INTRODUCTION

Immune thrombocytopenia (ITP) is an autoimmune blood disorder characterized by the production of antibodies against platelets antigens and therefore platelet destruction ***(Payandeh et al., 2013).***

It is a hematologic disorder affecting children with an incidence of four to five cases per 100,000 children per year. Both the natural course of ITP and the risk of life-threatening bleeding are unpredictable. The clinical outcome, whether acute or chronic, is also unclear ***(Elalfy et al., 2009)***.

Helicobacter pylori (*H. pylori*) is a gram- negative, micro-aerophilic, slowly-growing, spiral shaped and flagellated organism. It is believed that this organism infects more than half of world's human population ***(Wen and Moss, 2009)***.

The association between *H. pylori* infection and peptic ulcer, chronic gastritis and gastric lymphoma has been established ***(Mishra, 2013)***. Also, *H. pylori* has been implicated in the pathogenesis of many extra- gastric disorders including growth retardation, cardiovascular diseases (e.g. coronary disease), haemtological disorders (e.g. iron deficiency anaemia, Henoch Schonein purpura) and both organ specific and non-organ specific autoimmune diseases ***(Franceshi et al., 2015)***.

Many studies confirmed the role of *H. pylori* in adult chronic ITP, while others denied. However, evidence suggesting

that *H. pylori* infection may play role in pediatric persistent and chronic ITP is not yet established and published studies are not conclusive and empirical treatment of *H. pylori* in children with persistent and chronic ITP remains controversial ***(Ferrara et al., 2009)***.

AIM OF THE WORK

To evaluate the role of active *H. pylori* infection on persistence and chronicity of ITP in children and adolescents.

Chapter One

IMMUNE THROMBOCYTOPENIA

Definition and classification:

ITP is an autoimmune disorder characterized by a low circulating platelet count caused by the destruction of antibody-sensitive platelets in the reticuloendothelial system. ITP can be classified as childhood or adult, acute or chronic, and primary or secondary ***(Elalfy, 2011).***

Traditionally, **'acute ITP'** has been used to describe a self-limited form of the disease whereas **'chronic ITP'** identified ITP lasting for more than 6 months. Recently, the term **'newly diagnosed ITP'** is adopted for all cases at diagnosis. A new category, called **'persistent ITP'**, was introduced for patients with ITP to define the period lasting between 3 and 12 months from diagnosis. This category includes patients not achieving spontaneous remission or not maintaining their response after stopping treatment between 3 and 12 months from diagnosis. The term **'chronic ITP'** is used for patients with ITP lasting for more than 12 months ***(Rodeghiero et al., 2009).***

Historical point of view:

Before platelets were identified, the identification of ITP was based exclusively on the presence of purpura in an otherwise healthy individual. Purpura was recognized as a clinical symptom

as early as the Greco-Roman period by physicians such as Hippocrates and Galen ***(Stasi and Newland, 2011).***

It was not until 1025 that the Persian physician and philosopher Abu Ali al-Hussain Ibn Abdallah Ibn Sina briefly described chronic purpura that could fit the diagnosis of ITP ***(Jones and Tocantins, 1933).***

In 1556, the Portuguese physician Amatus Lusitanus described a boy who developed dark macules and bloody discharges for several days in the absence of fever and eventually recovered spontaneously. In 1735, the German physician and poet Paul Gottlieb Werlhof provided the classic clinical description of ITP, calling it **'morbus maculosus haemorrhagicus'**. He reported the case of a 16-year-old girl with cutaneous and mucosal bleeding that occurred after an infectious disease. It appeared around the neck and on the arms, spots partly black, partly violaceous or purple ***(Stasi and Newland, 2011).***

In 1887, the histologist Denys, called attention to the fact that platelets were diminished during the active phase of the purpura and increased when the hemorrhages ceased ***(Denys, 1887).***

In 1899, Henoch in Berlin differentiated purpura simplex with bleeding signs of the skin only (today known as dry purpura) from purpura haemorrhagica with mucosal bleeding (today known as wet purpura) ***(Henoch, 1899).***

Epidemiology:

In children, the disease has an incidence of 1.9 to 6.4 cases per 100.000 a year, compared to 3.3 cases per 100.000 a year in adults. Pediatric ITP has a peak incidence between 2 and 5 years of age, with no difference or a slight predominance of males ***(Laarhoven, 2015).***

It was reported in a large Egyptian study a 30% rate of chronic ITP, matching the international reports ***(Khalifa et al., 1993).***

There was no gender preference in most acute ITP studies; however, chronic ITP was more frequent in females in Egypt ***(Elalfy, 2013).***

A history of preceding viral infection was reported in 71% of acute and 63% of chronic ITP cases ***(Al-Mulla et al., 2009).***

Much of the research related to the aetiology of childhood ITP has centered on the possible association of ITP with recent vaccination, particularly with the MMR vaccine ***(Farrington et al., 1995; Black et al., 2003; Smeeth et al., 2004).***

A mass measles/mumps/rubella (MMR) vaccination for Egyptian secondary school adolescents after an epidemic of German measles in 2007 was followed by an increased incidence of ITP in the following few weeks ***(Elalfy, 2012).***

It was noticed that ITP has a seasonal variation, whereby the majority of cases who had an ITP diagnosis following an infection were diagnosed in the winter and the minority of cases with preceding infections were diagnosed in the summer months. This time-trend correlation between paediatric ITP patients and the known seasonal variation in influenza and other infections suggests a casual role of infections in paediatric ITP ***(Yong et al., 2010).***

Pathogenesis:

Immune-mediated mechanisms were first suggested in 1905 by Marino, who produced an antiplatelet antibody by injecting rabbit platelets into guinea pigs ***(Marino, 1905).***

In 1915 the German physician Ernest Frank proposed that it resulted from toxic suppression of the megakaryocyte by a substance produced in the spleen ***(Frank, 1915).***

In 1916 a medical student in Prague, Paul Kaznelson challenged Frank's idea and proposed that essential thrombocytopenia resulted from increased platelet destruction in the spleen. Kaznelson convinced his tutor, Professor Doctor Schloffer, to perform a splenectomy in a 36-year-old woman with a history consistent with our current definition of chronic ITP. The platelet count was 2000/L prior to splenectomy and rose to 500,000/L within 4 weeks from surgery with complete resolution of the purpura ***(Kanzelson, 1916).***

In 1946, ***Dameshek and Miller*** observed that the numbers of megakaryocytes and platelets were correlated across a variety of disorders, including leukemia and pernicious anaemia, and that the platelet count was consistently low when megakaryocytes were reduced in number. However, while platelet numbers were decreased in ITP, megakaryocyte numbers were normal or increased, but only a third or fewer of megakaryocytes showed evidence of platelet production. They concluded that the decrease in blood platelets was the result of a severe reduction in platelet production by megakaryocytes ***(Dameshek and Miller, 1946).***

In 1951, Harrington injected himself with plasma obtained from an ITP patient and developed a transient thrombocytopenia within hours, demonstrating that ITP is characterized by reduced platelet survival due to a humoral factor ***(Laarhoven, 2015).***

In 1953, Pisciotta hypothesized that, in ITP, megakaryocytes suffer from a defect in maturation as well as from abnormalities in platelet formation and release and that a powerful platelet agglutinin present in ITP was equally capable of attacking megakaryocytes and the platelets surrounding and budding from megakaryocytes ***(Pisciotta et al., 1953).***

In 1965, Shulman delineated that the thrombocytopenic factor in the plasma of ITP patients was associated with immunoglobulin G (IgG). The nature of the IgG producing

thrombocytopenia was eventually unraveled in 1982 by the experiments of van Leeuwen et al. They noted that sera or eluates of platelets from patients with ITP would, in each case, bind to normal platelets but only about one quarter would bind to the platelets of patients with Glanzmann thrombasthenia. They speculated that ITP patients produce autoantibodies against either platelet glycoprotein (GP) IIb or GPIIIa because thrombasthenic patients lack these proteins ***(Stasi et al., 2011).***

These antibodies are responsible for Fc-gamma receptor (FcγR) mediated platelet destruction by phagocytosis, which mainly occurs in the spleen. Two studies in particular, by ***Chang et al. (2003) and McMillan et al. (2004)*** support the view that autoantibodies in ITP suppress megakaryocyte production and maturation and platelet release ***(Laarhoven, 2015).***

In this disease model, platelets are opsonized by autoantibodies, leading to activation of FcγR bearing phagocytes and antigen presenting cells (APC), potentially resulting in recognition of autoantigen specific T cells. These T cells in turn interact with B cells, thereby modulating, perhaps boosting, autoantibody production, both closing the continuous pathogenic loop and strengthening it due to the process of somatic hypermutation. However, this mechanism of disease fails to account for all ITP patients ***(Cines et al., 2002).***

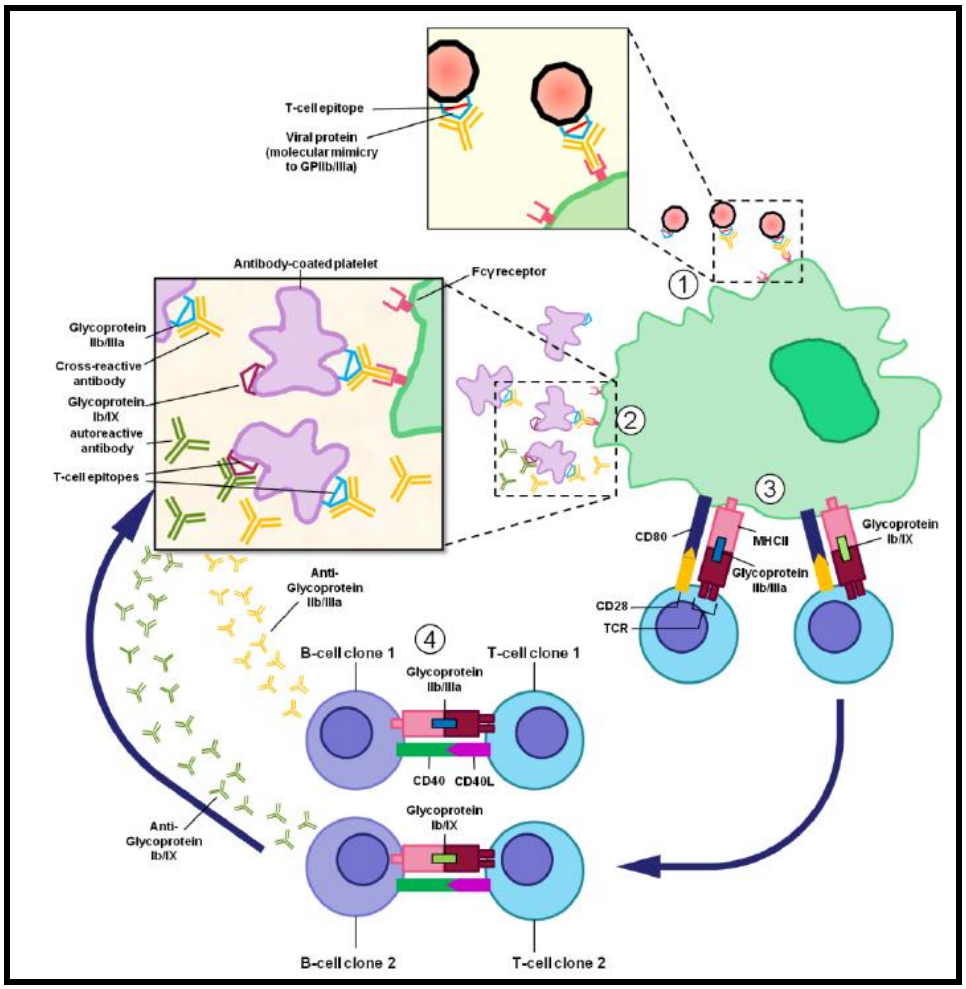

Figure (1): Pathogenesis model of ITP. Molecular mimicry, antibodies against a viral epitope are formed which also interact with glycoproteins on the platelets' surface (1). These antibodies opsonize platelets leading to increased uptake by FcγR bearing cells that can function as antigen presenting cells (APC) (2).This results in an augmented platelet antigen presentation (3). Autoantigen-specific T cells can interact with these antigens, and become activated. In turn, activated autoantigen specific T cells can stimulate B cells, which will continue producing antibodies and likely even undergo somatic hypermutation (4) ***(Cines, NEJM, 2002).***

The second immune mediated mechanism consists of exclusively T cell mediated platelet lyses.

In 1991 ***Semple et al.*** suggested that disorder may be the result of an abnormal T helper (Th) -cell defect that could direct autoreactive B cells to differentiate and secrete IgG autoantibodies ***(Semple et al., 1994).***

Olsson et al. made the intriguing observation that in patients with ITP, devoid of platelet autoantibodies, thrombocytopenia can be mediated by CD 8+ cytotoxic T lymphocytes (Tc). Patients with active disease had peripheral blood Tc that could bind to platelets in vitro and cause significant lysis of the platelets, whereas those patients in remission had little antiplatelet Tc reactivity ***(Olsson et al., 2003).***

In addition, these patients exhibited an increased expression of several cytotoxic and TH1 related genes such as IFNγ and IL-2, while members of the killer cell immunoglobulin-like receptor (KIR) were down regulated in patients with active ITP in comparison with controls and patients in remission. The latter potentially sensitizes autoreactive T cells toward destruction of self as KIR are associated with down regulation of cytotoxic T cells. Both findings underline CD8+ cytotoxic T cell involvement in ITP. Interestingly, ***Zhao et al.*** confirms the increased platelet lysis in presence of CD8+ cytotoxic T cells in ITP patients in comparison to controls. Moreover, they found that this

mechanism of action is found in the majority of 'autoantibody-negative' ITP patients (80%) in contrast to half of 'autoantibody-positive' ITP patients ***(Laarhoven, 2015).***

Finally, an important line of research encompasses genetic variations underlying ITP.

Among others, associations are known between genes influencing cytokines and/or its receptors involved in evoking a pro-inflammatory response, including T cell activation ***(McKenzie et al., 2013).***

Genetic variation within the FcγRII and FcγRIII genes leads to subtypes with higher affinity (e.g. FcγRIIa-H131, FcγRIIIa-V158, FcγRIIIb-HNA1a), functional defects (FcγRIIb-T232) or to increased expression levels (FcγRIIc-C-ORF) and copy number variations (CNV). Thus, the balance and function of FcγR might determine the means of processing and presentation of opsonized platelets in ITP, which may influence antigen-presentation in such a way that it results in autoreactive T cell activation ***(Laarhoven, 2015).***

In addition, the FcγRIIb-T232, which is associated with impaired inhibitory FcγRIIb function, and its heterozygous FcγRIIb-I/T232genotype was shown to predict chronic disease outcome in pediatric ITP. Thus, genetic variation within the FcγR genes is associated with ITP, but whether this reflects a role of FcγR in pathogenesis or in the clinical course of ITP is not yet known ***(Laarhoven, 2015).***

Thrombopoietin in ITP

TPO levels in ITP are low compared to those with thrombocytopenia from aplastic anemia, suggesting inappropriate thrombopoiesis in most of patients with ITP ***(Emmons et al., 1996).***

In ITP, thrombopoietin levels are normal in 75% of the cases rather than increased. Levels of TPO lower than expected in ITP may be caused by binding to TPO-receptor c-Mpl on the increased megakaryocyte mass with subsequent internalization and degradation or secondary to TPO bound to platelets targeted for destruction ***(Mokhtar et al., 2012).***

Current view of the pathophysiology of ITP

New thinking about the pathophysiology of ITP has been stimulated by recent epidemiological studies suggested that ITP might be related to emerging chronic infections, such as hepatitis C virus (HCV), human immunodeficiency virus (HIV), and *Helicobacter pylori* ***(Stasi et al., 2011).***

Clinical picture:

- The symptoms and signs of ITP is highly variable ***(Provan et al., 2010).***
- The most common presentation of ITP was **ecchymosis and petechiae** in 80%–100% of cases. However, absence of purpura does not exclude subclinical disease ***(Rodeghiero et al., 2009).***

- **Hematemesis and hematuria** are rare manifestations, but the latter could be a warning sign for an associated intracranial hemorrhage ***(Elalfy et al., 2010).***
- **Epistaxis** is a common problem in pediatric ITP ***(Hijazi et al., 1995).***
- **Intracranial Hemorrhage in ITP:** The risk of life-threatening bleeding in chronic ITP is very small, but clinically significant bleeding manifestations have been reported in 13-27% of such patients, and restrictions on daily activities are often imposed because of parental fears about bleeding. Intracranial hemorrhage can be neither anticipated nor prevented; however, some factors, such as a low platelet count, are considered more risky for this complication than others ***(Elalfy, 2011).***
- Patients with secondary ITP are less likely to bleed than those with primary ITP and might have higher platelet count at diagnosis. This could be associated with evidence of a different or combination of autoimmune diseases ***(Al-Sayes et al., 2012).***
- Splenomegaly is not a common feature of primary ITP.
- The mortality rate of ITP is very low in Arab countries, matching the international standards of less than 1% ***(Elalfy, 2013).***

Assessment of disease severity

Scoring of disease severity is an important tool in classification, not only to monitor disease activity, but also as a guideline to determine the need of medical intervention. Nowadays, disease severity is categorized based on bleeding tendency rather than the degree of thrombocytopenia ***(Laarhoven, 2015).***

A commonly used tool in assessing bleeding tendency is the modified Buchanan hemorrhage grading scale, which enables categorization of bleeding tendency ranging from none(score 0) to life-threatening/ fatal (score 5) (Table 1).

Table (1): Modified Buchanan hemorrhage grading scale ***(Laarhoven, 2015).***

Grade	Overall bleeding severity	Description
0	**None**	No new hemorrhage of any kind
1	**Minor**	Few petechiae (≤ 100 total) and/or ≤ 5 small bruises (≤ 3 cm diameter); no mucosal bleeding
2	**Mild**	Many petechiae (> 100 total) and/or > 5 large bruises (> 3 cm diameter); no mucosal bleeding
3	**Moderate**	Overt mucosal bleeding (epistaxis, gum bleeding, oropharyngeal blood blisters, menorrhagia, gastrointestinal bleeding, others) that doesn't require immediate medical attention or intervention
4	**Severe**	Mucosal bleeding or suspected internal hemorrhage (in the brain, lung, muscle, joint, elsewhere) that requires immediate medical attention or intervention
5	**Life-threatening / fatal**	Documented intracranial hemorrhage or life-threatening or fatal hemorrhage in any site