INTRODUCTION

Immune thrombocytopenia (ITP) is defined as an autoimmune disorder which leads to destruction of platelets peripherally, damage of megakaryocytes and inhibition of platelet production in marrow ***(Bolton-Maggs, 2000; Bussel et al., 2006).***

Febrile illness was found to precede disease onset in two third of children with ITP ***(Suvajdzic et al., 2006).***

ITP in children is usually a short self-limiting disease without any late sequelae. However, the condition become chronic in 5% to 30% of affected children ***(Cuker and Cines, 2010; Elalfy et al., 2010; Imbach et al., 2006; Kuhne et al., 2003).***

Human cytomegalovirus (HCMV) is a member of beta Herpesviridae family, HCMV infection in healthy children and adolescents is asymptomatic, only 10 percent of acquired HCMV infections result in symptoms. HCMV may lead to mononucleosis–like syndrome; fever, fatigue, pharyngitis, adenopathy (especially cervical adenopathy), and hepatitis**.** Confirmation of an acquired HCMV infection in a healthy child or adolescent is best accomplished by documenting a HCMV IgG seroconversion with HCMV IgM antibody ***(Kimberlin, 2015)***.

Several reports have implicated HCMV in the pathogenesis of infrequent cases of ITP. In a study in 1992, 3 of

28 children and 3 of 80 adults with ITP revealed HCMV in their urine ***(Wright, 1992)*** while a study that was conducted in China showed that majority of the children diagnosed with ITP, had HCMV infection, and those patients were more prone to exacerbation of ITP ***(Sheng et al., 2008).***

T cells are important cells in the immune system to control primary HCMV infection ***(Hislop et al., 2007; Waller et al., 2008).*** CD 8+ cytotoxic and CD4+ helper are the T cells responsible for the immune response in primary HCMV infection ***(Jackson et al., 2014; Day et al., 2007; Wills et al., 2002; Sissons, 2015).*** Ninety one patients were enrolled in a study to detect the effect of HCMV in the immune system, they found that HCMV carriers had high levels of IFN-γ, and mechanism of CMV reactivation occurs by differentiation of myeloid precursors, furthermore, patients infected with HCMV showed increase in the circulating levels of Th1 and Th2 cytokines ***(David et al., 2015).***

According to American society of hematology, a review about pathogenesis of ITP showed that chronic primary ITP patients, developed high Th1/Th2 ($CD4^+$cells) ratio and decrease in $CD4^+CD25^+$ T-regulatory cells (Tregs) which regulate T cell response, More decrease in Tregs related to severe disease in ITP ***(Johnson, 2012; Sakakura et al., 2007).***

AIM OF THE WORK

- To evaluate the frequency of human CMV infection among pediatric patients with chronic ITP.
- To assess the effect of HCMV infection on the course and treatment response of ITP.
- To evaluate the effect of HCMV positivity on the INF gamma and its input on bleeding manifestations and outcome in patients with ITP.

CHRONIC IMMUNE THROMBOCYTOPENIA

Definitions and classifications:

Immune thrombocytopenic purpura (ITP) is defined as an autoimmune disorder lead to destruction of platelets peripherally, damage megakaryocytes and inhibit platelet production in marrow ***(Bussel et al., 2006).***

Diagnosis of ITP is made by A platelet count less than 100 x 10 ˆ9/L, this cutoff point is used instead of the level of less than 150 x 10 ˆ9/L, since in a prospective cohort study, a platelet count between 100 and 150 x 10 ˆ9/L, was found in otherwise healthy people ***(Stasi et al., 2011; Adibi et al., 2007).***

ITP criteria were standardized by the International Working Group (IWG) ***(Rodeghiero et al., 2009).***

The phases of ITP is classified, according to prognosis into **"acute"** which has been used to describe a self-limited form of the disease, however the absence of reliable predictive clinical or laboratory parameters of disease duration, this term have been replaced with the term **"newly diagnosed ITP"** for all cases at diagnosis.

Another category **"persistent ITP"** was described 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 chances of spontaneous remissions are still significant during this period.

Another category **"chronic ITP"** need criteria to be fulfilled, the platelet threshold, the failure of the initial therapy, and the length of the disease, so it is diagnosed by low platelet count persisting for ≥12 months ***(Rodeghiero et al., 2009).***

According to several studies, the percentages of acute ITP ranged between 70 % and 80% and chronic ITP between 20% and 30% ***(Glanz, 2008; Kuhne, 2003; Watts, 2004).***

Refractory ITP is defined as the presence of severe ITP occurring after splenectomy. Non splenectomized patients are defined as responders and non-responders to various drug therapies, but should not be considered refractory ***(Neunert et al., 2011).***

Epidemiology:

ITP is a heterogeneous disorder with a diverse natural history and diverse pattern of treatment response ***(Elafy, 2013).***

Incidence of ITP, four cases per 100,000 children per year ***(Buchanan and Adix, 2001)*** but according to ***Terrell et al.*** the true incidence is not known, since individuals with mild

disease may be asymptomatic and undiagnosed ***(Terrell et al., 2010).***

The peak age of onset is between 2 and 6 years of age ***(Shirely, 2004).***

Male and female children were affected approximately equally ***(Kuhne et al., 2001; Terrell et al., 2010).*** Although there is no gender preference in most acute ITP studies, chronic ITP affected females more frequent in Egypt ***(ElAlfy et al., 2010).*** In contrast, reports from the Arabian Gulf region ***(Al-Mulla et al., 2009; Trad et al., 2011)*** and Lebanon ***(Moussalem and Yassine, 2003)*** have shown that nearly 80% of patients with chronic ITP were males. Also other studies reported that boys under 10 years of age were more affected with ITP ***(Bolton, 1997; Sutor et al., 2001).***

Clinical picture:

The signs and symptoms of ITP are highly variable. Major hemorrhage with prolonged severe thrombocytopenia is rare in ITP patients ***(Provan et al., 2010).***

According to Rodeghiero, 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).***

The majority of ITP patients underwent remission and severe thrombocytopenia was infrequent during long term follow up of children with ITP. Long-term data from the Intercontinental Cooperative ITP Study (ICIS) Group Registry II focusing on natural history, bleeding manifestations, and management, reported that 32% of patients remitted between 28 days and 6 months, 21 % between 6 and 12 months, and 29 % between 12 and 24 months. There were no reports of intracranial hemorrhage, and the most common site of bleeding was skin ***(Neunert et al., 2013).***

Serious bleeding, most commonly in the form of epistaxis or gastrointestinal tract bleeding reported in only 3% or fewer children ***(Bolton-Maggs, 2003).***

Although intracranial hemorrhage is the most serious complication, it is rare; occurring only in 0.3% of cases ***(Elalfy et al., 2010).***

Fluctuating clinical course is usually reported in patients with chronic ITP. Episodes of bleeding may last days or weeks and may be intermittent or even cyclic. Spontaneous remissions are uncommon and are likely to be incomplete. Relapses in some cases are associated with vaccination ***(Thienelt and Calverley, 2009).***

Bolton-Maggs and Moon in (1997) showed a scale to categorize bleeding as (1) none or mild—no bleeding at all or

bruising, petechiae, occasional mild epistaxis with very little or no interference with daily life. (2) Moderate—more severe skin manifestations with some mucosal lesions and more troublesome epistaxis or menorrhagia. (3) Severe—bleeding episodes (epistaxis, melena, menorrhagia, and/or intracranial hemorrhage) requiring hospital admission and/or blood transfusions, that is, symptoms interfering seriously with quality of life.

Bleeding Assessment tool:

The IWG on ITP in 2013, proposed Bleeding assessment tool (ITP-BAT), to be used in different phases of the disease in both children and adults with ITP, This ITP- BAT characterized by precise definition of the bleeding manifestations and provide a scale to grade their severity ***(Rodeghiero et al., 2013).***

Bleeding symptoms and signs are classified into: skin (S), visible mucosa (M), and organ (and internal mucosa) (O), SMO grade (SMOG) index.

A physician or trained nurse should record the bleeding grade at presentation and at each follow-up visit.

For each type of bleeding, the incident bleeding manifestation occurring during the interval since the previous evaluation should be recorded.

Grading ranges from 0 to 4 for epistaxis and for bleeding in the organ domain, except ocular and intracranial bleeding (grade 0 and 2 to 4). For the remaining bleeding sites (in skin and mucosal domains) four grades (0 to 3) were determined sufficient. Grade 5 is assigned to any fatal bleeding.

As recommended by IWG, all intracranial bleedings should be reported, irrespective of their grade. For example, if a child had S2 (subcutaneous hematoma) M2 (epistaxis) O3 (menorrhagia) and an intracranial bleeding grade 2 (post trauma, requiring hospitalization), the SMOG index is S2M2O3 (intracranial 2) ***(Rodeghiero et al., 2013).***

Diagnosis:

Diagnosis of ITP is usually done by exclusion; as there is no standard test for ITP ***(Provan et al., 2010).***

Examination of peripheral blood films stained with Wright's or May-Grunwald-Giemsa is the most informative test for diagnosing and distinguishing ITP from other causes of thrombocytopenia. Platelet size, count, and appearance are of critical diagnostic importance, especially in identifying genetic thrombocytopenia ***(Geddis and Balduini, 2007).***

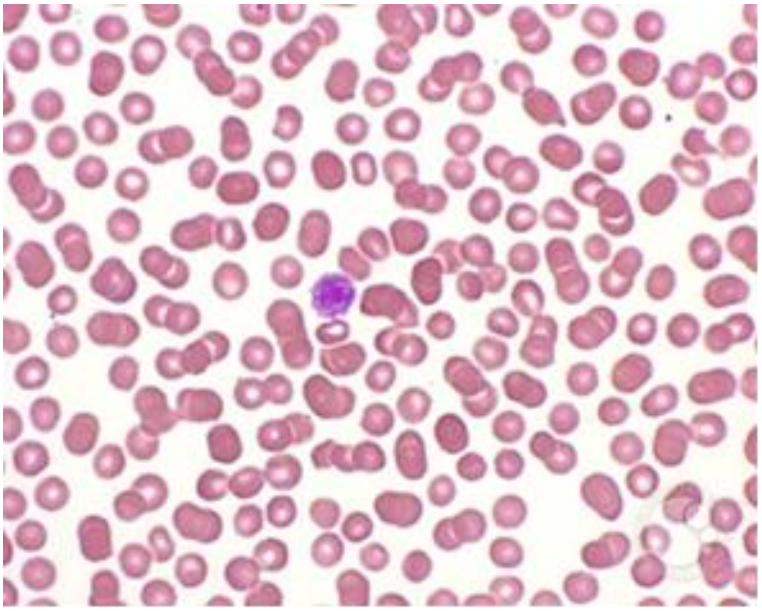

Figure (1): Shows peripheral smear in a patient with ITP showed total absence of platelets. A large, young platelet is seen in the center of the smear.

The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia stated that bone marrow examination is unnecessary in children and adolescents with the typical features of ITP ***(Neunert et al., 2011).***

Management of ITP:

A safe platelet count (e.g., one that prevents major bleeding) rather than correcting the platelet count to normal levels is the primary goal. That's to ensure an acceptable quality of life with minimal treatment-related toxicity ***(Neunert et al., 2011; Cines and Bussel, 2005; Stasi et al., 2004; Proven and Newland, 2015).***

Most of the available treatments can result in potential toxicity, that's why we should avoid unnecessary treatment in asymptomatic patients with milder degrees of thrombocytopenia. Platelet count threshold that necessitate treatment remains controversial.

The recommendation of the Intercontinental Cooperative ITP Study Group is not to start therapy for children without bleeding manifestations regardless their platelet count.

The goal in treatment in Chronic ITP is defined by the desire to avoid the risks of more toxic treatments such as splenectomy or immunosuppression.

"On-demand" treatment at the time of or in anticipation of high-risk bleeding situations or surgical procedures is another approach that is often warranted.

ITP patients with minor or moderate symptoms can be managed as outpatients with use of supportive care (e.g., antifibrinolytic agents, oral contraceptives) and weekly or less-frequent outpatient visits. Limiting activities in severe thrombocytopenia is usually done and treatment may be started ***(Provan et al., 2010).***

Table (1): Proposed criteria for assessing response to ITP treatments

Quality of response ▪ Complete remission (CR): platelet count ≥100x 10^9/L and absence of bleeding ▪ Response (R): platelet count ≥30x10^9/L and at least 2-fold increase the baseline count and absence of bleeding ▪ Time to response: time from starting treatment to time of achievement of CR or R ▪ No response (NR): platelet count ≤30 x 10 ^ 9/L or less than 2-fold increase of baseline platelet count or bleeding ▪ Loss of CR or R: platelet count below 100 x10^9/L or bleeding (from CR) or below 30 x 10^9/L or less than 2-fold increase of baseline platelet count or bleeding (from R)
Timing of assessment of response to ITP treatments ▪ Variable, depends on the type of treatment
Duration of response ▪ Measured from the achievement of CR or R to loss of CR or R ▪ Measured as the proportion of the cumulative time spent in CR or R during the period under examination as well as the total time observed from which the proportion is derived
Corticosteroid-dependence ▪ The need for ongoing or repeated doses administration of corticosteroids for at least 2 months to maintain a platelet count at or above 30 x 10 ^9 /L and/or to avoid bleeding (patients with corticosteroid dependence are considered non responders)

(Rodeghiero et al., 2009)

Corticosteroids

Corticosteroids are the most commonly used first-line therapy for ITP. The dosage of prednisolone or prednisone (1–2 mg/kg per day, P.O., single or divided doses). Complete or partial response with corticosteroids was achieved within the first week of treatment ***(Stasi et al., 2004).***

According to the American Society of Hematology in 2011, short course of corticosteroid is recommended but long-term corticosteroids should be avoided in children with ITP because of its side effects ***(Neunert et al., 2011).***

High-dose dexamethasone in chronic ITP patients was studied in several prospective and retrospective studies ***(Chen et al., 1997; Kühne et al., 1997; Borgna-Pignatti et al., 1997; Wali et al., 2002).***

Hedlund-Treutiger et al. conducted a randomized trial that included 23 chronic ITP children treated with 6 cycles of high-dose dexamethasone (0.6 mg/kg/d for 4 days every 4 weeks) and IVIG (800 mg/kg), About 25% of patients developed complete or partial remission regardless they were initially treated with corticosteroids or started them after failure to respond to IVIG ***(Hedlund-Treutiger et al., 2003).***

Proven et al. in ***2010*** recommended dexamethasone in treatment of persistent and chronic ITP patients. In refractory ITP, dexamethasone (28-40 mg/m2/d) has been suggested as

treatment since the response rate in previously untreated patients aged 18 years or younger was 86%, with 67% of all evaluable patients reaching platelet levels of at least 50 x 10 9/L lasting for a median time of 26 months. Sleeplessness, aggressive behavior, and loss of concentration are common side effects ***(Ku¨hne et al., 1997; Borgna-Pignatti et al., 1997).***

High-dose methylprednisolone. HDMP (given as an oral7-day course of 30 mg/kg/d for 3 days, then 20 mg/kg/d for 4 days) has been used as an alternative to IVIG, the number of platelets of all patients received HDMP increased during therapy, with a peak number at the 7th day, then decreased until the 14th day, and remained relatively stable until 12 months. HDMP therapy was described as a safe, easy and effective therapy in refractory chronic ITP children, and it may result in long-term remission in about two thirds of the patients ***(Ozer et al., 2000).***

Intravenous immunoglobulin (IVIG):

IVIG therapy is administered to patients requiring rapid or urgent elevation of platelet count (e.g., intraoperative or life-threatening bleeding). IVIG increases the platelet count in 70%–80% of treated patients, often within days. The American Society of Hematology in 2011, recommended a single dose of IVIG (0.8 to 1 g/kg) infusion for 1–2 days. IVIG also affects humoral and cellular immunity by influencing the expression and activity of Fc receptors ***(Leontyev et al., 2012).***

A systematic review and meta-analysis of randomized controlled trials comparing corticosteroids with IVIG, revealed that children received corticosteroids were 26% less likely to achieve platelet count > 20 × 10^9/L at 48 hour, moreover, in 9 studies, with a total of 586 patients, 3 of them had intracranial hemorrhage, 2 in patients treated with corticosteroids, both of whom improved, and 1 in a patient treated with IVIG who subsequently died ***(Beck et al., 2005).***

Anti-D immunoglobulin (Anti D):

Anti-D immunoglobulin binds to Rh (D) antigen on erythrocytes, thereby leading to clearance of antibody-coated cells and inhibiting the clearance of opsonized platelets by the reticuloendothelial system ***(Cooper and Bussel, 2006).***

Therefore, anti-D is effective only in Rh D positive individuals. Anti-D has been reported effective in approximately 50%–70% of patients treated with this agent ***(Crow et al., 2008).***

As recommended by the American association of hematology, Anti D therapy is not used in children with autoimmune hemolysis or decreased hemoglobin concentration because of bleeding. Also they suggest to use anti-D in single dose in Rh positive non splenectomized children that necessitate to start treatment ***(Neunert et al., 2011).***