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Interleukin-17 Serum Level and Its Prognostic Significance in Children with Immune Thrombocytopenia

Tamer Hasan Moustafa Hassan[1], Mohamed Mohamed Abdel Salam[2], Asmaa Diaa Eldin Ali Abdeen[1*], Nermin Raafat Abdel Fattah[1]

Departments of [1]Pediatrics and [2]Medical Biochemistry, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

***Corresponding author:** Asmaa Diaa Eldin Ali Abdeen, **Mobile:** (+20)01146451021, **E-mail:** asmaadiaaeldin2@gmail.com

ABSTRACT

Background: Immune thrombocytopenia (ITP) is an acquired hematological disorder that is developed secondary to the production of auto-antibodies against platelets leading to isolated thrombocytopenia, in the absence of other causes of thrombocytopenia such as drugs, infections, malignancy, or other autoimmune diseases.

Objective: This study aimed to determine serum levels of IL-17 and evaluate its prognostic significance in children with immune thrombocytopenia.

Subjects and methods: A case control study carried out in Pediatric Hematology Outpatient Clinic, Zagazig University Hospitals during the period from September 2019 to August 2020. It included 100 subjects who were divided into 2 group; 50 children with ITP and 50 age and sex matched healthy children as a control group.

Results: In this study, newly diagnosed patients with ITP had significantly higher levels of IL-17 compared to persistent and chronic patients with ITP (554.3, 259.9 and 158.6 pg/ml in in newly diagnosed ITP, persistent ITP and chronic ITP respectively, $p<0.001$).

Conclusion: We concluded that serum IL-17 predicts susceptibility to ITP in Egyptian children with ITP.

Keywords: Immune thrombocytopenia, Interleukin 17, Interleukin 17 in immune thrombocytopenia.

INTRODUCTION

One of the most prevalent hemorrhagic autoimmune illnesses, primary immune thrombocytopenia (PIT) is defined by solitary thrombocytopenia that is unaccompanied by any other conditions that might trigger thrombocytopenia. Increased platelet breakdown and insufficient platelet synthesis can lead to decreased platelet counts. The most frequent autoimmune cytopenia in children is PIT. PIT is diagnosed in 2.2–5.3 per 100,000 kids who are 18 years old or younger per year. PIT in children is thought to have a good prognosis [(1)].

Interleukin 17 (IL-17) is a pro-inflammatory cytokine that has recently been shown to have a significant role in the onset of autoimmune disorders. IL-17 also stimulates the development of a variety of cytokines and adhesion molecules [(2)].

In addition to being a vital component of the defence system against pathogenesis and autoimmune disease, the Th 17 cells' characteristic cytokine, IL-17, has been demonstrated to behave differently from other IL-17 cytokine family members, particularly IL-17A. As a result, IL-17F is regarded as a key modulator of cellular immunity because it maintains the production of crucial cytokines that cause pro-inflammatory reactions [(3)]. IL-17 genetic polymorphism has been associated to a number of autoimmune disorders, including inflammatory bowel diseases, asthma, ITP, and psoriasis [(4)].

Different cells are recruited and activated by IL-17 to promote inflammation. IL-17 is protective against infection, but excessive synthesis of the protein increases inflammation in autoimmune illnesses such psoriasis, multiple sclerosis, and rheumatoid arthritis [(5)].

This study aimed to determine serum levels of IL-17 and evaluate its prognostic significance in children with immune thrombocytopenia (ITP).

SUBJECTS AND METHODS

A case control study carried out in Pediatric Hematology Outpatient Clinic, Zagazig University Hospitals during the period from September 2019 to August 2020. It included 100 children.

Ethical considerations:

Written informed consent was obtained from all participants parents and the study was approved by the Research Ethical Committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

All patients were divided into 2 groups:

Patient group: 50 children with ITP who were recruited from Pediatric Hematology Outpatient Clinic of Zagazig University Hospitals.

Control group: 50 age and sex matched healthy children.

All patients were subjected to full history taking, thorough clinical examination bleeding symptoms in the absence of hepatosplenomegaly, lymphadenopathy, or stigmata of congenital conditions and routine laboratory investigations.

Complete blood count isolated thrombocytopenia (platelet count <100 X 109/L). Anemia only if due to significant bleeding-otherwise normal red cell indices, white blood cell count and differential.

Peripheral blood smear identified platelets should be normal to large in size. Red and white blood cell morphology should be normal.

Bone marrow examination was felt to be unnecessary in children with typical ITP prior to

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initiation of treatment with corticosteroids, prior to splenectomy, or in patients who fail intravenous immunoglobulin (IVIg) therapy.

Measurement of serum level of IL-17 was performed for all patients and controls using Enzyme Linked Immunosorbent Assay (ELISA) catalog number 201-12-0143 (Shanghai Sunred Biological Technology Co., Ltd, China).

Statistical Analysis

The data were checked, entered, and analyzed using SPSS version 20 (Armonk, NY: IBM Corp). Results were expressed as mean±standard deviation and range for quantitative variables, and as number and percentage for qualitative ones. Unpaired Student t-test, Chi-square test (X^2), ANOVA (F test) and Pearson coefficient of correlation (r) were used when appropriate. P values ≤0.05 qualify as significant results and those ≤0.001 as highly significant results.

RESULTS

Table 1 shows that patients and controls were matched as regards age and sex.

Table (1): Demographic characteristics of studied groups

Variable	Patients	Controls	Test	P
• **Age (years)** ○ **Mean ± SD** ○ **Range**	9.4±3.5 (5-16)	9.6±3.5 (3-16)	t= -0.31	0.38
• **Sex** ○ **Males** ○ **Females**	28 (56%) 22 (44%)	25 (50%) 25 (50%)	X^2=0.36	0.55

Table 2 shows the clinical presentation of patients.

Table (2): Clinical presentation of patients

Clinical presentation	N (%)
• **Purpura**	46 (92%)
• **Ecchymosis**	43 (86%)
• **External bleeding**	32 (64%)

Table 3 shows that the mean age at diagnosis and the mean initial platelet count in our patients.

Table (3): Age at diagnosis and initial platelet count in patients

Variable	Patients
• **Age at diagnosis (years)** ○ **Mean ± SD** ○ **Range**	6.9 ± 2.14 (3-12)
• **Platelet count (10^3/uL)** ○ **Mean ± SD**	12± 2.62

Table 4 shows that patients had significantly higher levels of serum IL-17 than controls.

Table (4): Serum IL-17 levels in studied groups

Serum IL-17	Patients	Controls	T test	p
Mean ± SD (pg/ml)	331.4± 65.3	106.7 ±7.6	5.7	< 0.00001

Table 5 shows that newly diagnosed patients with ITP had significantly higher levels compared to persistent and chronic patients with ITP.

Table (5): Serum IL-17 levels in our patients in relation to ITP classification

Serum IL-17	Newly diagnosed N=18	Persistent N=15	Chronic N=17	F test	P
Mean ±SD (pg/ml)	554.3±47.2	259.9±29.0	158.6±16.3	17.0	< 0.00001

Table 6 shows that there was significant negative correlation between serum IL-17 levels and age of patients. No significant correlation between serum IL-

17 levels and any of age at diagnosis or platelet count was found.

Table (6): Correlation between serum IL-17 level and each of age, age at diagnosis and initial platelet count

	r	p
• **Age (years)**	-0.30	0.03
• **Age at diagnosis (years)**	-0.03	0.8
• **Platelets counts (10^3/uL)**	0.21	0.1

DISCUSSION

The mean age of our patients was 9.4 years. They were 28 (56%) males and 22 (44%) females. The mean age at diagnosis was 6.9 years. Purpura, ecchymosis and external bleeding were present in 46 (92%), 43(86%) and 32 (64%) of our patients respectively. We did not find any severe or life threatening bleeding in our patients.

Our data was consistent with that published in the literature where ITP in children typically affects a previously healthy young child who is between two to seven years of age. Males and females are equally affected. However, recent studies reported a higher male/female ratio during infancy with a decreasing trend toward older age. The disease onset is abrupt with bruises and petechial rashes affecting almost all patients[6]**.** Severe life threatening bleeding is rare (0.2–0.9%) in children with ITP[7]**.** This difference in results can be attributed to difference in the study population where the previous studies performed on adults with ITP where our study investigated only children with ITP.

The mean initial platelet count in our patients was 12 $X10^3$ /uL. Patients with newly diagnosed ITP had significantly lower platelets count compared to patients with persistent and chronic ITP (6.7, 13.2 and 17.4 $X10^3$/uL respectively). This should be viewed in light of that higher platelet count at diagnosis is a risk factor predicting chronicity in ITP children. Our results were strongly supported by **Grimaldi-Bensouda *et al.*** [8] where they found that the sole possible predictor of chronicity at 12 months was a higher platelet count at baseline [Odds Ratio 1.03; 95% CI: 1.00, 1.06]. Similarly, **Heitink-Pollé *et al.*** [9] found a significantly higher platelet count at diagnosis in patients who developed chronic ITP, with a mean difference 5.27 (95% CI 2.69-7.86).

Though female gender was higher in chronic ITP compared to persistent and newly diagnosed patients, yet the difference didn't reach a statistically significantly level. Female gender was one of predictors of chronicity in a large meta-analysis conducted by **Heitink-Pollé *et al.*** [9] (Odds ratio [OR] 1.17, 95% confidence interval [CI] 1.04-1.31).

In our study, patients had significantly higher levels of serum IL-17 than controls (331.4 pg/ml versus 106.7 pg/ml respectively). In 2009, **Zhang and colleagues**[10] first described up-regulation of Th17 cells along with Th1 in patients with ITP and suggested that Th17 cytokines promoted an imbalance favoring a more Th1-type immune response in ITP. Our results are also consistent with **Ghallab *et al.*** [11] where there was a statistically significant difference between untreated ITP patients and controls as regards serum IL-17 levels (91.5 versus 59.9 pg/ml respectively, p<0.0001).

In our study, newly diagnosed patients with ITP had significantly higher levels of IL-17 compared to persistent and chronic patients with ITP (554.3, 259.9 and 158.6 pg/ml in newly diagnosed ITP, persistent ITP and chronic ITP respectively). Our results are in agreement with **Huang *et al.*** [12] where they found that the levels of IL-17 were lower in patients with chronic ITP than those with newly diagnosed ITP and comparable to the control group. **Ghallab *et al.*** [11] found the level of IL-17 was increased in patients with untreated ITP (p=0.0001) when compared with controls. However, there was statistically significant reduction in the level of IL-17 in responder patients (p=0.0001) while IL-17 level was insignificantly changed in non-responder patients (p=0.394).

In 2009, **Zhang and colleagues**[10] found that among the ITP patients, there were no statistical differences of the three kinds of cells (Th 17, Th1 and Tc1) tested between primary and recurrent ITP patients (p=0.18 for Th17, p=0.36 for Th1, p=0.35 for Tc1).

Our results showed that there was significant negative correlation between serum IL-17 levels and age of patient. No significant correlation between serum IL-17 levels and any of age at diagnosis or platelet count. Also, no significant association between serum IL-17 levels and any of clinical characteristics, first or second treatment lines.

There are very few studies investigating the relationship between serum IL-17 levels and demographic, clinical or laboratory parameters in childhood ITP. **Ma *et al.*** [13] found no significant association between plasma IL-17 levels and any of age, sex or platelet counts. **El Husseiny *et al.*** [14] observed insignificant correlation between IL-17 levels and platelet counts.

CONCLUSION

We concluded that serum IL-17 predicts susceptibility to ITP in Egyptian children with ITP. Measurement of serum IL-17 level could be added to the routine work up of children with ITP at initial diagnosis as it can predicts the natural course of ITP and offers new insights into the pathogenesis of childhood ITP. Larger multicenter studies are still needed to support our findings.

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