

**Circulating Endothelial Microparticles
(CD144+) and von Willebrand Factor Antigen
as Markers of Endothelial Dysfunction in
 β -Thalassemia Patients**

Thesis

*Submitted for partial fulfillment of M.Sc degree
in Paediatrics*

By

Mohammed Mahmoud SamyWahba

M.B., B. Ch.

Faculty of Medicine -Ain Shams University

Under Supervision of

Dr. Amira Abd El Moneam Adly

Assistant Professor of Pediatrics

Faculty of Medicine –Ain Shams University

Dr. Eman Abd El Rahman Ismail

Assistant Consultant of Clinical Pathology

Faculty of Medicine –Ain Shams University

Dr. Nayera Hazaa Khalil El Sherif

Lecturer of Pediatrics

Faculty of Medicine –Ain Shams University

Faculty of Medicine
Ain Shams University

2013

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَأَنْزَلَ اللَّهُ عَلَيْكَ
تَابِوُ الْحِكْمَةِ وَعَلَّ
مَكَّمَا لَمْ تَكُنْ تَعْلَمُ
كَانَ فَضْلُ اللَّهِ عَلَيْكَ
كَعَظِيمًا

صدق الله العظيم

سورة النساء

آية (113)

LIST OF CONTENTS

Title	Page No.
List of Abbreviations	i
List of Tables.....	iii
List of Figures.....	vii
Introduction.....	1
Aim of the work.....	3
Review of Literature	
Normal endothelium.....	4
Vascular Dysfunction In β -Thalassemia.....	32
Subjects and methods	48
Results.....	58
Discussion.....	98
Conclusion.....	118
Recommendations.....	119
References.....	120
Arabic Summary.....	—

LIST OF ABBREVIATIONS

Abbrev.	Full term
AF	: Atrial fibrillation
APC	: Activated protein C
BMI	: Body mass index
CAM	: Cell adhesion molecule
CBC	: Complete blood count
CECs	: Circulating endothelial cells
CEPCs	: Circulating endothelial progenitor cells
cGMP	: Cyclic guanylate monophosphate
DDAVP	: 1-desamino-8-D-arginine vasopressin
DMT1	: Divalent metal transporter 1
EC	: Endothelial cells
ED	: Endothelial dysfunction
EDHF	: Endothelium derived hyperpolarizing factor
EMPs	: Endothelial derived microparticles
eNOS	: Endothelial NO synthase
EPCR	: Endothelial protein C receptor
ET-1	: Endothelin-1
GSTM1	: Glutathione S-transferase-M1
HMEC-1	: Human microvascular endothelial cell-1
HPLC	: High performance liquid chromatography
ICAM-1	: Intracellular cell adhesion molecule-1
IL-1K	: Interleukin-1K
IQR	: Interquartile Range
IRAK1	: Interleukin receptor-associated kinase 1
LDH	: Lactate dehydrogenase
LPS	: Lipopolysaccharide
MDCT	: Multidetector computed tomography
MMP	: Matrix metalloproteases
MPs	: Microparticles
NF	: nuclear factor

LIST OF ABBREVIATIONS*(Cont...)*

Abbrev.	Full term
<hr/>	
NO	: Nitric oxide
PAI-1	: Plasminogen activator inhibitor
PAR-1	: Proteinase-activated receptor1
PE	: Phycoerythrin
PNH	: Paroxysmal nocturnal haemoglobinuria
PS	: Phosphatidylserine
PSGL-1	: P-selectin glycoprotein ligand 1
RBC	: Red blood cell
RTKs	: Receptor tyrosine kinases
SCD	: Sick cell disease
SD	: Standard deviation
SPSS	: Statistical Program for Social Science
sTM	: Soluble thrombomodulin
TFPI	: Tissue factor pathway inhibitor
TF	: Tissue factor
TM	: Thrombomodulin
TNF	: Tumor necrosis factor
t-PA	: Tissue plasminogen activator
TRAIL	: Tumor Related apoptosis-inducing ligand
uPAR	: Urokinase plasminogen activator receptor
VCAM-1	: vascular cell adhesion molecule-1
VEGF	: Vascular endothelial growth factor
VEGFR-1	: Vascular endothelial growth factor receptor-1
vwFag	: von Willebrand factor antigen
WBC	: White blood cell

LIST OF TABLES

Tab. No.	Title	Page No.
Table (1):	Demographic data of beta thalassemia patients	58
Table (2):	Demographic data of beta thalassemia patients	59
Table (3):	Demographic data of control.....	59
Table (4):	Clinical data of beta thalassemia patients	60
Table (5):	Laboratory data of beta thalassemia patients	61
Table (6):	Echocardiographic data of beta thalassemia patients.....	62
Table (7):	Comparison between β -thalassemia Patients and control as regard quantity of endothelial microparticles (CD144 ⁺ cells) & serum VWF ag level.....	62
Table (8):	Comparison between β -thalassemia major and β -thalassemia intermedia Patients as regard quantity of endothelial microparticles (CD144 ⁺ cells)& serum VWF ag level.	64
Table (9):	Comparison between both groups as regard demographic & clinical data.....	65
Table (10):	Comparison between both groups as regard Frequency of Blood Transfusion, Type of PRBCs and Transfusion index	66
Table (11):	Comparison between both groups as regard type of iron chelation & compliance to chelation.	67
Table (12):	Comparison between both groups as regard anthropometric measurements and Puberty.	68
Table (13):	Comparison between both groups as regard Laboratory finding.....	69
Table (14):	Comparison between both groups as regard echocardiographic findings	70

LIST OF TABLES *(Cont...)*

Tab. No.	Title	Page No.
Table (15):	Comparison between between both groups as regard quantity of endothelial microparticles (CD 144 ⁺ cells) & serum VWF ag level.....	71
Table (16):	Comparison between splenectomized & non splenectomized β -thalassemia Patients as regard demographic & clinical data.....	73
Table (17):	Comparison between splenectomized & non splenectomized β -thalassemia patients as regard frequency of blood transfusion, type of PRBCs, transfusion indexand mean pretransfusion Hb in last 2 years.	74
Table (18):	Comparison between splenectomized & non splenectomized β -thalassemia patients as regardtype of iron chelation & compliance to chelation.	75
Table (19):	Comparison between splenectomized & non splenectomized β -thalassemia patients as regard quantity of endothelial microparticles (CD144 ⁺ Cells) & serum VWF ag levels.	76
Table (20):	Comparison between β -thalassemia patients on monotherapy and combined chelation therapy as regard mean serum ferritin in last 2 years prior evaluation.	78
Table (21):	Comparison between β -thalassemia patients on monotherapy and combined chelation therapy as regard quantity of circulating endothelial micro-particles (CD144 ⁺ Cells) & serum VWF ag levels.	79
Table (22):	Comparison between β -thalassemia patients on monotherapy and combined chelation therapy as regard echocardiographic findings.	81

LIST OF TABLES *(Cont...)*

Tab. No.	Title	Page No.
Table (23):	Correlation between quantity of endothelial microparticles (CD144 ⁺ cells) and age at evaluation, disease duration and Age of diagnosis among studied beta thalassemia patients.....	82
Table (24):	Correlation between quantity of endothelial microparticles (CD144 ⁺ cells) and mean pre-transfusion Hb in last 2 years prior to evaluation, transfusion index and frequency of transfusion among studied beta thalassemia patients.	83
Table (25):	Correlation between quantity of endothelial microparticles (CD144 ⁺ cells) and anthropometric measurements (weight SDS, height SDS, BMI) among studied beta thalassemia patients.....	85
Table (26):	Correlation between quantity of endothelial microparticles (CD144 ⁺ cells) & finding among studied beta thalassemia patients.....	85
Table (27):	Correlation between quantity of endothelial microparticles (CD144 ⁺ cells) & laboratory findings among studied beta thalassemia patients.	87
Table (28):	Association between quantity of endothelial microparticles (CD144 ⁺ cells) & type of iron chelation and compliance to chelation among studied beta thalassemia patients.....	88
Table (29):	Association between quantity of circulating endothelial microparticles (CD144 ⁺ cells) & history of hepatitis C virus infection.	88
Table (30):	Correlation between quantity of endothelial microparticles (CD144 ⁺ cells) & serum VWF ag level and mean serum ferritin last 2 years prior evaluation among studied beta thalassemia patients.....	89

LIST OF TABLES *(Cont...)*

Tab. No.	Title	Page No.
Table (31):	Correlation between quantity of endothelial microparticles (CD144 ⁺ cells) and serum bilirubin (total and indirect) among studied beta thalassemia patients	91
Table (32):	Correlation between serum VWF ag level & patients age at evaluation, disease duration and age at diagnosis among studied beta thalassemia patients.....	91
Table (33):	Association between serum VWF ag level & history of hepatitis C virus infection	93
Table (34):	Correlation between serum VWF ag level and mean pretransfusion Hb in last 2 years prior to evaluation, transfusion index and frequency of transfusion among studied beta thalassemia patients.	93
Table (35):	Correlation between serum VWF ag level & anthropometric measurements (weight SDS, height SDS, BMI) among studied beta thalassemia patients.	94
Table (36):	Correlation between serum VWF ag level & echocardiographic finding among studied beta thalassemia patients.	95
Table (37):	Correlation between serum VWF ag level & patients laboratory finding among studied beta thalassemia patients.	95
Table (38):	Associations between quantity of serum VWF ag level & type of iron chelation and compliance to chelation among studied beta thalassemia patients.	96
Table (39):	Correlation between serum VWF ag and mean serum ferritin in last 2 years prior evaluation among studied beta thalassemia patients.....	96
Table (40):	Correlation between serum VWF ag and markers of hemolysis (total & direct bilirubin) among studied beta thalassemia patients.....	97

LIST OF FIGURES

Fig. No.	Title	Page No.
Figure (1):	Mechanisms of detachment of endothelial cells	13
Figure (2):	Pathogenetic implications of CECs.....	13
Figure (3):	Pathways and modes of vWF secretion from endothelial cells.....	17
Figure (4):	Schematic representation of the panel of molecules conveyed by EMP and the associated biological effects.	23
Figure (5):	Mechanismsof EMP formation. Thrombin stimulation of endothelial cells induces a complex release of EMP	25
Figure (6):	Endothelial Microparticles released after endothelial activation are at the crossroad of thrombosis and angiogenesis and participate in a cross-talk between inflammation and coagulation.	28
Figure (7):	Pathophysiology of hypercoagulable state and platelet activation in thalassaemia and sickle cell disease (SCD).....	37
Figure (8):	Diagram of the roles of von Willebrand factor in platelet adhesion to the damaged wall of the blood vessel and subsequent platelet aggregation.....	40
Figure (9):	pathophysiology of iron cardiomyopathy, artificially divided into iron uptake, iron storage, and iron toxicity.....	44
Figure (10):	the complex pathophysiology of pulmonary hypertension in thalassemia.....	46
Figure (11):	Association of arginase activity with hemolytic rate.	47

LIST OF FIGURES *(Cont...)*

Fig. No.	Title	Page No.
Figure (12):	Flow cytometric analysis of CD144+ endothelial microparticles (CD144+ EMPs). A-Increased EMPs (1.1%) in a thalassemia patient B- Decreased EMPs (0.33%) in a healthy control.	54
Figure (13):	Plots of median of circulating endothelial microparticles (CD144+cells) & serum VWF ag.....	63
Figure (14):	Comparison between both groups as regard quantity of endothelial microparticles (CD 144+cells)	72
Figure (15):	Comparison between between both groups as regard serum VWF ag level.....	72
Figure (16):	Comparison between splenectomized & non splenectomized β -thalassemia patients as regard quantity of endothelial microparticles (CD144+Cells).....	77
Figure (17):	Comparison between splenectomized & non splenectomized β -thalassemia patients as regard serum VWF ag levels	77
Figure (18):	Comparison between β -thalassemia patients on monotherapy and combined chelation therapy as regard mean serum ferritin.	78
Figure (19):	Comparison between β -thalassemia patients on monotherapy and combined chelation therapy as regard quantity of circulating endothelial microparticles (CD144+Cells)	80
Figure (20):	Comparison between β -thalassemia patients on monotherapy and combined chelation therapy as regard serum VWF ag levels.....	80
Figure (21):	Correlation between quantity of endothelial microparticles (CD144+cells) and frequency of transfusion	84

LIST OF FIGURES *(Cont...)*

Fig. No.	Title	Page No.
Figure (22):	Cut off value of anti CD144+cells for cardiac complications.....	86
Figure (23):	Correlation between quantity of endothelial microparticles (CD144+cells) & HbF.	87
Figure (24):	Correlation between quantity of endothelial microparticles (CD144+cells) & serum VWF ag level among studied beta thalassemia patients.	90
Figure (25):	Correlation between quantity of endothelial microparticles (CD144+cells) mean serum ferritin last 2 years prior evaluation among studied beta thalassemia patients.....	90
Figure (26):	Correlation between serum VWF ag level & patients age at evaluation	92
Figure (27):	Correlation between serum VWF ag level & disease duration.	92
Figure (28):	Correlation between serum VWF ag level & body mass index	94



Acknowledgement

First of all praise and thanks to **ALLAH** providing me with time and effort to accomplish this thesis.

It is a pleasure to express my deepest thanks and profound respect to my honored professor, **Assistant Professor Dr. Amira Abd El Monem Adley**, Assistant Professor of Pediatrics, Faculty of medicine, Ain Shams University for her continuous encouragement and support that she gave me throughout the whole work. It has been an honor and a privilege to work under her generous supervision.

A special tribute and cordial thanks are paired to **Dr. Eman Abdel Rahman Ismail**, Assistant Consultant of Clinical Pathology, Faculty of Medicine, Ain Shams University for her authentic guidance, meticulous supervision. She gave me a lot of her time, effort and experience to accomplish this work.

I want to take this chance to express my than I would like to express my true thanks to **Dr. Nayera Hazaa Khalil El Sherif**, Lecturer of Pediatrics, Faculty of Medicine - Ain Shams University, for her great guidance and advices throughout this work and fruitful suggestions without which this work would have never been accomplished.

Thanks, respect and love are to all professors and medical staff of **Pediatric Cardiology Unit** for their warm support, help and encouragement.

At last but certainly not least, my special thanks to **my patients and their families** for their cooperation without which this work would have never been accomplished.

Mohammed Mahmoud Samy

INTRODUCTION

Transfusion-related iron overload in β -thalassemia has been associated with the onset of cardiovascular complications, including cardiac dysfunction and vascular anomalies (*Stoyanova et al., 2012*). Endothelial function is impaired in young thalassemia patients, and this dysfunction is associated with oxidant stress (*Kukongviriyapan et al., 2008*).

In the last 10 years, identification of endothelial derived microparticles (EMPs) has raised considerable interest as non invasive markers of vascular dysfunction (*Sabatier et al., 2009*). EMPs are small vesicles released from disturbed endothelial cells that have recently been reported as a marker of endothelial injury and systemic vascular remodelling (*Horstman et al., 2004*). EMPs are biologically active and stimulate pro-inflammatory responses in target cells. Thus, EMPs can promote a prothrombogenic and pro-inflammatory vicious circle leading to vascular dysfunction (*Boulanger et al., 2008*).

EMPs contain membrane, cytoplasmic, and nuclear constituents, characteristic of their precursor cells that confer to EMPs the properties of circulating multifunctional effectors, promoting inflammation of the arterial wall and thrombogenicity through cellular cross-talk (*Boulanger et al., 2008*). Leukocyte adhesion and migration are dependent on a range of cellular adhesion molecules (CAMs) that are up regulated in the endothelium during atherosclerosis (*Blankenberg et al., 2003*).

Because microparticles are fragments of endothelial cell membranes, they also express CAMs (*Hunter MP et al., 2008*).

EMPs can be characterized by their expression of different cell surface antigens including CD31, CD34, CD54, CD62E, CD51, CD105, CD106, CD144 and CD146 (*Burnier et al., 2009*).

von Willebrand factor antigen (vWF Ag) has been purposed as a biomarker of endothelial dysfunction as it is important in the aggregation of platelets and their adhesion to subendothelial cells contributing to inflammatory and atherosclerotic vascular diseases and represent a link between hemostasis and angiogenesis (*Horvath et al., 2004; Papassotiriou and Kattamis, 2012*). Increased activated circulating endothelial cells, enhanced vWF Ag and adhesion molecules were observed in patients with β -thalassemia/hemoglobin E (*Buttke et al., 2004*).