	*Liver iron concentration*

List of Abbreviations (Cont...)

Abb.	Full term
MCH	*Mean cell hemoglobin*
MCV	*Mean cell volume*
MDA	*Malondialdehyde*
MI	*Myocardial infarction*
MRI	*Magnetic resonance imaging*
P5N-I	*Pyrimidine-5 nucleotidase-I*
PAH	*Pulmonary arterial hypertension*
PAMPs	*Pathogen-associated molecular patterns*
ROS	*Reactive Oxygen Species*
SMCs	*Smooth muscle cells*
TBARS	*Thiobarbituric Acid Reactive Substances*
TGF-β	*Transforming growth factor-β*
TLR	*Toll-like receptors*
TNF-α	*Tumor necrosis factor*
UAP	*Unstable angina pectoris*
WBC	*White blood cells*
β	*Beta*

Abstract

Background: Vascular endothelial damage starts at an early age in β-thalassemia patients and leads to serious cardiovascular diseases in further years. Pentraxin 3 (PTX-3) can be an indicator of vascular endothelial damage occurring with oxidative stress. **Aim:** To test the hypothesis that vascular endothelial dysfunction could occur in newly diagnosed minimally transfused as well as chronic transfusion-dependent young patients with β-thalassemia major, this study assessed the level of pentraxin-3 as a potential early marker for oxidative stress and assess its relation to markers of hemolysis, iron overload, lipid peroxidation as an index of oxidative stress and subclinical atherosclerosis. **Methods**: Sixty β-TM patients (30 newly diagnosed cases and 30 chronic transfusion dependent ones) without symptoms of heart disease were compared to 30 healthy controls and studied stressing on splenectomy, transfusion history, chelation therapy and mean serum ferritin in last year prior to the study. PTX-3 levels were measured by enzyme linked immunosorbent assay (ELISA) and MDA was assessed by measuring the production of Thiobarbituric Acid Reactive Substances (TBARS). Carotid intima media thickness (CIMT) was measured using Doppler ultrasound. **Results:** Serum MDA, PTX-3 and CIMT were significantly higher in all the studied β-TM patients than the control group ($p<0.05$). MDA and PTX-3 levels were significantly higher in both newly diagnosed and chronic β-TM patients compared with healthy controls with the highest levels found among chronic transfusion-dependent patients ($p<0.05$). CIMT was significantly higher in chronic transfusion-dependent β-TM patients compared with newly diagnosed and healthy controls ($p<0.05$) while no significant difference between the latter two groups ($p>0.05$). Comparison between the clinical data of newly diagnosed and chronic β-TM patients revealed significantly lower blood pressure, disease duration and transfusion index among newly diagnosed patients. The incidence of splenectomy and hepatitis C was higher among chronic β-TM patients. As regard laboratory and radiological data, it was found that bilirubin, liver enzymes, LDH, ferritin, MDA, PTX-3, and CIMT were significantly higher among chronic TM patients compared with newly diagnosed ones while other variables including blood counts were comparable between both groups. Pentarxin-3 levels were significantly elevated in splenectomized patients ($p=0.047$). Chronic β-TM patients with good compliance to chelation had lower pentarxin-3 levels than non-compliant patients ($p=0.036$). Both MDA and pentraxin-3 were positively correlated.

Significant correlations were found between pentraxin-3 and disease duration, transfusion index, total and indirect bilirubin, LDH and serum ferritin as well as CIMT among newly diagnosed β-TM patients. As regards chronic transfusion-dependent β-TM patients, significant correlations were found between pentarxin-3 levels and disease duration, WBC count, LDH, ferritin and CIMT. Multivariable linear regression analysis revealed that LDH, serum ferritin, MDA and CIMT were the significant independent variables related to increased pentraxin-3 levels in newly diagnosed or chronic patients with β-TM. **Conclusion:** We suggest that oxidative stress starts early in pediatric patients with β-TM even in newly diagnosed ones as defined by high levels of PTX-3 and MDA. This process aggravates in chronic transfusion-dependent patients. PTX-3 is a promising marker of oxidative stress in β-TM patients. The positive correlation between CIMT and PTX-3 suggests that it could be used as a marker of vascular dysfunction and subclinical atherosclerosis in β-TM patients. Thus, it could be useful for screening of patients at risk of cardiac complications later in adult life because this alteration occurs in early stage subclinical disease. Good selection of chelation therapy based on the patients' compliance, iron overload status is needed in order to decrease oxidative stress and the incidence of endothelial dysfunction among β-TM patients and consequently, lower PTX-3 levels.

INTRODUCTION

β-thalassemia is an inherited hemoglobin disorder caused by the impaired synthesis of the b-globin chain, resulting in chronic hemolytic anemia ***(Higgs et al., 2001)***. Iron overloading is a prominent feature in hemolytic anemia as β-thalassemia major owing to hemolysis of impaired erythrocytes as well as increased iron intestinal absorption and erythrocyte transfusion. This results in excessive free iron that represents an oxidative stress to the cells especially vascular endothelial cells. Vascular endothelial damage has a great impact on cardiovascular system with consequent diseases. This starts at an early age in thalassemia patients and leads to serious cardiovascular diseases in further years ***(Veerapol et al., 2008)***.

All of the newly diagnosed thalassemia major at one year later become transfusion dependent thalassemia major patients with ferritin level that keep up increasing ***(Hamizah et al., 2017)***.

It has been demonstrated that an increase in lipid peroxidation products such as malondialdehyde (MDA) as a result of oxidative stress is related to the occurrence of atherogenic vascular complications in thalassemia patients ***(Esposito et al., 2003; Amer and Fibach, 2005)***. In addition, the increased iron load has an impact on the thrombotic response to arterial injury, vascular production of reactive

oxygen species, and endothelium-dependent vasoreactivity ***(Kukongviriyapan et al., 2008)***.

Therefore, the assessment of endothelial functional impairment is a useful prognostic tool for monitoring cardiovascular diseases ***(Esposito et al., 2003; Amer and Fibach, 2005)***. It has been shown that one of the markers of vascular endothelial damage is pentraxin 3 (PTX-3) protein ***(Verma et al., 2003; Mantovani et al., 2003)***. PTX-3, the prototype of long pentraxins, is a discovered marker of the acute-phase inflammatory response, and plays an important role in innate immunity ***(Bottazzi et al., 2006; Garlanda et al., 2005)***. Similar to C reactive protein (CRP), PTX3 belongs to the PTX protein family ***(Hirschfield and Pepys, 2003)***. Unlike CRP, which is produced in the liver in response to interleukin-6 (IL-6), PTX3 is mainly produced at extrahepatic sites; endothelial cells, smooth muscle cells, mononuclear phagocytes, and dendritic cells in response to the inflammation and oxidative stress ***(Introna et al., 1996)***.

It is suggested that PTX-3 increases in response to oxidant stress and can be used as an early diagnostic marker for inflammation. PTX3 is a sensitive and specific biomarker for the diagnosis of cardiovascular diseases such as acute coronary syndrome (ACS) ***(Kume and Mitsuoka, 2011)*** and also in coronary artery disease patients with inflammatory rheumatic disease (IRD) ***(Hollan et al., 2010)***. On this basis, PTX-3 can be an indicator of vascular endothelial damage occurring with