INTRODUCTION

Childhood acute Idiopathic thrombocytopenic purpura (ITP) is a common pediatric hematologic disorder characterized by increased destruction of antibody-sensitized platelets with normal to increased megakaryocytes in the bone marrow, as well as the presence of thrombocytopenia with otherwise normal red cells and leukocytes, absence of splenomegaly and the absence of other causes of thrombocytopenia ***(Akin et al., 2011)***. Until recently, a diagnosis of chronic ITP required persistence of thrombocytopenia for ≥ 6 months after diagnosis. This definition has now been changed to persistence of thrombocytopenia for ≥ 12 months after diagnosis ***(Nogaard et al., 2011).***

The decrease of platelets is caused by increased autoantibodies against self-antigens, particularly IgG antibodies against GPIIb/IIIa. The production of these autoantibodies by B cells depends on a number of cellular mechanisms that form a network of modulation, with T cells playing a pivotal role in pathophysiology ***(Zhou, 2005).*** Recent studies paint a broader picture of immune dysregulation leading not only to accelerated platelet destruction, but to abnormalities in megakaryocyte growth and development and poorly compensated thrombopoiesis ***(Gernsheimer, 2008)***.

Although the etiology of ITP remains unclear, it is generally accepted that both environmental and genetic factors play an important role in the development of the disease ***(Pehlivan et al., 2011)***. Many recent studies have focused on

the association between some non cytokine gene polymorphisms and susceptibility to ITP ***(Okulu et al., 2010).*** Gene polymorphisms of non cytokines are associated with several diseases including ITP ***(Suzuki et al., 2008).***

Recently, it has been shown that the conservative mutations in the regions, where non-cytokines are encoded, and the nucleotide changes in the regulatory region might be the causes of individual differences in non-cytokine production and that these genetic polymorphisms affect the release of non-cytokines both in vivo and in vitro media. It was reported that there was an association between the non cytokine gene polymorphisms affecting the non cytokine production and secretion and infectious diseases, allergic diseases, autoimmune diseases, and malignant diseases both at the stage of formation of disease and in the course of disease and their responses to treatment ***(Pehlivan et al., 2011).***

The genomic revolution in medicine has not escaped attention of clinicians and scientists involved in medical management and research studies of immune thrombocytopenic purpura (ITP). In principle, ITP biology and care will benefit greatly from modern methods to understand the patterns of gene expression and genetic markers associated with fundamental parameters of the disease including predictors of remission, risk factors for severity, determinants of response to various therapies, and possibly biological sub-types ***(Bergmann et al., 2010).***

Multidrug resistance-1 (MDR-1) is characterized by the overfunction of P-glycoprotein (P-gp), a pump molecule that decreases intracellular drug concentration by effluxing them from the intracellular space. P-gp is expressed in the apical membrane of cells with excretory functions, such as those in the liver, kidney, small intestine, stomach, and the blood–brain barrier ***(Lum and Gosland, 1995)***. Functional P-gp is found in several types of human leukocytes and stem cells. Among hematological cells, P-gp expression is highest in natural killer cells, CD4+ and CD8+ lymphocytes, and bone marrow progenitor cells ***(Klimecki et al., 1994)***. Exon 26 3435CNT polymorphism was found to correlate significantly with intestinal P-gp expression levels as well as the bioavailability of some drugs. Individuals who are homozygous for 3435T have significantly decreased intestinal P-gp expression and increased digoxin serum levels after oral administration ***(Wang et al., 2005)***. Natural killer (NK) cell activation is regulated by the balance in expression of a series of activating and inhibitory receptors. These include the human killer cell immunoglobulin-like receptor (KIR) family, of which individual members can transduce either activating or inhibitory signals ***(Campbell et al., 2011)***. KIR repertoire is determined by KIR genotype, with individuals exhibiting extensive haplotypic variation in gene number and content, and allelic polymorphism for individual genes ***(Wilson et al., 2000)***.

AIM OF THE WORK

The aims of this study are to:

1. To detect characterization of the different gene polymorphisms in:
    a. Human killer cell immunoglobulin-like receptor (KIR2) gene.
    b. The multi-drug resistance (MDR1) gene, among childhood ITP Egyptian patients.
2. To study the potential role of these polymorphisms in relation to chronicity and response of ITP to treatment modalities.

Chapter 1

PLATELET PHYSIOLOGY AND FUNCTION

Platelet physiology:

Platelets are small (2-µm-diameter), non-nucleated blood cells produced in the bone marrow from megakaryocytes. Platelets are activated rapidly after blood vessel injury or blood exposure to the artificial surfaces of implanted devices, and they are a crucial component of the primary hemostatic response. In their inactivated state, platelets are roughly discoid in shape and contain cytoplasmic organelles, cytoskeletal elements, invaginating open-canalicular membrane systems, and platelet-specific granules, called alpha and dense granules ***(Kottke-Marchant and Corcoran, 2002)***.

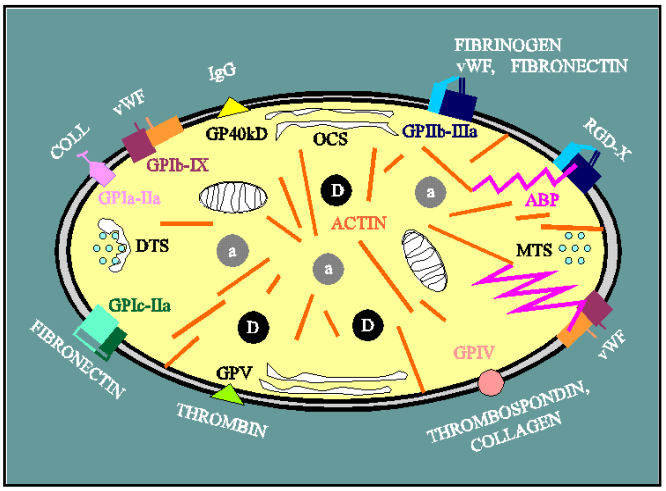

Figure (1): The "Anatomy" of Human Blood Platelets ***(Brewer, 2006)***.

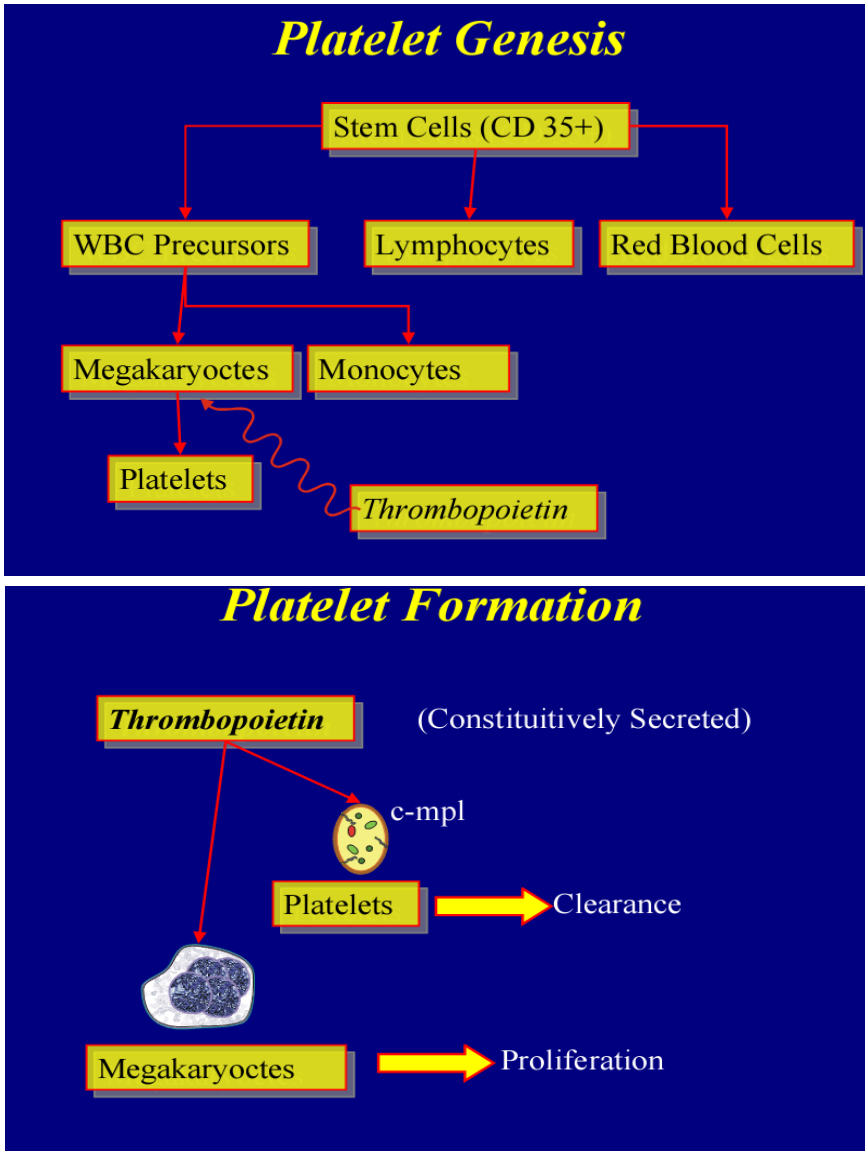

Figure (2): Platelet genesis and formation ***(Kelton, 2003)***.

Platelet Function:

Platelets mediate the body's clotting response to injury and day-to-day blood vessel repair.

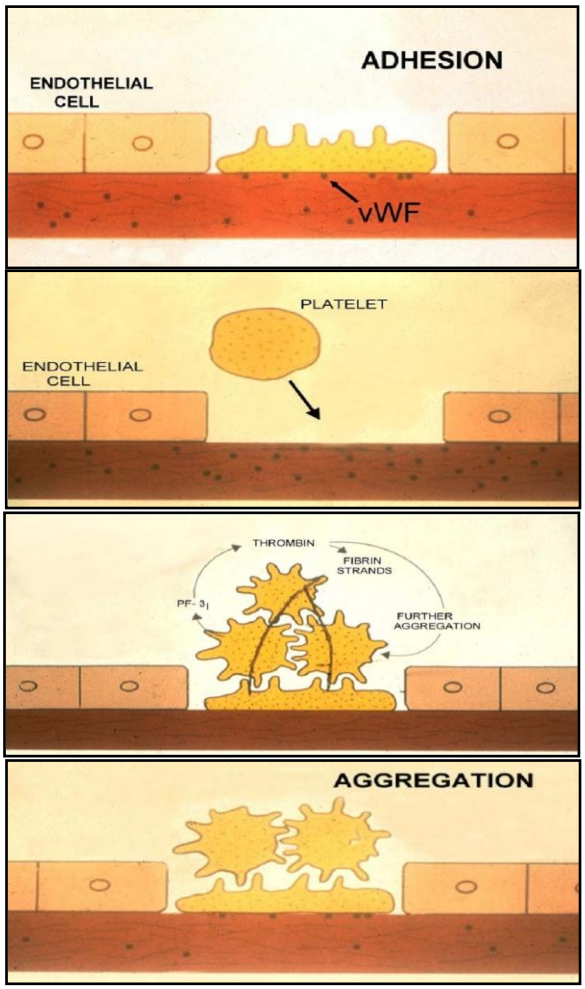

Figure (3): Platelet function at the wall of the blood vessels ***(Kelton, 2003)*.**

Platelets promote hemostasis by four interconnected mechanisms:

1- Adhering to sites of vascular injury or artificial surfaces.

2- Releasing compounds from their granules.

3- Aggregating together to form a hemostatic platelet plug, and providing a pro coagulant surface for activated coagulation protein complexes on their phospholipid membranes.

***(Kottke-Marchant and Corcoran, 2002)*.**

Platelet Regulation:

Thrombopoietin levels are inversely related to the peripheral blood platelet count. Moreover, in a number of other thrombocytopenic disorders (including idiopathic thrombo-cytopenic purpura), plasma thrombopoietin levels are elevated ***(Kaushansky, 1996)*.**

The precise inverse relationship between plasma level of thrombopoietin and the platelet count has led some investigators to propose that platelets regulate the levels of the hormone responsible for their production. Thrombopoietin is regulated by some of the same mechanisms proposed for the regulation of macrophage CSF, the cytokine responsible for monocyte and macrophage development ***(Kaushansky, 1996)***.

Causes of thrombocytopenia:

Two main mechanisms are involved in the pathogenesis of thrombocytopenia:

(a) **Decreased platelet production** in the bone marrow, as in the case of hypo productive thrombocytopenia due to bone marrow suppression after chemotherapy for hematological malignancies.

(b) **Increased platelet destruction** which is the case in idiopathic thrombocytopenic purpura ***(George et al., 2008)*.**

Immune Thrombocytopenia:

Immune thrombocytopenic purpura (ITP), also known as idiopathic thrombocytopenic purpura, is an immune-mediated acquired disease of adults and children characterized by transient or persistent decrease of the platelet count and, depending upon the degree of thrombocytopenia, increased risk of bleeding ***(Cooper and Bussel, 2006).***

The Intercontinental Childhood ITP Study group decided to avoid the term "idiopathic," preferring "immune," to emphasize the immune-mediated mechanism of the disease and to choose "primary" (as opposed to idiopathic) to indicate the absence of any obvious initiating and/or underlying cause. The term "purpura" was felt inappropriate, because bleeding symptoms are absent or minimal in a large proportion of cases ***(Rodeghiero et al., 2009)***.

The acronym ITP (now proposed to stand for immune thrombocytopenia) was preserved because of its widespread and time honored use and taking into account its utility for literature searches ***(Adibi et al., 2007).***

Platelet-count threshold of ITP:

Thrombocytopenia, generally defined as a platelet count of less than 150,000/L ***(Blanchette and Bolton-Maggs, 2008).***

A platelet count less than 100 x 10^9/L was established as the threshold for diagnosis ***(Stasi et al., 1995).*** A uniform predefined cutoff, instead of local normal ranges or other thresholds based on frequency distribution, is more convenient for practical use and comparisons across studies. This threshold was preferred to the more commonly used level of less than 150 x 10^9/L, based upon a prospective cohort of otherwise healthy subjects with a platelet count between 100 and 150x10^9/L, showing that the 10-year probability of developing more severe thrombocytopenia (persistent platelet count below 100x10^9/L is only 6.9% ***(Stasi et al., 1995).***

The cutoff level avoids inclusion of most women with pregnancy-related thrombocytopenia, a well known physiologic phenomenon not requiring specific follow-up in the absence of additional clinical features ***(Kelton, 2003).***

Moreover, in some non-Western populations, platelet count values between 100 and 150 x 10^9/L are frequently found in apparently healthy people ***(Adibi et al., 2007).***

ITP can be classified based on patient age (childhood versus adult), duration of illness (acute versus chronic), and presence of an underlying disorder (primary versus secondary) ***(Blanchette and Bolton-Maggs, 2008).***

Classification of ITP based age (childhood versus adult):

Table (1): The features of childhood and adult ITP are compared in table (1)

	Child	**adult**
Age most likely to occur (years)	2-6	2-6
Gender ratio of disease (M:F)	1:1	1:3
Start of disease subtle	acute	
Preceding infection	common	unusual
Platelet count (x 109/L)	often <20	often > 20
Spontaneous remission rate%	>80	< 20
Usual duration months/years	2-4 weeks	

(Harrison and Machin, 2006)

Classification of ITP based on presence of underlying disorder (primary versus secondary): The distinction between primary and secondary immune thrombocytopenia is clinically relevant because of their different natural histories and distinct treatments

Primary ITP:

Primary ITP is characterized by isolated thrombo-cytopenia in the absence of other causes or disorders that may be associated with thrombocytopenia. The diagnosis of primary

ITP remains one of exclusion; no robust clinical or laboratory parameters are currently available to establish its diagnosis with accuracy. The main clinical problem of primary ITP is an increased risk of bleeding, although bleeding symptoms may not always be present ***(Blanchette and Bolton-Maggs, 2008)*.**

Icidence:

The peak age of diagnosis for childhood ITP is between 2 and 6 years of life and ITP affects roughly 1 in every 10,000 children. Incidence in boys and girls is roughly the same. Many patients give a history of a preceding viral infection within a few weeks of presenting with thrombocytopenia, raising the possibility that the aberrant autoimmune response of ITP may be triggered by infection. Indeed, seasonal fluctuation in ITP incidence has been described, highest in the spring and lowest in the autumn, perhaps reflecting seasonal variation in viral illnesses ***(Kremer Hovinga et al., 2010)*.**

Secondary ITP:

All forms of immune-mediated thrombocytopenia except primary ITP which include chronic infections (including HIV, CMV) ***(Liebman and Stasi, 2007)***, bone marrow failure (Fanconi anemia, myelodysplastic syndrome), collagen vascular disorders (systemic Lupus erythematosus) ***(Blanchette and Bolton-Maggs, 2008)***, von Willebrand disease type 2B, thrombotic thrombocyto-penic purpura, Evans syndrome and other autoimmune or

immunodeficiency disorders (common variable immuno-deficiency, Wiskott- Aldrich syndrome ***(Laws et al., 2009).*** The percentage distribution of these disorders are illustrated in figure (4)**.**

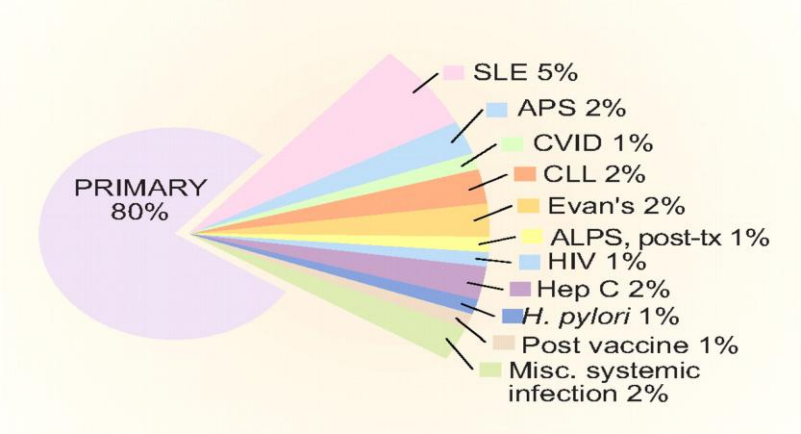

Figure (4): Secondary cause of ITP ***(Cines et al., 2009)*.**

For cases possibly initiated by or associated with Helicobacter pylori infection, considering the high prevalence of the infection in some countries, a diagnosis of "secondary ITP (Helicobacter pylori-associated)" would require the demonstration of complete resolution of ITP after proven eradication of the bacteria ***(Visco et al., 2008)*.**

In the case of thrombocytopenia related to drug exposure (with the exclusion of myelo suppressive chemotherapy), the term "drug-induced" was preferred. The name of the incriminated drug should be indicated when known. For example: "Secondary ITP (quinine-induced)." Heparin-induced thrombocytopenia will maintain its designation and acronym (HIT) because of its unique features.

For thrombocytopenias secondary to an ongoing medical condition, treatment is often targeted toward the underlying disorder. On the other hand, drug-induced ITP often remits quickly after the withdrawal of the inciting drug, and most severe cases may require transfusion of platelets alone as initial treatment, as opposed to the application of immunomodulation often used in primary ITP ***(Aster and Bougie, 2007).***

The coexistence of antinuclear antibodies and/or anti-phospholipid antibodies (aPL) on their own, in the absence of distinctive clinical manifestations suggestive of SLE and/or antiphospholipid syndrome, does not qualify these cases as secondary ITP ***(Miyakis et al., 2006).*** The increased risk of thrombosis in aPL antibody-positive cases reported in some studies is controversial ***(Liebman, 2007)***.

The available evidence does not warrant consideration of the coexistence of thrombocytopenia with aPL antibodies as a distinct clinical entity ***(Miyakis et al., 2006)***.

Classification of ITP Based on duration of illness (acute versus chronic).

ITP can be classified according to how long symptoms have persisted. Thus, ITP is referred to as "newly diagnosed" within 3 months of initial diagnosis, "persistent" from 3 to 12 months after diagnosis, and "chronic" if it persists beyond 12 months ***(Rodeghiero et al., 2009)***.

In the absence of reliable predictive clinical or laboratory parameters of disease duration, the term "newly diagnosed ITP" was suggested for all cases at diagnosis. Anew category, called "persistent ITP," was introduced for patients with ITP to define the period lasting between 3 and 12 months from diagnosis ***(Sailer et al., 2006)***.

This category includes patients not achieving spontaneous remission or not maintaining their response after stopping treatment between 3 and 12 months from diagnosis. The chances of spontaneous remissions are still significant during this period making deferral of more aggressive therapeutic approaches (such as splenectomy) worthy of consideration ***(Imbach et al., 2006)***.

Acute ITP:

Acute ITP starts suddenly and usually follows a viral illness in a child. Acute ITP may require no treatment, especially if the platelet count does not fall too low and there is little bleeding. It usually improves spontaneously and, in children at least, rarely comes back ***(George and Raskob, 1998)*.**

The history is short with the appearance of purpura and bruising over a 24-48 hour period. With such symptoms the count is usually less than 10-20×109/l. Children with higher counts rarely shows any symptoms. The presenting platelet