Introduction

Immune thrombocytopenia **(ITP)**, also known as immune or idiopathic thrombocytopenic purpura, is characterized by decreased platelet counts resulting from auto antibody mediated peripheral platelet destruction and suboptimal platelet production. A normal platelet count is between 150,000 and 400,000/microliter of blood. It is diagnosed by exclusion of other causes of low platelet count. There is no accurate definitive test to diagnose ITP ***(Rodeghiero et al., 2009).***

Immune thrombocytopenia is usually manifested by bleeding tendency for example: Easy or excessive bruising (purpura), superficial bleeding into the skin that appears as a rash of pinpoint-sized reddish-purple spots (petechiae), epistaxis, bleeding per gums, hematuria, heavy menstruation, intra cranial hemorrhage which is one of the leading causes of death in patients with ITP ***(Sarpatwari et al., 2010).***

Treatment is recommended for a platelet count $<30\times10^9$/L. The ASH recommends that if treatment is needed and corticosteroids are given, longer courses (eg, prednisone 1 mg/kg orally for 21 days then tapered) are preferred over shorter courses of corticosteroids or IVIG as first-line treatment. IVIG be used with corticosteroids in patients who require a more rapid increase in platelet count. If corticosteroids are contraindicated, either IVIG (initially, 1 g/kg in a single dose) or IV RhIG (in appropriate patients) may be used as a first-line treatment. The ASH

suggests consideration of thrombopoietin receptor agonists for patients at risk of bleeding when splenectomy is contraindicated and at least one other therapy has failed, and recommends thrombopoietin receptor agonists in adult patients who relapse after splenectomy and are at risk for bleeding. The ASH suggests consideration of rituximab in patients at risk of bleeding when one line of therapy (eg, corticosteroids, IVIg, splenectomy) has failed ***(Neunert et al., 2011).***

Thrombosis is the process of a blood clot, also known as a thrombus, forming in a blood vessel. This clot can block or obstruct blood flow in the affected area, as well as cause serious complications if the clot moves to a crucial part of the circulatory system, such as the brain or the lungs. Thrombosis can be broadly classified as either venous thrombosis or arterial thrombosis, according to where the thrombus presents in the body. There are three main causes of venous thrombosis; hypercoagulability (thrombophilia), injury to the endothelial cells of the blood vessel wall and abnormal flow of the blood, while the main cause of arterial thrombosis is hyperlipidemia leading to atherosclerosis ***(Bruce et al., 2008).***

In most patients with **ITP**, the increased risk of **thrombosis** is small (relative risk < 2) and not sufficient to influence management. However, more recent studies suggest that certain subpopulations of patients with ITP may be at a significantly higher risk of thrombotic complications. These include patients who have undergone

splenectomy, Patients older than 60 years are also at increased risk of thromboembolism, The presence of antiphospholipid antibodies (aPL) has been observed in a substantial proportion of ITP patients and there is increased thrombotic risk associated with thrombopoietin receptor agonists, some studies do suggest increased risk with these agents ***(Keith et al., 2011; Rodeghiero et al., 2016).***

Aim of the Work

The aim of this study is to assess the thrombotic complications in Egyptian immune thrombocytopenic purpura patients and to identify the possible relationship of thrombosis with other patients as well as disease related factors.

Chapter I

Platelets

Introduction:

A megakaryocyte is a large bone marrow cell with a lobated nucleus responsible for the production of blood thrombocytes (platelets), which are necessary for normal blood clotting. Megakaryocytes usually account for 1 out of 10,000 bone marrow cells in normal people. In general, megakaryocytes are 10 to 15 times larger than a typical red blood cell, averaging 50–100 μm in diameter. During its maturation, the megakaryocyte grows in size and replicates its DNA without cytokinesis in a process called endomitosis. As a result, the nucleus of the megakaryocyte can become very large and lobulated, which, under a light microscope, can give the false impression that there are several nuclei. In some cases, the nucleus may contain up to 64N DNA, or 32 copies of the normal complement of DNA in a human cell. Megakaryocytes are derived from hematopoietic stem cell precursor cells in the bone marrow. They are produced primarily by the liver, kidney, spleen, and bone marrow. These multipotent stem cells live in the marrow sinusoids and are capable of producing all types of blood cells depending on the signals they receive. The primary signal for megakaryocyte production is thrombopoietin or TPO. TPO is sufficient but not absolutely necessary for inducing differentiation of progenitor cells in the bone marrow towards a final megakaryocyte phenotype. Other molecular signals for megakaryocyte differentiation include granulocyte

macrophage colony stimulating factor, interleukin 3 (IL-3), interleukin 6(IL-6), interleukin 11(IL-11), chemokines (stromal cell drived factor 1, fibroblast growth factor 4) and erythropoietin ***(Yang et al., 2012).***

Platelets, also called thrombocytes, are a component of blood whose function, along with the coagulation factors, is to stop bleeding by clumping and clotting blood vessel injuries. Platelets have no cell nucleus, they are fragments of cytoplasm that are derived from the megakaryocytes of the bone marrow. Major part of cytoplasm is composed of a contractile protein called Thrombosthenin ***(Mercy et al., 2017).***

These unactivated platelets are biconvex discoid (lens-shaped) structures 2–3 μm in greatest diameter. Platelets are found only in mammals ***(Machlus et al., 2014).***

Platelet production:

The megakaryocyte develops through the following lineage:

Pluripotential hemopoietic stem cell or hemocytoblast → Megakaryoblast → promegakaryocyte → megakaryocyte. The cell eventually reaches megakaryocyte stage and loses its ability to divide. However, it is still able to replicate its DNA and continue development, becoming polyploid. The cytoplasm continues to expand and the DNA complement can increase up to 64N in human and 256N in mouse ***(Yang et al., 2012).***

Platelets are produced during hematopoiesis in a sub-process called thromopoiesis, or production of

thrombocytes. Thrombopoiesis occurs from common myeloid progenitor cells in the bone marrow, which differentiate into promegakaryocytes and then into megakaryocytes. Megakaryocytes stay in the bone marrow and are thought to produce protoplatelets within their cytoplasm, which are released in cytoplasmic extensions upon cytokine stimulus. The protoplatelets then break up into hundreds of platelets that circulate throughout the bloodstream, while the remaining nucleus of the ruptured megakaryocyte is consumed by macrophages. Each megakaryocyte produces between 1,000 and 3,000 platelets during its lifetime. An average of 10^{11} platelets are produced daily in a healthy adult. The average life span of circulating platelets is 8 to 9 days. Life span of individual platelets is controlled by the internal apoptotic regulating pathway ***(Tianyu et al., 2015).***

Megakaryocyte and platelet production is regulated by thrombopoietin, a hormone produced by the liver and kidneys. Thrombopoietin stimulates differentiation of myeloid progenitor cells into megakaryocytes and causes the release of platelets. Thrombopoietin is regulated by a negative feedback mechanism based on platelet levels in the body so that high levels of platelets result in lower levels of thrombopoietin, while low levels of platelets result in higher levels of thrombopoietin. There are many cytokines that affect megakaryocytes. Certain cytokines such as IL-3, IL-6, IL-11, leukemia inhibitory factor (LIF), erythropoietin, and thrombopoietin all stimulate the maturation of megakaryocytic progenitor cells ***(Jonathan et al., 2010; Afdhal et al., 2012).***

Structure of the platelets:

Structurally the platelet can be divided into four zones, from peripheral to innermost:

1. Peripheral zone - is rich in glycoproteins (Glycoprotein Ib-IX-V complex, Glycoprotein VI, Glycoprotein Ia / IIa complex, Glycoprotein IIb / IIIa complex, glycoprotein V / IIIa) required for platelet adhesion, activation, and aggregation ***(Lepage et al., 2012).***
2. Sol-gel zone-is rich in microtubules and microfilaments, allowing the platelets to maintain their discoid shape ***(Machlus et al., 2014).***
3. Organelle zone-is rich in platelet granules. Alpha granules contain clotting mediators such as factor V, factor VIII, fibrinogen, fibronectin, platelet-derived growth factor, and chemotactic agents. Delta granules, or dense bodies, contain ADP, calcium, serotonin, which are platelet-activating mediators ***(Jones et al., 2012).***
4. Membranous zone – contains membranes derived from megakaryocytic smooth endoplasmic reticulum organized into a dense tubular system which is responsible for thromboxane A2 synthesis. This dense tubular system is connected to the surface platelet membrane to aid thromboxane A2 release ***(Machlus et al., 2014).***

Platelet functions:

Platelets circulate in the plasma and are primarily involved in hemostasis, by causing the formation of blood

clots, also known as coagulation. The adhesive surface proteins of platelets allow them to accumulate on the fibrin mesh at an injury site to form a platelet plug that clots the blood ***(Sanjeev et al., 2014).***

Three stages of platelets activation:

1- Platelet Adhesion:

Disruption of the endothelial layer leads to anchoring of the platelets to the subendothelium by collagen and Von willebrand factor (vWF).

Platelet glycoprotein (GP1b-IX-V) receptor binds with vWF and GPVI receptor and integrin alpha2beta1 binds with collagen ***(Hosseini et al., 2012).***

2- Activation:

The binding of the subendothelial collagen to its receptors on the platelets leads to their activation, this occurs seconds after adhesion. Platelet activation is stimulated by bound platelet secretion products and local prothrombotic factors such as tissue factor. Multiple pathways can lead to platelet activation. There are two principle activating pathways in platelets. Glycoprotein Ib-IX-V receptor complex (GP Ib-IX-V), Glycoprotein VI (GP VI), or C-type lectin-like receptor 2 (CLEC-2) are all membrane glycoproteins exclusively expressed in platelets and megakaryocytes and have closely related signal transduction pathways. GP VI is thought to be the major signaling receptor involved in platelet activation on exposed collagen. Following GP VI interactions with collagen, platelets initiate strong activation and release the content of α- and dense granules ***(Jones et al., 2012).***

Platelets contain dense granules, lambda granules and alpha granules. As seen in figure (1):

1- Alpha granules containing P-selectin, platelet factor 4, transforming growth factor-β1, platelet-derived growth factor, fibronectin, B-thromboglobulin, vWF, fibrinogen, and coagulation factors V and XIII.

2- Delta (dense) granules containing Adenosine DiPhosphate (ADP) or Adenosine TriPhosphate (ATP), calcium, and serotonin.

3- Lambda granules-similar to lysosomes and contain several hydrolytic enzymes ***(Kakali et al., 2014).***

Activated platelets secrete the contents of these granules through their canalicular systems to the exterior. bound and activated platelets degranulate to release platelets chemotactic agents to attract more platelets to the site of endothelial injury. Platelets secrete thromboxane A2, which acts on the platelet's own thromboxane receptors on the platelet surface (it is called "out-in" mechanism), and those of other platelets. These receptors trigger intraplatelet signaling, which converts GPIIb/IIIa receptors to their active form to initiate aggregation ***(Seong-Hoon et al., 2016).***

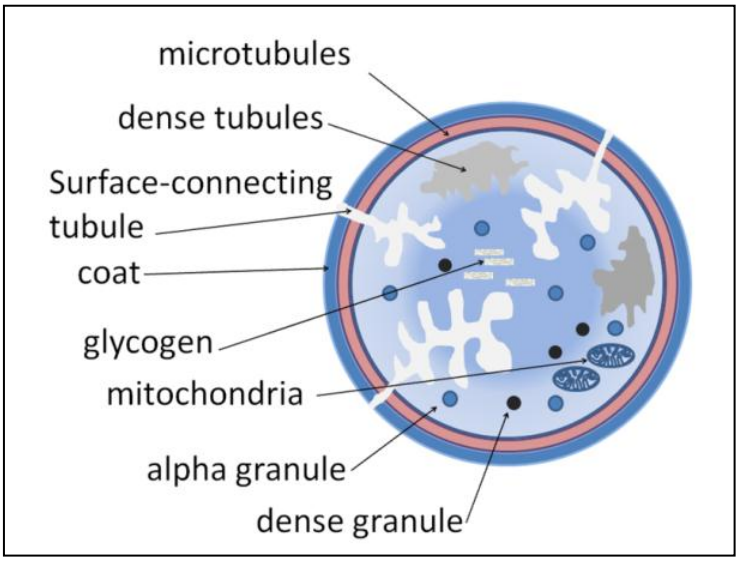

Figure (1): Structure of platelets

3- Aggregation:

Platelets aggregation starts minutes after the activation as a result of turning on the GPIIb/IIIa receptor allowing these receptors to bind with vWF or fibrinogen. Classically it was thought to be the only mechanism involved in platelet aggregation ***(Jones et al., 2012).***

Immune thrombocytopenic purpura (immune thrombocytopenia)

Definition:

The American Society of Hematology (ASH) defined the abbreviation in common use (ITP) to be Immune Thrombocytopenia (neither Idiopathic nor Purpura) because the pathophysiology is better understood and the majority of both adult and pediatric patients do not present with purpura, even if they have petechiae and bruising ***(Michel et al., 2013).***

It is defined as isolated thrombocytopenia in the absence of other causes of thrombocytopenia, diagnosed by exclusion, it is characterized by decreased platelets count $<100\times10^9$/L of blood resulting from auto antibody mediated peripheral platelet destruction and suboptimal platelet production ***(Neunert et al., 2011).***

It occurs in both adults and children, with a multimodal incidence with 1 peak in childhood and second and third peaks in young adults and the elderly respectively. The underlying disease process in childhood ITP and adult ITP may be fundamentally different, as evidenced by the discrepancy of the rates of chronicity in these patient populations**.** Although the majority of children have self-limited disease, in adults, ITP is more often a chronic disorder ***(Schulze et al., 2011).***

Phases of the disease:

The ASH removed the term "acute" ITP, as this diagnosis can only be made in retrospect, after the patient has recovered from the thrombocytopenia. Instead, they proposed standardized terminology:

1. Newly diagnosed: from time of diagnosis up to 3 months (<3 months duration).
2. Persistent ITP: from 3 months up to 12 months (3-12 months duration).
3. Chronic ITP: disease of more than 12 months duration (>12 months duration) ***(Michele et al., 2017).***

Epidemiology:

The incidence of primary ITP in adults is 3.3/100 000 adults per year with a prevalence of 9.5 per 100 000 adults and between 1.9 and 6.4/100,000 children per year ***(Terrell et al., 2010).***

The incidence of ITP increases with age and, among adults between the ages of 18 and 65 years as demonstrated in (Figure 2) ***(Gaurav et al., 2013).***

There is a predilection for female patients in younger adults, but the prevalence of ITP in men and women is fairly even in the elderly above 65 years ***(Moulis et al., 2014).***

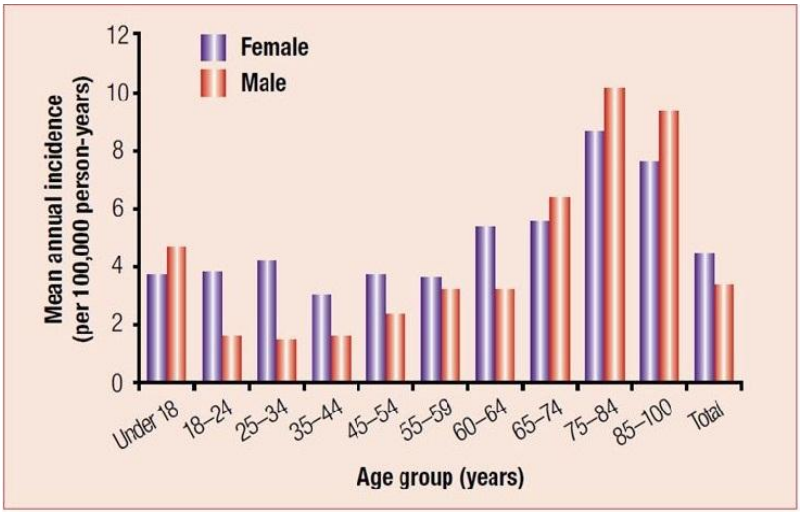

Figure (2): Incidence of ITP according to gender and age ***(Gaurav et al., 2013)***

Pathophysiology of primary immune thrombocytopenia:

Immune thrombocytopenia (ITP) is a disorder characterized by immune-mediated accelerated platelet destruction and suppressed platelet production. The etiology of ITP is not yet known. ITP may present either as primary (isolated) ITP or as secondary ITP in the context of other associated diseases ***(Neunert et al., 2011).***

It is now clear that Primary ITP is an acquired immune disorder where thrombocytopenia results from:

1) Enhanced platelets destruction:

a. Pathologic antiplatelet antibodies ***(Anne et al., 2017)***
b. T-cell–mediated destruction of platelets ***(Bakchoul et al., 2016)***
c. Antigen presenting cells in ITP ***(Anne et al., 2017)***
d. B regulatory cells abnormalities ***(Aslam et al., 2016)***

2) Impaired megakaryocytopoiesis (Khodadi et al., 2016)

a. Pathologic antiplatelet antibodies:

As many as 60% to 70% of patients with ITP have platelet-specific immunoglobulin G antibodies. These are generally directed at the most abundant platelet surface glycoproteins, GPIIb/IIIa and GP1b/IX/ V.

Platelets are targeted by the attachment of autoantibodies to their surface GP antigens, bound to Fcγ receptors expressed on tissue macrophages of the reticuloendothelial system and cleared from the circulation. Complement-induced lysis following antibody binding may also play a role ***(Michele et al., 2017).***

The type of epitope targeted by these autoreactive antibodies may influence the course of the disease. Studies reported that platelet associated anti-GPIIb/IIIa antibodies frequently bind to cation-dependent conformational antigens. The autoantigenic epitopes are localized mainly on the β-propeller domain of GPIIb ***(Hirokazu and Yoshiaki, 2013).***

Interestingly, in ITP patients the reactivity of autoantibodies was completely destroyed by only one amino acid substitution, suggesting that the target epitopes were localized on very restricted region on GPIIb ***(Kiyomizu, 2012).***

Some research has suggested that these different types of antibodies may differentially alter clearance, inhibit megakaryopoiesis, or induce platelet apoptosis ***(Cines et al., 2014).***