# Inherited Thrombocytopenia and Thrombasthenia Among Egyptian Children and Adolescents with Un-Diagnosed Bleeding or Mis-Diagnosed as ITP

Thesis

*Submitted in Partial Fulfillment of Doctorate Degree in Pediatrics*


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بسم الله الرحمن الرحيم

﴿وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا﴾

**صدق الله العظيم**

**سورة النساء آية (١١٣)**

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# Dedication

*Words can never express my sincere thanks to* ***My Family*** *especially* ***My Mother*** *for their generous emotional support and continuous encouragement, which brought the best out of me. I owe them all every achievement throughout my life.*

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# List of Abbreviations

| Abb. | Full term |
|---|---|
| ADP | Adenosine 5'-diphosphate |
| ARC | Arthyrogryposis, renal dysfunction and cholestasis |
| CAMT | Congenital amegakaryocytic thrombocytopenia |
| CD | Cluster of differentiation |
| DITP | Drug-induced immune thrombocytopenia |
| Gp | Glycoprotein |
| GT | Glanzmann`s thrombasthenia |
| HOX | Homeobox |
| ITP | Idiopathic thrombocytopenia |
| IVIG | Intravenous immunoglobulins |
| MEP | Megakaryocyte-erythroid progenitor |
| MK-BFU | Megakaryocyte burst forming unit |
| MK-CFU | Megakaryocyte colony forming unit |
| MPL | Myeloproliferative |
| MPV | Mean platelet volume |
| NR | Platelet count lower than 30 × $10^9$/L or less than doubling of the baseline count |
| PCR | Polymerase chain reaction |
| PRBCS | Packed red blood cells |
| QPD | Quebec platelet disorder |
| R | Platelet count between 30 and 100 × $10^9$/L and at least doubling of the baseline count |
| TAR | Thrombocytopenia absent radii |
| TPO | Thrombopoietin |
| $TxA_2$ | Thromboxane A2 |
| VWF | Von Willebrand factor |
| WAS | Wiskott–Aldrich syndrome |

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## Abstract

**Background:** Childhood hereditary thrombocytopathies are an undiagnosed group of disorders that are mis-attributed to immunological etiology. In this study we aimed at identifying misdiagnosed cases of hereditary thrombocytopenia and thrombasthenia.

**Patients and methods**: This cross sectional study included 67 patients with chronic thrombocytopenia and undiagnosed bleeding followed at Ain Shams University pediatrics hematology clinic. They were divided into; group I (n=47) with low platelet count and group II (n=20) with normal platelet count. Diagnostic approach was applied consisting of primary level of investigations included complete blood counts (CBC) with mean platelet volume (MPV) and light microscopic study of platelet morphology and granules and white blood cell count (WBCs) granules. Secondary level of investigations included flow cytometric analysis for platelet surface glyco-proteins, Tertiary level of investigations included bone marrow aspirate, electron microscope and genetic study when required.

**Results:** 25% of the patients were diagnosed as inherited thrombocytopenia and 25% as thrombasthenia. Probable diagnosis was reached in 34 patients (50.7%). Platelet glycoproteins diagnosed 24 patients (16 patients (23.9%) as Glanzmann`s thrombasthenia, 7 patients (10.4%) as Bernard Soulier syndrome and 1 patient (1.5 %) was diagnosed as collagen receptor defect with no significant correlation with their bleeding severity. Genetic testing confirmed diagnosis of 6 patients (4 patients (5.9%) as congenital amegakaryocytic thrombocytopenia (CAMT), 2 patients (3%) as Wiskott Aldrich syndrome).

**Conclusion:** Applying a diagnostic approach showed the importance of platelet glycoproteins and targeted gene testing in identifying the underdiagnosed cases of hereditary thrombocytopenia and thrombasthenia.


**Keywords:** Idiopathic thrombocytopenia, wiskott–aldrich syndrome, mean platelet volume

# INTRODUCTION

Idiopathic thrombocytopenia (ITP) is considered one of the most common encountered causes of low platelet count occurring in approximately 1 in 10,000 of the general population ***(ElAlfy et al., 2003).***

Inherited thrombocytopenias and thrombasthenia are group of rare diseases that are often misdiagnosed as chronic idiopathic thrombocytopenia (ITP) ***(Gohda et al., 2006).*** An atypical clinical course should serve as a red flag and be followed by proper studies to exclude heritable conditions ***(Picu et al., 2005).***

Diagnosing inherited thrombocytopenias will not just prevent unnecessary treatment to this group of patients(when treated as ITP), but will also allows detecting patients at risk of developing additional disorders more dangerous than thrombocytopenia itself during life ***(Noris and Pecci, 2017).***

Developing a systematic approach for diagnosis of bleeding disorders and identifying the underlying etiology in patients with chronic ITP as well as other cases with bleeding tendency despite normal platelet count and normal coagulation profile is challenging ***(D'Andrea et al., 2009)***.

# Aim of the Study

To detect inherited thrombocytopenias and or thrombasthenia with low platelet count in patients diagnosed with persistent or chronic ITP with no response to immune-modulatory therapy as well as studying patients with unexplained bleeding disorders with normal platelet number and coagulation profile.

Chapter 1

# THROMBOCYTOPENIA

## Introduction

### *Thrombopoiesis:*

Platelet counts range from 150000 to 400000 cell per microliter, however, it is normally kept within a narrow range for every person, This is done by a balance between thrombopoiesis, which is primarily regulated by thrombopoietin (TPO), and platelet consumption ***(Johnson et al., 2016).***

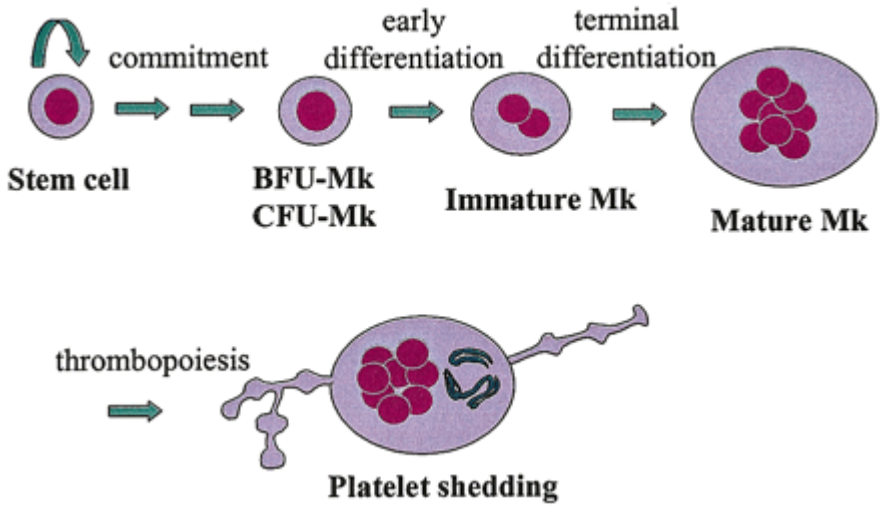


**Figure (1):** Shows the essential developmental steps of megakaryocytes, from a pluripotent stem cell to a fully differentiated, polyploid, platelet-shedding cell, MK: megakaryocytes, BFU: burst forming unit, CFU: colony forming unit ***(Drachman, 2004).***

Hematopoietic stem cells have the capacity to proliferate; self renew, and differentiate into cells of all the blood lineages

to maintain hematopoiesis. Megakaryocytes are derived from the megakaryocyte-erythroid progenitor (MEP), that gives rise to cells of both megakaryocytic and erythroid lineages and controlled by many transcription factors, including GATA-binding factor 1 (GATA-1) ***(Fan et al., 2017).***

The average human platelet life span is 7–10 days. After they are formed from bone marrow megakaryocytes, platelets are divided into two compartments: (1) the stationary compartment in the spleen accounting for about one third of the whole platelet mass and (2) circulating platelets representing the remaining two thirds. The final platelet count in the peripheral blood is the result of platelet production, distribution into the two compartments, consumption due to adhesion to vessel wall damage or formation of platelet aggregates, and clearance ***(Bakchoul and Schulze, 2016).***

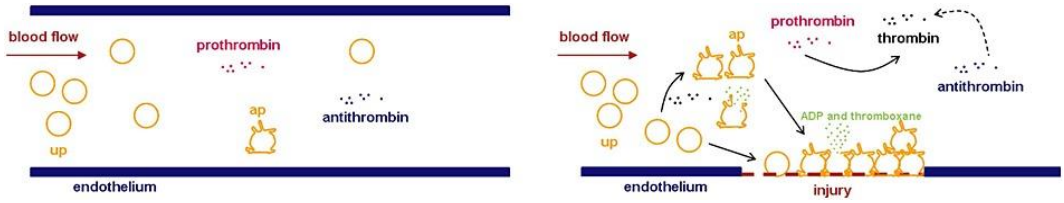


**Figure (2):** Passive transport of platelets and chemicals in healthy blood vessels (left); reactions happening in case of vessel wall injury that leads to the formation of a platelet plug (right) ***(Storti et al., 2014).***

## ***Platelets plug formation:***

Platelets plug formation passes into main four phases; adhesion of platelet glycoprotein (Gp) 1b receptor to

subendotheliam, vasoconstriction which is mediated by activated platelets producing thromboxane A2 ($TxA_2$), swelling and inter-platelet binding by fibrinogen cross links to GpIIb/IIIa receptors on platelet membrane, and maturation in which fibrinogen converts to fibrin with cross-linking to increase the strength of the plug ***(Galioto et al., 2011).***

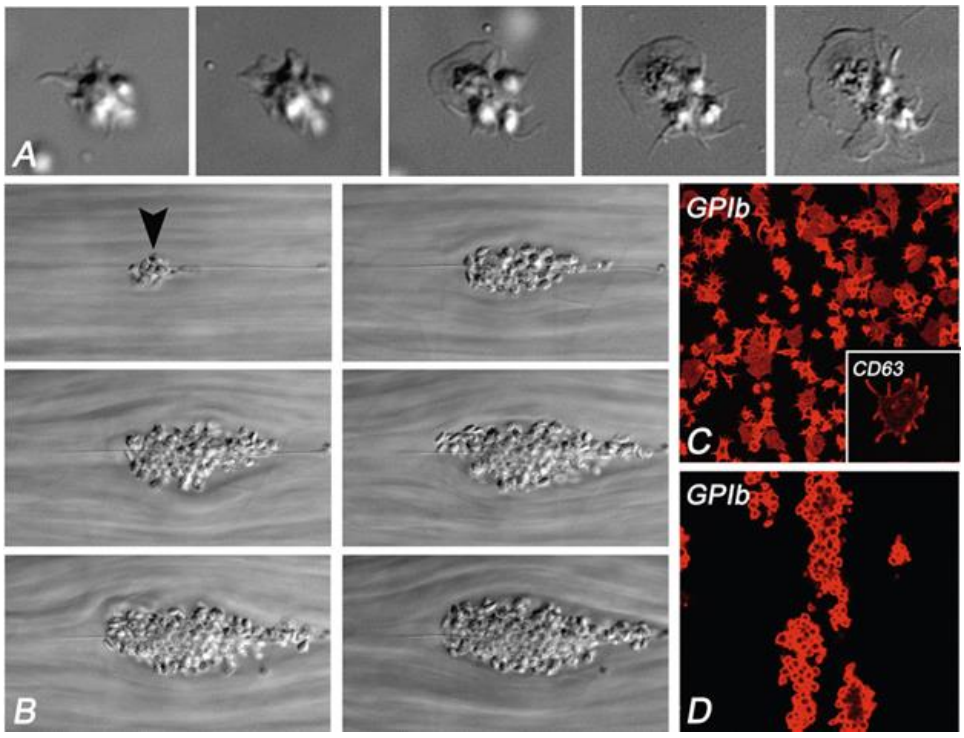


**Figure (3):** Real-time and confocal visualization of platelet adhesion. (a) Series of still images from video recordings of platelet adhesion and spreading on fibrinogen under flow (DIC), shear rate 100/s. (b) Still images taken from live recordings of platelet thrombus formation (arrow head) on a collagen surface, shear rate 1600/s. (c) Confocal images after whole blood perfusion over fibrinogen, immunolabeling with anti-GPIb or anti-CD63 antibody (inset). (d) Confocal image after whole blood perfusion over collagen; immunolabeling with anti-GPIb antibody ***(Heijnen and Korporaal, 2017).***