Vascular dysfunction and its impact on coagulopathy in adult Egyptian thalassemic patients

Thesis

Submitted for partial fulfillment of master degree In Clinical hematology

Presented by

Magda Mamdouh Fahmy Hanna

M.B., B.Ch

Supervised by

Prof. Dr. Sozan Kamal Eldin Hussein

Professor of Internal Medicine & Clinical Hematology

Faculty of Medicine, Ain Shams University

Prof. Dr. Gihan Kamal Shams Eldin

Professor of Internal Medicine & Clinical Hematology
Faculty of Medicine, Ain Shams University

Dr. Hanaa Fathey Abdelsamee

Assistant Professor of Internal Medicine & Clinical Hematology

Faculty of Medicine, Ain Shams University

Faculty of Medicine
Ain Shams University
2018

الخلل الوعائى وتأثيره على الاعتلال الخثرى في مرضى انيميا□ البحر المتوسط المصريين البالغين□

رسالة

توطئة للحصول علي درجة الماجستير في امراض الدم الإكلينيكيه

مقدمة من

الطبيبة/ ماجدة ممدوح فهمى حنا□

بكالوريوس الطب و الجراحة

تحت إشراف

أ.د/ سوزان كمال الدين حسين□

أستاذ الباطنه العامه وأمراض الدم الإكلينيكيه

كلية الطب -جامعة عين شمس

أ.د/ جيهان كمال شمس الدين□

أستاذ الباطنه العامه وأمراض الدم الإكلينيكيه

كلية الطب -جامعة عين شمس

د/ هناء فتحى عبد السميع□

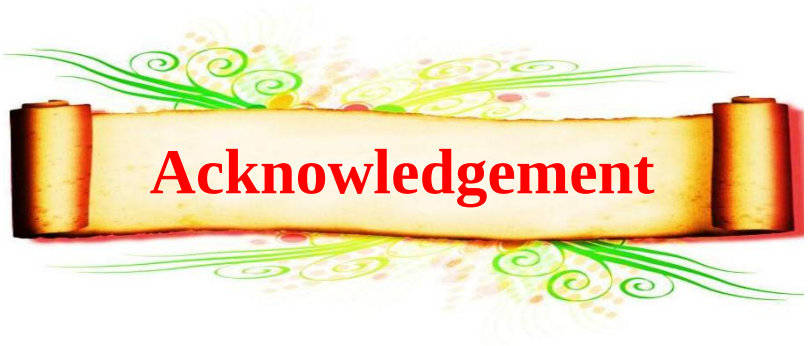
أستاذ مساعد باطنه عامه وأمرض الدم الإكلينيكيه

كلية الطب -جامعة عين شمس

كلية الطب

جامعة عين شمس

٢٠١٨



Acknowledgement

First and foremost thanks to God, the Most Merciful whose help is the main factor in accomplishing this work.

I wish to express my deep appreciation and sincere gratitude to my dear Professors for their kind supervision and great help especially,

Prof. Dr. Hanaa Fathey Abdelsamee, *Assistant Professor of Internal Medicine & Clinical Hematology, Ain Shams University, for her guidence , scientific assistance , close supervision, valuable instructions, continuous help, patience, advices and guidance. she has generously devoted much of her time and effort for planning and supervision of this study. It was a great honor to me to work under her direct supervision..*

Last and not least, I want to thank my dear husband , my wonderful mother, and my great father who gave me love, support ,and strength to continue this work .

Finally I would present all my appreciations to my patients without them, this work could not have been completed.

Contents

List of Abbreviations

ACS	:	Acute coronary syndrome.
ADAMT13	:	Disintegrin-like and metalloprotease with thrombospondin type-1motif 13.
AF	:	Atrial fibrillation.
CBC	:	Complete blood picture.
CD	:	Cluster of differentiation.
CEC	:	Circulating endothelial cells.
CVD	:	Cardio vascular disease.
DVT	:	Deep venous thrombosis.
ECs	:	Endothelial cells.
ELAM-1	:	E-selectin adhesion molecule-1.
EMPs	:	Endothelial microparticles.
EPCs	:	Endothelial progenitaor cells.
ERFE	:	Erythroferrone.
GDF15	:	Growth and differentiation factor 15.
GP	:	Glycoprotein.
Hb A	:	Adult hemoglobin.
Hb F	:	Fetal hemoglobin
HBB	:	Hemoglobin Beta
HCII	:	Heparin co -factor ii.
HIF	:	Hypoxia inducible factor.
HUVEC	:	Human umbilical vein endotelial cells.
ICAM-1	:	Intercellular adhesion molecule-1
MCV	:	Mean corpuscular volume.
mMps	:	Monocytic microparticles.
MPA	:	Monocyte platelets aggregate
Mps	:	Microparticles.
NO	:	Nitric oxide.
NTDT	:	Non transfusion dependant.
NVAF	:	Non valvular atrial fibrillation.
PAD	:	Peripheral arterial disease.
PAOD	:	Peripheral arterial occlusive disease.
PC	:	Platelet count.
PE	:	Phosphatidyl ethanoleamine.

PG	:	Prostacyclin.
PHT	:	Pulmonary hypertension.
Ps	:	Phosphatidyl serine.
PSGL1	:	P-selectin glycoprotein ligand-1.
RBCs	:	Red blood cells.
RDW	:	Red cell diameter width.
TEE	:	Thromboembolic events.
TEG	:	Thromboelastography.
TF	:	Tissue factor.
TGT	:	Thrombin generation test.
TI	:	Thalasemia intermediate.
TIBC	:	Total iron binding capacity.
TM	:	Thalasemia major.
TSP-1	:	Thrombospondin.
TTP	:	Thrombotic thrombocytopenic purpura.
TWSG1	:	Twisted gastrulation protein homolog 1.
ULvWF	:	Ultra large von willebrand factor.
VCAM-1	:	Vascular cell adhesion molecule-1
vWFa	:	Von willebrand factor antigen.
WBCS	:	White blood cells.

List of Table

List of Figures

Abstract

This study aimed to clarify and update the role of endothelial dysfunction and Monocytes among adolescents with transfusion dependant β- thalassemic patients using von Willebrand factor antigen (VWF:Ag) and flow cytometric analysis of circulating CD14 monocyic Micropartiles (mMps) and CD11b for monocytes activation and assess their relation to hypercoagulopathy and thrombosis in these patients.This study showed that VWF Ag was higher in patients than controls and was positively correlated to thrombotic events in thalassemic patients,.There was no significance difference for CD14 between patients and controls and CD11b was higher in controls.Additionally, splenectomized patients had positive correlation with thrombosis. Iron overload caused monocytic dysfunction that could participate in decresed CD11b level and affected its role in blood coagulation.

Key Words:

Endothelial dysfunction, Monocytes, β- thalassemic, VWF: Ag, CD14, CD11b, thrombosis

Introduction

Thalassemias described as a group of inherited hemolytic anemia due to differential expressions of α or β globin genes. In β -thalassemia β globin synthesis is disturbed leading to excess α chain in red blood cell (RBC) cytosol ***(Karmakar et al., 2016)***.

Beta thalassemia is classified into three types depending on severity of symptoms : thalassemia major (Cooly's anemia) which is a transfusion dependant disorder, thalassemia intermediate which encompasses a wide spectrum of clinical severities, and thalassemia minor that is symptomless ***(Thein, 2013)***.

In 2012 Cappellini et al., found that there is a high incidence of thromboembolic events (TEE) in thalassemic patients and that has led to the identification of a hypercoagulable state in these patients.

Although, Thalassemia intermediate (TI) patients have a milder clinical phenotype than patients with thalassemia major (TM) , they have the highest incidence of TEE ***(Musallam et al., 2012)*.**

The endothelium has a key role in vascular homeostasis by the releasing a variety of factors that interact with platelets, inflammatory cells and the vessel wall. Patients receiving regular blood transfusions have increased iron load that has an impact on the thrombotic response to arterial

injury, and endothelium-dependent vasoreactivity ***(Anderson, 2006).***

Von Willebrand factor antigen (VWF: Ag) can be used as a marker of endothelial dysfunction in many vascular diseases.It is important in the aggregation and the adhesion of platelets to subendothelial cells, when levels of circulating VWF are incresed , this may promote atherosclerosis and contribute to hypercoagulability events ***(Horvath et al., 2004).***

Microparticles (MPs) are small plasma membrane vesicles which have procoagulant function that is related to the presence of Phosohatidylserine (PS) on the outer membrane and they release tissue factor (TF) that plays an important role in coagulation ***(Mooberry and Key, 2016).***

In 2014, ***Trzepizur*** and his collegues found that endothelial dysfunction was associated with elevated levels of patelets-Microparticles(CD41,CD42,CD61),endothelial-Microparticles(CD31,CD34,CD146)and leukocytic derived Microparticles (CD19,CD20,CD3,CD5,CD16,CD14).

MPs had been reported to be associated with an increased risk for both arterial and venous thrombosis and elevated levels of MPs have been reported in thalassemia ***(van Beers et al., 2015)***.

Endothelial microparticles (EMPs) are small vesicles released from disturbed endothelial cells and ***(van Ierssel et***

al., 2012) and have recently been reported as a marker of endothelial injury and systemic vascular remodeling.

Endothelial microparticles can promote a prothrombogenic and proinflammatory effect leading to vascular dysfunction ***(Sabatier et al., 2014)***.

Aim of the work

Aim of this study was to clarify the role of monocytes, platelets and endothelial activation in the pathogenesis of prothrombotic and hypercoagulable state among transfusion dependant adult Egyptian β-thalassemic patients in different types of ß-thalassemia with or without splenectomy by measuring level of vWFA as a marker of endothelial dysfunction, level of cd14 as marker for monocyte microparticles and cd11b as marker for monocyte activation .

Thalassemia

Beta-thalassemia is an autosomal recessive hemoglobinopathy diorder, it affects the production of the β-globin chains of the hemoglobin. Beta globin gene mutations on chromosome 11 leads to impairment in the production of β-globin chains with accumulation of excess α-globin chains and formation of insoluble hemichromes ***(Makis et al., 2016)***.

The **globins** are a superfamily of heme-containing globular proteins, involved in binding and/or transporting oxygen. These proteins all incorporate the globin fold, a series of eight alpha helical segments. Two prominent members include myoglobin and hemoglobin. Both of these proteins reversibly bind oxygen via a heme prosthetic group. They are widely distributed in many organisms **(Vinogradov et al., 2007)**.

All normal human globins found in adults have one pair of α-chains.The α-chains can combine with β-chains ($\alpha_2\beta_2$), δ-chains ($\alpha_2\ \delta_2$ **)** and γ-chains ($\alpha_2\gamma_2$) (**Hardison and Ross, 2012**).

The β globin chains are encoded by a single gene on chromosome 11;α globin chains are encoded by two closely linked genes on chromosome 16 (**Petrou and Mary**, **2010**). So,in a normal person with two copies of each chromosome,