PLATELET MICROPARTICLES IN TYPE 1 DIABETES MELLITUS

Thesis

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By

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Hosam Adly Kamel

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List of Abbreviations

Abb.	Meaning

ACE.....: Angiotensin converting enzyme
ACEI .....: Angiotensin converting enzyme inhibitors
ACR.....: Albumin-to-creatinine ratio
ADA .....: American Diabetes Association
AHA .....: American Heart Association
ANOVA.....: Analysis of Variance
APC.....: Activated protein C
aPL.....: Antiphospholipid antibodies
APS .....: Antiphospholipid antibody syndrome
ATP.....: Adenosine triphosphate
AUC .....: Area under the curve
bFGF.....: basic fibroblast growth factor
BMI.....: Body mass index
cDCs.....: Conventional dendritic cells
CTLA-4 .....: Cytotoxic T lymphocyte associated-4
CVD .....: Cardio vascular disease
DKA .....: Diabetic ketoacidosis
DBP.....: Diastolic blood pressure
DM .....: Diabetes mellitus
DNA .....: Di nucleic acid
DSME .....: Diabetes self-management education
ECs.....: Endothelial cells
EDTA.....: Ethylene-Diamine-Tetra-Acetic acid
ELISA.....: Enzyme-linked immunosorbent assay
EMPs .....: Endothelial MPs
eNOS.....: endothelial NO synthase
EPCR .....: Endothelial protein C receptor

List of Abbreviations (Cont...)

Abb.	Meaning

ErMPs.....: Erythrocytes-derived MPs
ESRD .....: End-stage renal disease
FBS .....: Fasting blood sugar
GAD .....: Glutamic acid decarboxylase
GFR.....: Glomerular filtration rate
GP .....: Glycoprotein
HbA 1c .....: Hemoglobin A1c
HDL .....: High density lipoprotein
HIT.....: Heparin-induced thrombocytopenia
hs-CRP.....: high sensitivity C-reactive protein
ICAM .....: Intercellular adhesion molecule
ICAs .....: Islet Cell Autoantibodies
IDDM.....: Insulin-dependent diabetes mellitus
IFN-γ.....: Interferon gamma
iNKT .....: Invariant natural killer T
iNOS .....: inducible NO-synthase
IQR.....: Interquartile range
LDL.....: low density lipoprotein
MMPs.....: Monocyte-derived microparticles
MNT.....: Medical Nutrition Therapy
MPs .....: Microparticles
mRNA.....: messenger ribo nucleic acid
NO.....: Nitric oxide
NPV .....: Negative predictive value
OGTT.....: Oral glucose tolerance test
PAF .....: Platelet activating factor
PAI-1.....: Plasminogen activator inhibitor-1

List of Abbreviations (Cont...)

Abb.	Meaning
PAR1	Protease activated receptor 1
pDCs	plasmacytoid DCs
PDGF	Platelet-derived growth factor
PDR	Proliferative diabetic retinopathy
PE	Phosphatidyl-ethanolamine
PFP	Platelet-free plasma
PMNs	Polymorphonuclear leukocytes
PMPs	Platelet microparticles
PPV	Positive predictive value
PS	Phosphatidylserine
PSGL-1	P-selectin glycoproteinligand-1
PrPc	Prion protein
RANTES	Regulated on activation, normal T-cells expressed and secreted
RBC	Red blood cells
ROC	Receiver operating characteristic
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SCD	Sickle cell disease
SDS	Standard deviation scores
SLE	systemic lupus erythematosus
T1DM	Type 1 diabetes mellitus
TF	Tissue factor
Th1	T helper 1
TMPs	Tumor cells-derived MPs
TNF-α	Tumor necrosis factor-α

List of Abbreviations (Cont...)

Abb.	Meaning

TSH.....: Thyroid-stimulating hormone
UACR.....: Urinary albumin-to-creatinine ratio
UAE .....: Urinary albumin excretion
VEGF.....: Vascular endothelial growth factor
VWF.....: Von Willebrand factor

ABSTRACT

Background: Diabetes complications represent a huge burden for patients and health services.Diabetic nephropathy (DN) is one of the most serious complications in patients with type 1 diabetes. It is considered the primary cause of mortality in type 1 diabetics and the most common cause of end-stage renal failure, also, a major predictor of premature death. Although microalbuminuria is considered the best available non-invasive marker for DN, it has inadequate specificity and sensitivity. The development of vasculopathies in diabetes involves multifactorial processes including pathological activation of vascular cells. Release of microparticles mainly derived from platelets by activated cells has been reported in diseases associated with thrombotic risk, but few data are available in diabetes. **Objectives:** This study aimed to explore the level of platelets microparticles in children and adolescents with type 1 diabetic patients and its relation to inflammation, glycemic control and microvascular complications. **Patients and methods:** Sixty children and adolescents with type 1 diabetes were recruited from pediatrics diabetes clinic, Ain shams university and compared with 40 age- and sex-matched healthy controls. Patients were subjected to detailed medical history, thorough clinical examination and routine work up including; CRP, HbA1c and urinary albumin excretion. In addition, flow cytometric analysis was done for platelets microparticles using anti-CD41b. **Results:** PMPs levels were significantly elevated in all diabetic patients compared with controls.PMPs levels were significantly increased in patients with micro-vascular complications (3.46 ± 1.11%) and non-complicated patients (2.37 ± 1.28%) compared with healthy control group (1.28 ± 0.64%) with highest levels found in patients with complications ($p<0.001$). PMPs levels were significantly increased in relation to nephropathy (microalbuminuria) Although PMPs were increased in patients with peripheral neuropathy than those without, the difference did not reach a significant level ($p>0.05$).Correlation studies showed significant positive correlations between PMPs levels and BMI, HbA1c, serum creatinine, total cholesterol, UACR and hs-CRP ($p<0.05$).Multiregression linear analysis showed that HbA1c, UACR, hs-CRP and total cholesterol were independently related to PMPs levels in type 1 diabetic patients.ROC curve analysis revealed that the cutoff value of PMPs at 2.48% could differentiate patients with and without micro-vascular complications with a sensitivity of 80% and specificity of 73.3%

Conclusions: Platelets microparticles were elevated in type 1 diabetic patients than controls and can be considered as an early marker of microvascular complications. It is related to inflammation, glycemic control and albuminuria level of patients. Regular measurement of platelets microparticles especially in poorly controlled patients would help to identify those at high risk of developing vascular complications later in life.

INTRODUCTION

Diabetes is a group of metabolic diseases characterized by chronic hyperglycemia that is associated with long-term damage, dysfunction, and failure of different organs ***(Fowler, 2008; ADA, 2012)***. Type 1 diabetes mellitus (T1DM) is one of the most common endocrine and metabolic conditions in childhood. Incidence of T1DM is rapidly increasing especially among the youngest children but the overall annual increase is estimated around 3%. These very young children face long prepubertal years of hyperglycemia with the risk of early development of micro- and macro-vascular complications ***(Soltesz et al., 2009)***.

Diabetes complications represent a huge burden for patients and health services. The fight against each single complication has led to significant improvements in diabetes care, especially for microvascular complications, yet macroangiopathy remains a major source of morbidity and mortality. A common approach for the prevention and treatment of diabetes complications relies on the understanding of their complex pathophysiology ***(Fadini et al., 2007)***.

The development of vasculopathies in diabetes involves multifactorial processes including pathological activation of vascular cells. Release of microparticles by activated cells has been reported in diseases associated with thrombotic risk, but few data are available in diabetes ***(Sabatier et al., 2002)***. These

vesicles have also been implicated to play a role in inflammation, coagulation and diseases associated with impairment of vascular function, e.g. atherosclerosis, diabetes and hypertension ***(Tushuizen et al., 2011)***.

Microparticles are intact vesicles derived from cell membranes following activation or apoptosis; they vary in size from 0.2-2.0 μm. They originate from blebbing and shedding from cell membrane surfaces in physiological and pathological conditions and are present in low concentrations in normal plasma ***(Piccin et al., 2007)***. Platelet-derived MPs (PMPs) are the most abundant, representing about 70-90% of all circulating MPs and were originally studied because of their strong procoagulant activity ***(Owens and Mackman, 2011)***.

Increased levels of microparticles, mainly derived from platelets and to a lesser extent from leukocytes and endothelial cells, have been described in several pathologies associated with prothrombotic and proinflammatory tendencies like heparin-induced thrombocytopenia, thrombotic thrombo-cytopenic purpura, paroxismal nocturnal hemoglobinuria, HIV infection, hemolytic anemia and acute coronary syndromes ***(Mallat et al., 2000; Burnier et al., 2009; Tantawy et al., 2013)***. However, the clinical relevance of PMPs in type 1 diabetes remains to be fully elucidated.