INTRODUCTION

Immune thrombocytopenia (ITP) is one of the most common blood diseases as well as the commonest acquired bleeding disorder in childhood ***(Pesmatzoglou et al., 2012).***

It is characterized by immune-mediated accelerated platelet destruction and suppressed platelet production ***(Neunert et al., 2011)***. The autoantibodies frequently appear to be directed against GpIb/IX and GPIIb/IIIa, although specificity for other platelet antigens can occur ***(Semple et al., 2010)***.

Although the development of autoantibodies by B cells remains central in the pathophysiology of ITP, a multi-dysfunction in cellular immunity and cytokine response may take place in the pathogenetic mechanisms of the disease ***(Stasi et al., 2008)***.

ITP is usually a self-limiting disease in children ***(Provan et al., 2010).*** However, approximately 20% of childhood newly diagnosed ITP progress to a chronic form ***(Rodeghiero et al., 2009).***

The clinical differences between newly diagnosed and chronic ITP suggest the existence of different pathophysiological mechanisms in the two forms ***(Provan et al., 2010).***

Some authors have investigated the role of genetic and immunologic factors in the development of this disorder ***(Foster et al., 2001; Wang et al., 2005; Wu et al., 2005).*** They

failed in identifying specific characteristics of children with ITP who will likely develop the chronic form of the disorder, mainly because of the study design and differences in patients' immunomodulating therapy ***(Guo et al., 2007; Stasi et al., 2008).***

A recent study showed in a preliminary analysis that Interleukin10 (IL-10) expression was significantly increased in a group of 28 children presenting an acute course of ITP with respect to 11 children that had a chronic progression of the disease, even if it was not possible to define a clear cut-off value of IL-10 with a prognostic significance ***(Del Vecchio et al., 2011).***

AIM OF THE WORK

The purpose of the present study is to investigate the role of serum level of IL-10, a cytokine with broad immunoregulatory function and a product of T helper (Th) type 2 cells, as a marker for prediction of ITP chronic progression in children.

Chapter I

IMMUNE THROMBOCYTOPENIC PURPURA (ITP)

Introduction:

Platelets are important components in the first phase of hemostasis known as platelet plug formation. Defects in platelet number or function may lead to bleeding. Bleeding due to platelet disorders usually involves skin and mucous membranes, including petechiae, purpura, ecchymosis, epistaxis, hematuria, menorrhagia and gastrointestinal and even intracranial hemorrhage ***(Bussel & Renaud, 2011).***

Immune thrombocytopenia (ITP) is an autoimmune syndrome involving antibody- and cell-mediated destruction of platelets and suppression of platelet production that may predispose to bleeding. Recent recommendations from an international working group suggest that ITP be used to designate all cases of immune-mediated thrombocytopenia, occurring as a whether component of another clinically evident disorder or drug exposure (secondary ITP) or in the absence of a clear predisposing etiology (primary ITP) ***(Cuker & Cines, 2010).***

Definition:

Idiopathic thrombocytopenic purpura, or ITP, is a bleeding disorder characterized by isolated thrombocytopenia with

peripheral blood platelet count < 100 × 10^9/L in the absence of any obvious initiating or underlying cause. Therefore, the diagnosis of ITP is one of exclusion and is based principally on patient history, physical examination, complete blood count, and review of the peripheral blood smear ***(Liebman & Pullarkat, 2011).***

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).***

Epidemiology:

North American studies reported that the incidence of ITP in children was 7.2–9.5 per 100,000. There is a seasonal pattern to ITP with a peak in winter and early spring time, presumably mimicking the pattern of viral illnesses ***(Bussel & Renaud, 2011).***

Male and female children were affected approximately equally with the caveat that boys outnumbered girls in young children, especially those less than 1 year of age ***(Kuhne et al., 2001)***.

In Egypt, sixty-five percent of the childhood ITP patients had acute course and 34.9% had chronic ITP. Patients with chronic ITP had a higher mean age at diagnosis. Females more

than 10 years of age were more susceptible than males to follow a chronic course ***(El Alfy et al., 2010).***

ITP is usually chronic in adults and the probability of durable remission is 20–40 percent. The male to female ratio in the adult group varies from 1:1.2 to 1.7 in most age ranges and the median age of adults at the diagnosis is 56–60 ***(Pallak et al., 2013)***.

Etiology of ITP:

Identification of factors that precipitate ITP is extremely difficult due to the likely transient nature of the provoking event ***(Johnsen et al., 2012).***

1- Acute infections:

An acute infectious event has long been suspected to be a trigger in the initiation of primary ITP. In newly diagnosed ITP, there is often a history of antecedent symptoms that may be attributed to infection in the days or weeks before diagnosis of ITP ***(Cines et al., 2009).***

In most acute ITP cases, a pathogen is not identified, and the vague constitutional symptoms predating or accompanying the diagnosis of ITP are difficult to distinguish from those which that be expected with inflammation from an ongoing autoimmune process. Therefore, unidentified acute infection remains a plausible candidate to induce ITP either by providing an opportunity for molecular mimicry or similar targeting of the

immune system to platelets or by the mere presence of an acute inflammatory response tipping the balance in a predisposed patient to break tolerance ***(Johnsen, 2012).***

2- Vaccination:

For decades, ITP has been known to be a rare complication of the measles-mumps-rubella vaccination. This is likely due to provocation of the immune system by the vaccine antigens in a manner similar to actual infection with these childhood diseases, each of which is also associated with ITP. In a recent study in a large network of managed care organizations, the association between measles-mumps-rubella and increased risk of ITP in young children was confirmed, although overall such events were still rare ***(O'Leary et al., 2012).*** The investigators also reported a possible (even more rare) increased risk of ITP after hepatitis A, Varicella, or dTap vaccination in older children ***(Johnsen, 2012).***

3- Chronic infections:

ITP is associated with several chronic infections, notably Helicobacter pylori (H Pylori), HIV, and hepatitis C virus (HCV) infections ***(Johnsen, 2012).***

The pathogenesis of H pylori–associated ITP likely includes elements inherent to both the bacterium and the human host. From the side of the H pylori bacteria, there is evidence that H pylorimay provoke antigens ***(Stasi et al., 2009).***

Moreover, some strains of H pylorican induce platelet aggregation and platelet expression of p-selectin and phosphatidylserine ***(Yeh et al., 2010).***

From the perspective of the human host, genetic factors such as Lewis type (a carbohydrate blood group antigen system) or HLA type are associated with H pylori– associated ITP ***(Asahi et al., 2008).***

Therapies to eradicate H pylorihave demonstrated success in treating H pylori–associated ITP, but the rates of success vary in different geographic populations ***(Cines et al., 2009).***

Similar to the antiplatelet Abs provoked during H pyloriinfection, HIV can provoke anti-HIV Abs that cross-react with platelet glycoproteins and form immune complexes, as can HCV ***(Semple et al., 2010).*** Additional mechanisms of platelet destruction also become apparent from studies in virus-associated ITP. For example, HIV can provoke anti-GpIIIa Abs, which lead to complement-independent platelet fragmentation ***(Li et al., 2005).***

Whereas in HCV, the virus itself can bind platelets directly, leading to circulating anti-HCV Ab-antigen-platelet complexes ***(Cines et al., 2009).*** In both HIV and HCV, suppression of viral replication can result in improvement of thrombocytopenia ***(Johnsen, 2012).***

4- Autoimmune disorders:

Patients with systemic autoimmune diseases, such as systemic lupus erythematosus, antiphospholipid antibody syndrome, and rheumatoid arthritis, are prone to developing ITP ***(Arkfeld et al., 2009).***

In these cases autoantibodies against platelets, leucocytes and erythrocytes can be detected. It can be in the form of specific-platelet autoantibodies or immune complex deposit on platelets ***(Levine et al., 2004).***

5- Immunodeficiency:

Patients with congenital immunodeficiency are at high risk for the development of immune-mediated thrombocytopenia. The main cause referred to an improper regulation of their immune system ***(Ware, 2001)***.

6- Drug induced immune thrombocytopenia:

Drug-induced thrombocytopenia (DITP) is a relatively common clinical disorder ***(Visentin and Liu, 2007).***

Accelerated platelet destruction in the presence of the offending drug is most often of immune origin. DITP is characterized by drug-dependent antibodies that bind to platelets and cause their destruction ***(Aster, 2007).***

Hundreds of drugs have been implicated in its pathogenesis, among those, drugs most often associated with

DITP are: heparin, cinchona alkaloid derivatives (quinine and quinidine), penicillin, sulfonamides, non-steroidal anti-inflammatory drugs, anticonvulsants, antirheumatic and oral antidiabetic drugs, gold salts, diuretics, rifampicin and ranitidine ***(Van den Bemt et al., 2004).***

7- Alloimmune Thrombocytopenia:

- *Neonatal Alloimmune Thrombocytopenia (NAITP)*

It occurs as a result of maternal immunization against fetal platelet alloantigens inherited from the father. Alloantibodies of IgG type, cross the placenta, cause immune destruction of platelets in utero. It was noticed that this immune thrombocytopenia occurs in 30% of cases in the first pregnancy ***(Koutsogianni, 2004)***.

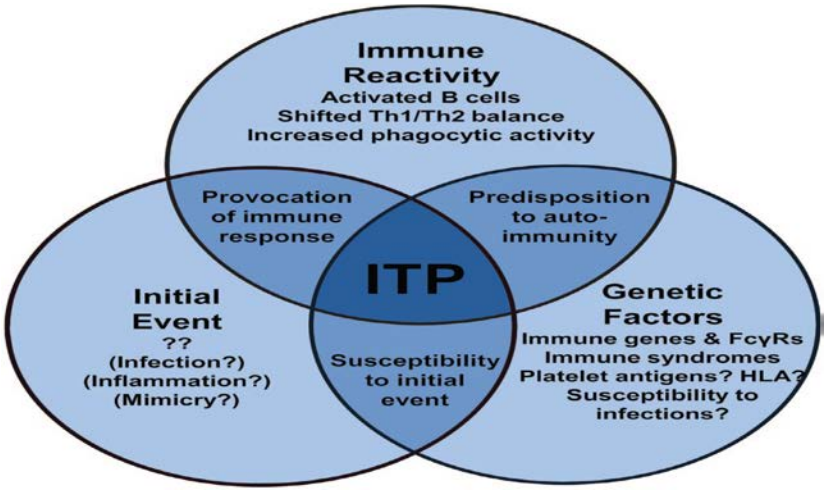

Figure (I): Model of relationship of contributing factors in ITP *(Johnsen, 2012).*

Pathophysiology:

The pathophysiology of ITP is a complex process with involvement of many players in the human immune orchestera including antibodies, cytokines, antigen-presenting cells, costimulatory molecules, and T and B lymphocytes (including T-helper, T-cytotoxic, and T-regulatory lymphocytes) ***(Blanchette & Bolton, 2008)*.**

Immune thrombocytopenic purpura (ITP) is characterized by autoantibody mediated platelet destruction. These autoantibodies opsonize platelets for splenic clearance, resulting in low levels of circulating platelets ***(Cuker & Cines, 2010).***

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, 2009).***

An acute infection often appears to be the initial trigger, but that may only potentiate an already-established immunologic disturbance ***(Bussel & Renaud, 2011)***.

Probably most of acute and / or chronic ITP may be caused by molecular mimicry, and antibodies formed in response to viral infection may cross-react with antigenic targets naturally present on platelets ***(Fujita, 2003)***.

However, increasing evidence indicates that an additional component of this disorder is immune-mediated decreased platelet production ***(Nugent et al., 2009).***

Studies supporting suppressed platelet production include: reduced platelet turnover in over 80% of ITP patients, morphological evidence of megakaryocyte damage, autoantibody-induced suppression of in vitro megakaryocytopoiesis, and increased platelet counts in most ITP patients following treatment with thrombopoietin receptor agonists ***(Cines & McMillan, 2005).***

The most commonly occurring autoantibodies in about 75% of ITP patients are directed against the platelet surface glycoprotein complexes gpIIb–IIIa and gpIb–IX ***(McMillan, 2000)***. Antibodies against other glycoproteins (Ia–IIa, IV, and V) have been identified ***(Nugent et al., 2009).***

A possible sequence of events in ITP is as follows. A trigger, possibly an infection or toxin, leads to the formation of antibodies /immune complexes that attach to platelets. Antibody-coated platelets then bind to antigen-presenting cells (macrophages or dendritic cells) through low-affinity Fcγ receptors (Fcγ RIIa/Fcγ RIIIa) and are internalized and degraded. Activated antigen-presenting cells then expose novel peptides on the cell surface and with co-stimulatory help facilitate the proliferation of platelet antigen specific CD4-positive, T-cell clones. These T-cell clones drive autoantibody production by platelet antigen-specific B-cell clones ***(Blanchette & Bolton, 2008)***.

As part of the platelet destructive process in ITP, cryptic epitopes from platelet antigens are exposed, leading to the formation of secondary platelet antigen-specific T-cell clones, with stimulation of new platelet antigen-specific B-cell clones and broadening of the immune response. The autoantibody profile of individual patients who have ITP reflects activity of polyclonal autoreactive B-cell clones derived by antigen-driven affinity selection and somatic mutation ***(Blanchette & Bolton, 2008)***.

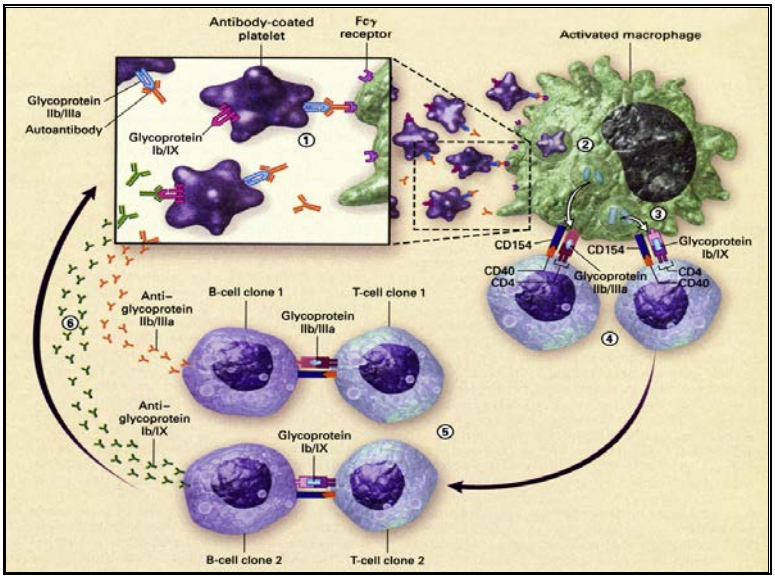

Figure (II): Pathogensis of epitope spread in ITP. The factors that initiate autoantibody production are unknown. Here, glycoprotein IIb/IIIa is recognized by autoantibody (orange), whereas antibodies that recognize the glycoprotein Ib/IX complex have not been generated at this stage (1). Antibody-coated platelets bind to antigen-presenting cells (macrophages or dendritic cells) through Fcg receptors and then are internalized and degraded (2). Antigen-presenting cells not only degrade glycoprotein IIb/IIIa (light blue oval), thereby amplifying the initial immune response, but also may generate cryptic epitopes from other platelet glycoproteins (light blue cylinder) (3). Activated antigen-presenting cells (4) express these novel peptides on the cell surface along with costimulatory help (represented in part by the interaction between CD154 and CD40) and the relevant cytokines that facilitate the proliferation of the initiating CD4-positive T-cell clones (T-cell clone 1) and those with additional specificities (T-cell clone 2) (5). B-cell immunoglobulin receptors that recognize additional platelet antigens(B-cell clone 2) thereby also are induced to proliferate and synthesize antiglycoprotein Ib/IX antibodies (green) in addition to amplifying the production of anti-glycoprotein IIb/IIIa antibodies(orange) by B-cell clone 1 (6) ***(Cines & Blanchette, 2002).***

It is increasingly clear that cellular immune mechanisms play a role in ITP. The production of antiplatelet antibodies by B cells requires antigen-specific, CD4-postive, T-cell help. It also is possible that in some ITP cases, cytotoxic T cells play a role in the destruction of platelets ***(Blanchette & Bolton, 2008)***.

Cytotoxic lymphocytes appear to be abnormally activated in patients with ITP, showing increased expression of cytotoxic genes such as tumor necrosis factor, perforin, granzyme A and granzyme B ***(Zhang et al., 2006, Olsson et al., 2003).***

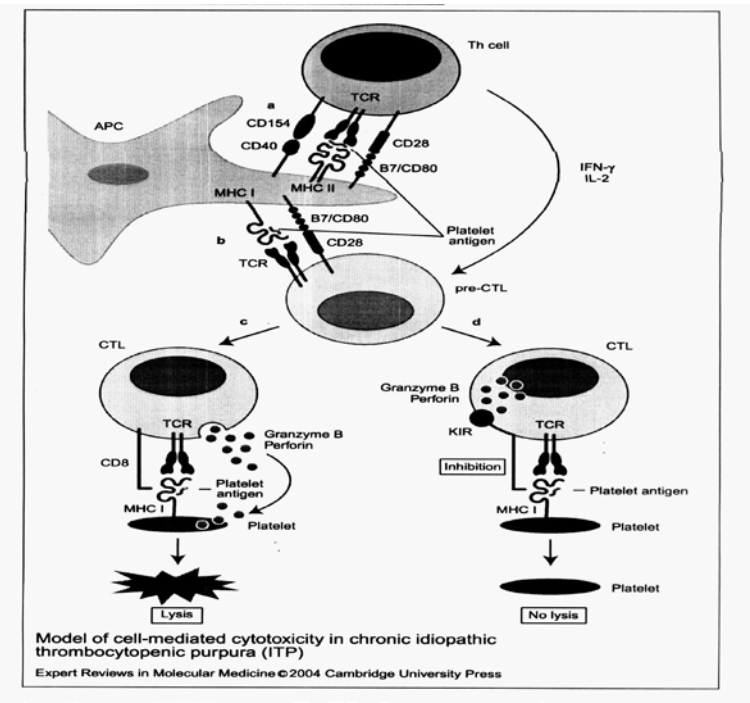

Figure (III): Model of cell-mediated cytotoxicity in chronic idiopathic thrombocytopenic purpura (ITP) *(Johnsen, 2012).*