INTRODUCTION

Primary immune thrombocytopenia (ITP) of childhood is an autoimmune disease characterized by production of pathogenic auto-antibodies, targeting platelet-specific antigen such as glycoprotein Ib/IX, glycoprotein IIb/IIIa ***(McMillan et al., 2009).***

These auto-antibodies bind not only to platelets, facilitating platelet destruction through variable mechanisms, including antibody-mediated lysis and T-cell-mediated platelet lysis ***(Olsson et al., 2003),*** but also to megakaryocytes, resulting in suppression of megakaryocyte activities and shortening of megakaryocytic survival, eventually leading to under-production of platelets ***(McMillan et al., 2004; Pan et al., 2011).***

Stromal-derived factor-1 (SDF-1) and its chemokine receptor CXCR4 are also involved in the migration, homing, mobilization, proliferation, and survival of hematopoietic stem cells ***(Broxmeyer et al., 1999).***

Of particular importance is the role of SDF-1 in enhancing megakaryopoiesis by regulating a different pathway to that used by Thrombopoietin and may be responsible for the platelet formation in the absence of TPO ***(Hodohara et al., 2000).***

Previous studies have shown single nucleotide polymorphisms (SNPs) in the human SDF-1 gene may influence SDF-1 function, such as serum SDF-1 level and its expression in hematopoietic stem cells in peripheral blood ***(Benboubker et al., 2001; Kimura et al., 2005; Soriano et al., 2002; Xiao et al., 2008).***

As the SDF-1 plays an important role in megakaryopoiesis, the role of SDF-1 SNPs in ITP patients has yet to be elucidated.

The study aimed to investigate the association between childhood ITP and polymorphisms in the SDF-1 gene.

AIM OF THE WORK

The study aims to investigate the relation of a single nucleotide polymorphism (= rs1801157) at position 801 in the 3′-untranslated region (UTR) of the SDF1 gene in Egyptian patients with ITP and its relation to disease susceptibility, severity of bleeding, response to treatment modalities and disease outcome.

CHAPTER 1

PRIMARY IMMUNE THROMBOCYTOPENIA (ITP)

Primary immune thrombocytopenia (ITP) is an acquired immune-mediated disorder characterized by isolated thrombocytopenia, defined as a peripheral blood platelet count less than 100×10^9/L, and the absence of any obvious initiating and/or underlying cause of the thrombocytopenia ***(Rodeghiero et al., 2009).***

Until recently, the abbreviation ITP stood for idiopathic thrombocytopenic purpura, but current awareness relating to the immune-mediated nature of the disease, and the absence or minimal signs of bleeding in a large proportion of cases have led to a revision of the terminology ***(Rodeghiero et al., 2009).***

Immune thrombocytopenic purpura (ITP) is one of the most common acquired bleeding disorders of childhood. The incidence is 1.9~6.4 per 105 children/year ***(Terrell et al., 2010)***.

Patients with ITP present with hemorrhage secondary to severe thrombocytopenia. The bleeding manifestations of ITP are highly heterogeneous; however, patients usually experience mild mucocutaneous hemorrhage (severe hemorrhage is uncommon when the platelet count is $> 30 \times 10^9$/L). Prospective data show that the risk of severe hemorrhage is extremely low for children with ITP regardless of the platelet count ***(Neunert et al., 2008).***

ITP is one of exclusion and antiplatelet antibody testing is not recommended because of high inter-laboratory variability and poor sensitivity ***(Davoren et al., 2005).***

The hallmark of immune thrombocytopenia (ITP) is autoimmune destruction of platelets in addition to suppression of platelet production by the bone marrow (BM) mega-karyocytes ***(Rodeghiero et al., 2009).***

Concepts surrounding the mechanisms of thrombocytopenia in ITP have shifted from the traditional view of increased platelet destruction mediated by autoantibodies to more complex mechanisms in which both impaired platelet production and T cell–mediated effects play a role ***(Olsson et al., 2003; Chang et al., 2003; McMillan et al., 2004; Houwerzijl, 2004 and Zhang et al., 2006).***

Recent advances in our understanding of the role of individual genetic risk factors and immune dysregulation in immune thrombocytopenic purpura (ITP) have opened the door to new avenues of research in the most common form of hematological autoimmune disease in adults and children ***(Neunert, 2008).***

Classification of ITP:

a) According to etiology:

In primary ITP, other causes of thrombocytopenia are not observed and the main problem is the bleeding. Secondary ITP involves immune-mediated forms of thrombocytopenia, such as systemic lupus erythematosus, human immunodeficiency virus (HIV), hepatitis C, drugs, and *Helicobacter pylori*, among others ***(Rodeghiero et al., 2009).***

Table (1): Types of ITP

Primary ITP	Primary ITP is an autoimmune disorder characterized by isolated thrombocytopenia (peripheral blood platelet count less than 100×10^9/L in the absence of other causes or disorders that may be associated with thrombocytopenia. The diagnosis of primary ITP remains one of exclusion; no 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.
Secondary ITP	All forms of immune-mediated thrombocytopenia except primary ITP

(Rodeghiero et al., 2009)

Table (2): Selected examples of medical conditions associated with secondary ITP.

- Systemic lupus erythematosus
- Antiphospholipid syndrome
- Autoimmune thrombocytopenia (eg, Evans syndrome)
- Common variable immune deficiency
- Drug administration side effect
- Infection with cytomegalovirus, *Helicobacter pylori*, hepatitis C, human immunodeficiency virus, varicella zoster
- Lymphoproliferative disorders
- Bone marrow transplantation side effect

(Neunert et al., 2011)

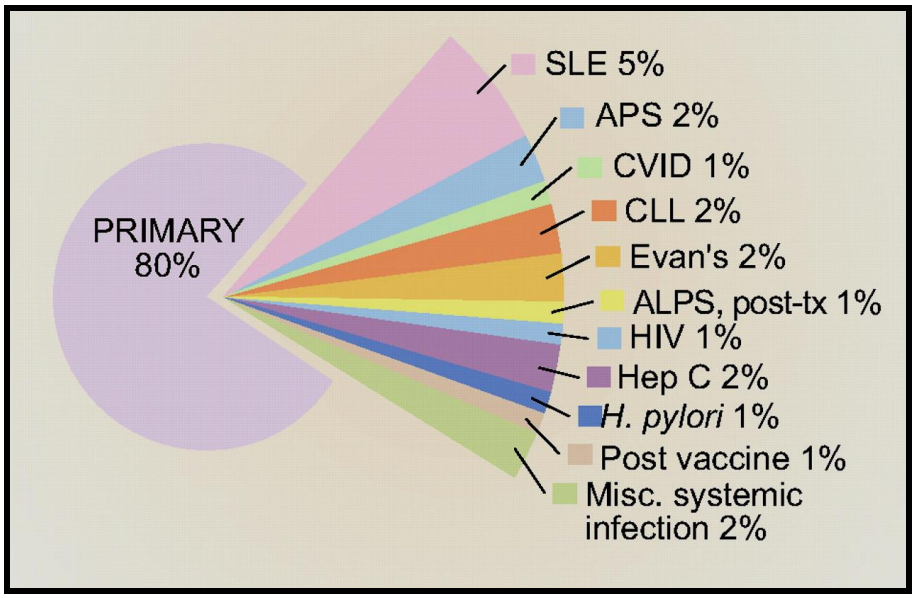

Figure (1): Types of ITP ***(Cines et al., 2009).***

SLE: Systemic lupus erythematosus; APLS: Antiphspholipid syndrome
CVID: Common variable immunodeficiency; CLL: Chronic lymphocytic leukemia
ALPS: Autoimmune lymphoproliferative syndrome; HIV: Human immunodeficiency virus
H pylori: Helicobacter pylori; HCV: Hepatitis C virus

b) According to disease phenotype:

The term acute ITP has recently been replaced by newly diagnosed ITP. It is defined when the platelet count is low for less than three months. Persistent ITP refers to patients who have not achieved remission or not maintained their response to treatment for a period between three and 12 months after diagnosis. When there is no remission of thrombocytopenia 12 months after diagnosis, it is considered chronic ***(Rodeghiero et al., 2009).***

Newly diagnosed ITP in children is usually preceded by viral infections and the patient exhibits thrombocytopenia ($<100x10^9$ platelets/L), petechiae and bruises. About 80% of these patients have spontaneous remission within the first six months after diagnosis; of the remaining 20% of cases, more than 50% have spontaneous remission within the first four years, i.e. platelet count normalizes each year in 10 to 15% of the patients ***(De Mattia et al., 2010).***

Thrombocytopenia persists for more than six months in 33% of children with ITP and for more than 12 months in 10% ***(Bennett et al., 2009).***

Ten to 30% of children who have chronic ITP at six months of follow-up achieve remission after this period ***(Imbach et al., 2003).***

The gradual onset of symptoms over more than 2 weeks, an initial platelet count of ≥ 20 × 109/L, and being aged more than 10 years at presentation are major predictors, while a negative history of preceding febrile illness and less-frequent mucosal bleeding are minor predictors for identifying the subset of patients more prone to developing chronic ITP ***(El-Alfy et al., 2010).***

Table (3): According to disease phenotype

Newly diagnosed ITP:	Within 3 months from diagnosis.
Persistent ITP:	Between 3 to 12 months from diagnosis. Includes patients not reaching spontaneous remission or not maintaining complete response off therapy.
Chronic ITP:	Lasting for more than 12 months.
Severe ITP:	Presence of bleeding symptoms at presentation sufficient to mandate treatment, or occurrence of new bleeding symptoms requiring additional therapeutic intervention with a different platelet-enhancing agent or an increased dose

(Rodeghiero et al., 2009)

c) According to severity of illness

ITP was defined as "severe" when the presence or recurrence of bleeding manifestations was sufficient to mandate treatment, regardless of the platelet count. Use of terms such as "mild" or "moderate" ITP was discouraged because of their vagueness ***(Rodeghiero et al., 2009).***

Three bleeding scores (SMOG index) that assess Bleeding symptoms, are grouped into three major domains: skin (S), visible mucosae (M), and organ (and internal mucosae) (O) ***(Rodeghiero et al., 2013),*** confirm that most children with ITP do not have serious bleeding problems despite very low platelet counts ***(Bolton-Maggs et al., 1997; Buchanan et al., 2002; Neunert et al., 2008).*** Severe bleeding is more likely in children with platelet counts less than 10 x 10^9/L ***(Butros et al., 2003).***

The incidence of ICH in children with ITP is approximately 0.1% to 0.5% and predicting with confidence which children will develop an ICH is not possible ***(Imbach et al., 2006; Lilleyman, 1994; Lilleyman, 1997).***

Risk factors for ICH in children with severe thrombocytopenia include head trauma and concomitant use of medications that adversely affect platelet function ***(Provan et al., 2010).***

Classification of children with ITP by severity of bleeding is useful to guide management ***(Provan et al., 2010)***.

Table (4): Grades of severity and management of patients with ITP

Bleeding/quality of life	Management approach
Grade 1: Minor bleeding, Few petechiae (<100 total) and/or < 5 small bruises (< 3-cm diameter); No mucosal bleeding	Consent for observation
Grade 2: Mild bleeding,. Many petechiae (>100 total) and/or >5 large bruises (>3-cm diameter); No mucosal bleeding	Consent for observation or for treatment in selected children
Grade 3: Moderate bleeding. overt mucosal bleeding, troublesome lifestyle.	Intervention to reach grade 1/2 in selected children
Grade 4: Mucosal bleeding or suspected internal hemorrhage	Intervention

(Bolton-Maggs et al., 2001; Buchanan et al., 2002; Imbach et al., 2006)

d) According to response to treatment

The definition of a treatment response should ideally reflect clinically important endpoints including bleeding and quality of life, rather than rely exclusively on surrogate end points (platelet count) with arbitrary thresholds. Nevertheless, the platelet count is a useful measure of response that is objective, clinically relevant, and easily compared (Table 5). Identical response criteria are proposed for splenectomy. Assessment of response should occur within 1 to 2 months and after withholding concomitant treatment(s), if any, for a time sufficient to reasonably exclude persistence of their effect ***(Rodeghiero et al., 2009).***

Table (5): According to responce to treatment

Complete response (CR)	A platelet count ≥ 100 × 10^9/L measured on 2 occasions > 7 days apart and the absence of bleeding.
Response (R)	A platelet count ≥ 30 × 10^9/L and a greater than 2-fold increase in platelet count from baseline measured on 2 occasions > 7 days apart and the absence of bleeding.
No response (NR)	A platelet count < 30 × 10^9/L or a less than 2-fold increase in platelet count from baseline or the presence of bleeding. Platelet count must be measured on 2 occasions more than a day apart.
Loss of complete response	A platelet count < 100 × 10^9/L measured on 2 occasions more than a day apart and/or the presence of bleeding.
Loss of response	A platelet count < 30 × 10^9/L or a less than 2-fold increase in platelet count from baseline or the presence of bleeding. Platelet count must be measured on 2 occasions more than a day apart.

(Rodeghiero et al., 2009)

Diagnosis:

ITP is a diagnosis of exclusion made in most cases through a careful history and physical exam, complete blood count (CBC), and review of the peripheral smear. Primary ITP requires only the finding of an isolated thrombocytopenia (< 100,000/L) with no obvious associated medical condition ***(Provan et al., 2010).***

There is no "gold standard" test that can reliably establish the diagnosis. Response to ITP-specific therapy, for example, intravenous immunoglobulin (IVIg) and intravenous anti-D, is supportive of the diagnosis, but a response does not exclude secondary ITP ***(Provan et al., 2010).***

Table (6): International ITP Working Group consensus recommendations for the diagnosis of ITP in children and adults

Basic evaluation	Tests of potential utility in the management of an ITP patient	Tests of unproven or uncertain benefit
• Patient history	• Glycoprotein-specific antibody	• TPO
• Family history	• Antiphospholipid antibodies (including anticardiolipin and lupus anticoagulant)	• Reticulated platelets
• Physical examination	• Antithyroid antibodies and thyroid function	• PaIgG
• Complete blood count and reticulocyte count	• Pregnancy test in women of childbearing potential	• Platelet survival study
• Peripheral blood film	• Antinuclear antibodies	• Bleeding time
• Quantitative immunoglobulin level measurement	• Viral PCR for parvovirus and CMV	• Serum complement
• Bone marrow examination (in selected patients)		
• Blood group (Rh)		
• Direct antiglobulin test		
• H pylori		
• HIV		
• HCV		

(Rodeghiero et al., 2009)

Basic evaluation:

Clinical presentation and natural history of ITP in children:

The peak age of presentation of ITP in children is between 5 and 6 years, with 70% of cases presenting between ages of 1 and 10 years ***(Kühne et al., 2003).***

Approximately 50%-60% of children will have a febrile illness that preceded the discovery of thrombocytopenia. Numerous viral infections, including rubella, varicella, mumps, rubeola, and EBV, as well as immunizations with measles-mumps-rubella vaccine, have been associated with the subsequent development of ITP in children ***(Kühne et al., 2003; Bennett et al., 2009; Cines et al., 2009).***

Signs of mucocutaneous bleeding most often occur without other systemic symptoms, and the child frequently does not appear ill ***(Bennett et al., 2009).***

Many patients have either no symptoms or minimal bruising, whereas others experience serious bleeding, which may include gastrointestinal hemorrhage (GI), extensive skin and mucosal hemorrhage, or intracranial hemorrhage (ICH). The severity of thrombocytopenia correlates to some extent but not completely with the bleeding risk ***(Neylon et al., 2003; Stasi et al., 2008).***

In a study of 863 children with newly diagnosed ITP, none or mild bleeding manifestations were reported in 77% of patients, moderate bleeding occurred in 20%, and severe bleeding in 3% ***(Neunert et al., 2008).***

Life-threatening bleeding is rare and the estimated risk of intracranial hemorrhage is between 0.1% and 0.5% in newly diagnosed cases ***(Kühne et al., 2003; Neunert et al., 2008).***

Approximately 65%-70% of children remit by 6 months and another 15%-20% by 12 months ***(Kühne et al., 2003; Bennett et al., 2009).***

The 5%-10% of children who develop chronic ITP tend to be older, are more often female, and usually present with a higher platelet count ***(Bennett et al., 2009).***

Inherited thrombocytopenia should be considered in patients with long-standing thrombocytopenia unaffected by treatment and in those with a family history of thrombocytopenia or bleeding disorders ***(Provan et al., 2010).***