Role of Urinary 11-Dehydro Thromboxane B2 Quantification in Evaluating Anti-Platelet Drugs Resistance

Thesis

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Presented by

Khaled Ahmed El-shourbagy

M.B.B.Ch.-Mansoura University

Supervised by

Professor / Tahany Ali Al-kerdany

Professor of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University

Professor / Soha Raouf Youssef

Professor of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University

Doctor / Hanan Mohamed Mahmoud

Lecturer of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University

Faculty of Medicine - Ain Shams University

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طبيب/ خالد احمد الشوربجى

بكالوريوس الطب و الجراحة- جامعة المنصورة

تحت إشراف

أستاذ دكتور / تهانى على الكردانى

أستاذ الباثولوجي الاكلينيكى و الكيمائي

كلية الطب –جامعة عين شمس

أستاذ دكتور / سهى رؤوف يوسف

أستاذ الباثولوجي الاكلينيكى و الكيمائي

كلية الطب –جامعة عين شمس

دكتور / حنان محمد محمود

مدرس الباثولوجي الاكلينيكى و الكيمائي

كلية الطب –جامعة عين شمس

كلية الطب - جامعة عين شمس

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Khaled Ahmed El-Shourbagy

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List of Abbreviations

Abb.	Full term
11dhTxB2	*11-dehydro thromboxane B2*
ADP	*adenosine diphosphate*
AMI	*acute myocardial infarction*
ASCET	*Aspirin non-responsiveness and Clopidogrel Endpoint Trial*
ATC	*Antiplatelet Trialists' Collaboration*
CABG	*coronary artery bypass surgery*
CAD	*coronary artery disease*
C-ADP	*collagen plus ADP*
C-EPI	*collagen plus epinephrine*
COX-1	*Cyclo-oxygenase enzyme 1*
CPA	*Cone-and-Plate(let) Analyzer*
ESC	*European Society of Cardiology*
GP	*Glycoprotein*
ISIS-2	*Second International Study of Infarct Survival*
LTA	*Light transmission aggregometry*
NSAIDs	*nonsteroidal anti-inflammatory drugs*
PCI	*percutaneous coronary intervention*
PFA	*platelet function analyzer*
RESISTOR	*Research Evaluation to Study Individuals who Show Thromboxane*
RPFA	*Rapid Platelet Function Assay*
TEG	*Thromboelastography*
TXA2	*thromboxane A2*
TXB2	*thromboxane B2*
VWF	*Von willbrand factor*

INTRODUCTION

Anti-platelet drugs are indicated for primary and secondary prevention of coronary and cerebral artery diseases. Aspirin is the most commonly used anti-platelet drug ***(Kour et al., 2006).***

Low dose aspirin blocks more than 95% of platelet Cyclo-oxygenase enzyme 1 (COX-1) activity. In activated platelets COX-1 acts on arachidonic acid to produce molecules, which under effect of thromboxae synthetase produce TXA2. TXA2 has a short half life and is rapidly hydrolyzed to thromboxane B2 (TXB2). TXB2 in turn is metabolized to 11-dehydro thromboxane B2 (11dhTxB2) and a number of other minor TxB2 metabolites which are excreted by the kidney. Thus 11dhTxB2 is a stable metabolite of TXA2 and an in-vivo indicator of platelet activity ***(Edelman et al., 2003).***

Through irreversible inhibition of Cox-1, Aspirin in turn inhibits the facilitator of platelet aggregation thromboxane A2 (TXA2), Thus offering protection against myocardial infarction (MI), stroke, and death ***(Guputa et al., 2007).***

Despite the demonstrated benefit of aspirin in primary and secondary prevention. There are some individuals who don’t derive the anticipated anti-platelet

response from low dose aspirin therapy and manifest with breakthrough atherothrombotic events ***(Kour et al., 2006).***

Based on this, the concept of aspirin resistance has emerged. Though no formal definition of aspirin resistance exists, it may involve clinical failure of therapeutic dose of aspirin or laboratory methods indicating the failure of aspirin to inhibit platelet activity which means biochemical resistance ***(Kour et al., 2006).***

The prevalence of platelet non responsiveness to aspirin has been reported with frequencies ranging from 5% up to 50% of treated patients. This wide range is in part because of clinical differences in methodology used to asses responsiveness to aspirin therapy ***(Gengo et al., 2008).***

Platelet resistance doesn't appear to be an all or non phenomenon. Some patients demonstrate nearby complete resistance to aspirin, clopidogrel, or both agents, while others have partial or incomplete response of their platelets to these agents ***(Guputa et al., 2007).***

Adverse cardiovascular outcomes in patients with known coronary artery disease are associated with aspirin resistance on several different platelet function assays, including the level of urinary 11-dehydrothromboxane B2 (11dhTxB2), platelet aggregation to arachidonic acid and

adenosine diphosphate, and closure time on the platelet function analyzer 100 (PFA) ***(Faraday et al., 2006).***

The three methods were evaluated for their usefulness in defining resistance in patients at risk. The aggregation criteria for resistance were found to be too infrequent to be clinically useful, and aspirin resistance detected by platelet function analyzer -100 (PFA) was associated with VWF, but not with more traditional cardiovascular risk factors or Framingham risk score ***(Faraday et al., 2006).***

Meanwhile aspirin resistance defined by urinary 11-dehydrothromboxane B2 (11dhTxB2) has been found to be strongly and significantly associated with cardiovascular risk factors and with total predicted coronary heart disease risk using the Framingham risk score ***(Johnson, 2009).***

AIM OF THE WORK

The aim of this work is to evaluate the use of quantitative measurement of urinary 11-dehydrothromboxane B2 (11dhTxB2) as an indicator for platelet unresponsiveness in patients receiving anti-platelet drugs for primary and secondary prevention of coronary and cerebral artery diseases.

PLATELETS

Platelets are discoid-shaped blood cells that circulate in a concentration of 150,000 to 450,000 cells/ml3. They are cytoplasmic fragments that do not have a nucleus. On peripheral blood smears stained with Wright- Giemsa stain, platelets appear as small and granular staining cells with a rough membrane. Of the total quantity of platelets in the body, 70% are present in the circulation and 30% in the spleen. Despite their simple appearance on the peripheral blood smear, platelets have a complex structure; their internal structure is divided into four zones: peripheral zone, sol-gel zone, organelle zone, and membrane zone (***Van Geet, 2004).***

Platelets Production

Megakaryocytes are highly specialized precursor cells that differentiate to produce blood platelets via intermediate cytoplasmic extensions known as proplatelets, that serve as assembly lines for platelet production ***(Thon et al., 2010).***

Megakaryocytes undergo a series of morphological changes during the 4 to 10 hour process of platelet production (figure 1) ***(Patel et al., 2005).***

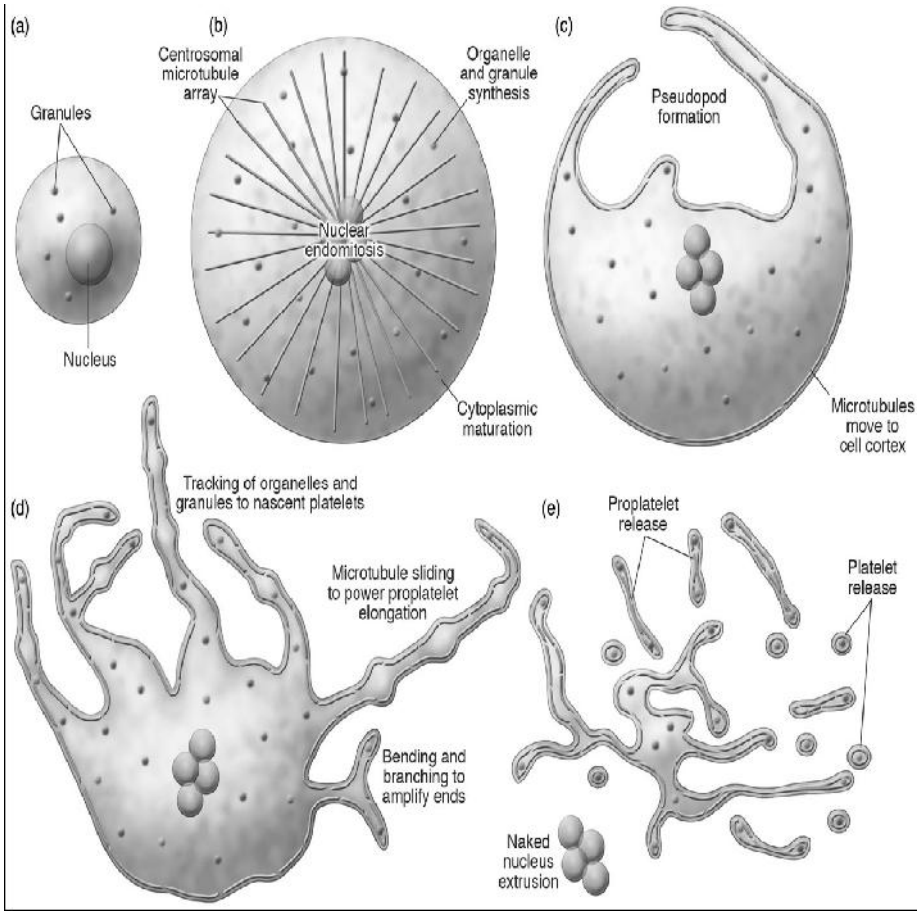

Fig (1): Megakaryocyte production of platelets. Immature megakaryocytes (a) first undergo (b) nuclear endomitosis, organelle synthesis, and cytoplasmic maturation and expansion, while a microtubule array merges from centrosomes (c) Before proplatelet formation, centrosomes disassemble and microtubules migrate to the cell cortex. Proplatelet formation begins with the development of pseudopods (d) Sliding of overlapping microtubules drives proplatelet elongation as organelles are tracked intoproplatelet ends, where nascent platelets assemble. Proplatelet formation/expansion continues throughout the cell, while bending and branching amplify existing proplatelet ends (e) The entire megakaryocyte cytoplasm is converted into a mass of proplatelets, which are released from the cell. The nucleus is eventually extruded from the proplatelet mass, and individual platelets are released from proplatelet ends ***(Patel et al., 2005)***.

Platelet structure:

A. The peripheral zone

It includes the outer membranes and closely associated structures. The platelet has a surface-connected system of channels called the open canalicular system (OCS). The release of platelet products through the OCS after platelet activation is called “the release reaction”. The membranes of the platelet are rich in glycoprotein ***(Wei et al., 2009).***

The peripheral zone is also where membrane phospholipids are found. Phospholipids are an important component of coagulation and serve as the initial substrate for platelet enzymes to produce thromboxane A2; the platelet membrane also has the ability to translate signals from the surface into internal chemical signals (***Italiano and Shivdasani, 2003).***

B. The sol-gel zone

It is beneath the peripheral zone and is the framework of the platelet, the cytoskeleton which forms the support for the maintenance of the platelet’s discoid shape as well as the contractile system that, upon activation, allows shape change, and release of granular constituents. These elements comprise somewhere between 30% to 50% of the total platelet protein (figure 2) ***(Thon et al., 2010).***

C. The organelle zone

It consists of the granules and cellular components, such as lysosomes, mitochondria, etc. The alpha and dense granules are included in this zone.

- The dense granules contain non-metabolic ATP and ADP, serotonin, and calcium.
- The alpha granules The alpha granules contain adhesive proteins, such as fibrinogen, fibronectin, von Willebrand factor, thrombospondin, and vitronectin. also contain growth-promoting products such as platelet-derived growth factor, platelet factor IV and transforming growth factor. Coagulation factors including factor V, high molecular weight Kininogen, factor XI, and plasminogen activator inhibitor-1 are also present in the alpha granules (table 1) (***Wei et al., 2009).***

Table (1): Contents or alpha & dense granules

	Dense granules	Alpha granules
Contents Of granules	Non-metabolic ATP ADP Serotonin Calcium.	Fibrinogen fibronectin von willebrand facor thrombospondin vitronectin Platelet factor IV Coagulation factors including factor V Kininogen Factor XI