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ORGINAL ARTICLE

T helper 17 cells and interleukin-17 contribute to the pathogenesis of primary immune thrombocytopenia in Egyptian children

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ABSTRACT

Background:Although the exact pathogenesis of primary immune thrombocytopenia (ITP) is still unclear, there are many research efforts directed toward the role of T helper 17 and interleukin 17 (IL -17) in the pathogenesis of this disease. The Th17 cell, which produces IL -17, is a subset of T helper cells. Interleukin 17 is a proinflammatory cytokine that has recently been shown to play a critical role in the development of autoimmune diseases. Our aim was to investigate the role of T helper cells 17 and interleukin-17 in the development of ITP in Egyptian children.

Methods and results:This study was performed on 100 children with ITP and 100 healthy children as a control group. Patients underwent a complete history, a thorough physical examination, and routine investigations according to our local standards. The percentage of Th17 cells was measured in the study groups by flow cytometry. Serum IL -17 was also measured by ELISA in the study groups. Th17 cells were significantly higher in patients compared with controls. In addition, serum levels of IL -17 were observed to be 3.1-fold higher in patients with ITP compared to controls. Newly diagnosed patients had a significantly higher percentage of Th-17 cells as well as higher IL -17 levels than patients with persistent or chronic ITP.

conclusion :We concluded that Th-17 cells and IL -17 contribute to the pathogenesis of ITP in Egyptian children.

Key words: ITP: T helper 17; IL-17; Children; Pathogenesis

INTRODUCTION

Primary ITP is the most common autoimmune cytopenia in children. It is characterized by isolated thrombocytopenia that is not accompanied by other diseases that may lead to thrombocytopenia. Thrombocytopenia usually results from increased platelet destruction along with impaired platelet production. 2.2-5.3 out of 100000 children ≤ 18 years of age are diagnosed with primary ITP each year [1]
The pathophysiology of ITP is not fully understood. Several mechanisms have been proposed, including antiplatelet antibodies secreted by autoreactive B lymphocytes, platelet destruction by T cells, and poor functionality of regulatory T cells associated with reduced numbers [2].
Innovative data have supported the hypothesis of plasticity of CD4+ve T cells, which in turn lead to different immune responses based on the nature of the influence of the inflammatory environment. One example of this is T helper 17 cells [3] Th17 cells are considered promoters of autoimmune diseases as they produce a number of pro-inflammatory cytokines, including IL -17, and accordingly cause remarkable tissue damage [3].
IL-17 belongs to the IL 17 cytokine family, which contains 6 different IL pro-inflammatory cytokines, from IL 17A to IL 17F. IL 17A is often referred to as IL 17. The high degree of similarity between IL 17A and IL 17F is significant and they also share a common biological function [4]
IL-17 is a characteristic cytokine secreted by Th 17 cells. Not only does it play a critical role in the development of autoimmune diseases, but it has been shown to function differently from other members of the IL 17 cytokine family. Interleukin 17 induces the expression of various cytokines and adhesion molecules [5]
IL-17 stimulates and activates various cells to increase inflammation. Although protective in infections, overproduction of IL 17 promotes inflammation in autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and

psoriasis [6]
Several studies have shown that a genetic polymorphism of IL -17 is associated with various autoimmune diseases such as inflammatory bowel disease, asthma, ITP, and psoriasis [7].

MATERIALS AND METHODS.

A case-control study was conducted in the pediatric hematology outpatient clinic of Zagazig University Hospitals from September 2019 to August 2020. The study included 100 children with ITP recruited from the pediatric hematology outpatient clinic and 100 age- and sex-matched healthy children
Criteria for participation:

1- Patients with primary immune thrombocytopenia 2- Ages 1-15 years 3- Both sexes
Exclusion criteria:
1- Patients with secondary immune thrombocytopenia 2- Patients with other causes of thrombocytopenia 3- Age < 1year or > 15years
Diagnosis of ITP and classification: ITP is diagnosed based on the 2011 clinical practice guidelines from ASH as a platelet count less than 100,000/µl in the absence of other causes or conditions that may be associated with thrombocytopenia. ITP was classified as newly diagnosed (diagnosed up to 3 months), persistent (3 to 12 months after diagnosis), or chronic (lasting longer than 12 months) [8].
Study design: Case-control study.

METHODS

1- All patients underwent a complete history, a thorough clinical examination, and routine laboratory tests for diagnosis and follow-up of ITP according to our local standards, including a blood count at diagnosis and during follow-up and determination of platelet trend.
2- Th-17 cells were measured in all patients and controls by flow cytometry using BD FACSCalibur (BD Biosciences, USA). Th-17 cells were detected by the expression of intracellular IL -17.
3- Measurement of serum level of IL -17 was performed in all patients and controls using Enzyme Linked Immunosorbent Assay (ELISA) catalog number 201-12-0143 (Shanghai Sunred Biological Technology Co., Ltd).

Test principle

The kit uses an Enzyme-Linked Immunosorbent Assay (ELISA) with two antibodies to determine the level of Human Interleukin 17 (IL -l7) in samples. Add Interleukin IL -17 to the well pre-coated with the human IL -l7 monoclonal antibody Enzyme and incubate. Then add IL -l7 antibody labeled with biotin and combined with streptavidin-HRP to form an immune complex; incubate again and wash to remove the unbound enzyme. Then add chromogen solutions A and B. The color changes to blue and under the action of acid, the color finally turns yellow
The chroma of the color and the concentration of the human substance IL -17 of the sample were positively correlated.

STATISTICAL ANALYSIS

Data were reviewed, entered, and analyzed using SPSS version 20 (Armonk, NY: IBM Corp). Results were expressed as mean ± standard deviation for quantitative variables and as number and percentage for qualitative variables. Unpaired Student t tests, chi-square tests (X2), ANOVA (F test), and Pearson correlation coefficients (r) were used as needed. P values ≤0.05 are considered significant results and those > 0.05 are considered nonsignificant results.

RESULTS

The mean age of patients was 9.4±3.5 years with a range of 3 to 15 years. The mean age at diagnosis was 6.9±2.1 years. There were 56 men and 44 women. According to ITP classification, 36 patients had newly diagnosed ITP, 30 patients had persistent ITP, and 34 patients had chronic ITP. Age at diagnosis did not differ significantly between the different groups of patients ($p = 0.25$). Patients with chronic ITP were significantly older than other patients (12.1, 9.3, and 7.0 for chronic, persistent, and newly diagnosed ITP, respectively, $p < 0.001$). Although female gender was higher in chronic ITP compared to persistent and newly diagnosed patients, the difference did not reach a statistically significant level (53%, 33.3%, and 39%, respectively, $p = 0.5$).
The mean age of the controls was 9.6±3.5 years with a range of 3 to 15 years. They were 50 female and 50 male. There was no significant difference between patients and controls in age and sex ($p = 0.38$ and $p = 0.55$, respectively)
Most patients (92%) had purpura as their initial clinical presentation, followed by ecchymosis in 86% of patients and external bleeding in 64% of patients. The mean initial platelet count was 12,000/µl. It was significantly higher in chronic ITP patients than in other patients (17400, 13200 and 6700/µl in chronic, persistent and newly diagnosed ITP, respectively, $p < 0.001$). The mean initial platelet count was also significantly higher in women than in men (14640 /µL versus 10200 /µL, $p = 0.03$) (Table 1)
As for first-line therapy, 14 patients were treated conservatively, 66 patients received steroids, 10 patients received intravenous immunoglobulin (IVIG), and 10 patients received a combination of steroids and IVIG. As for second-line therapy, 84

patients received thrombopoietin receptor agonists (TPO-RA), while 14 patients received azathioprine and only 4% underwent splenectomy

The percentage of Th 17 cells was significantly higher in patients than in controls (1.85% vs 0.82%, p < 0.0001). Newly diagnosed ITP patients had a significantly higher percentage of Th 17 cells than patients with persistent and chronic ITP (Table 2)

Significantly higher serum levels IL -17 were observed in patients compared to controls (331.4 pg/ml vs 106.7 pg/ml, p <.00001) (Figure 1). Newly diagnosed ITP patients had significantly higher serum levels IL -17 compared to persistent and chronic ITP patients (Table 3).

A significant negative correlation was observed between serum levels IL -17 and patient age (r = - 0.3, p = 0.03) (Figure 2). However, there was no significant correlation between serum levels IL -17 and age at diagnosis (r = - 0.03, p = 0.8) or platelet count (r = 0.2, p = 0.1). There was also no significant association between serum levels IL -17 and any of clinical presentation (p=0.07), first-line therapy (p=0.25), or second-line therapy (p=0.6).

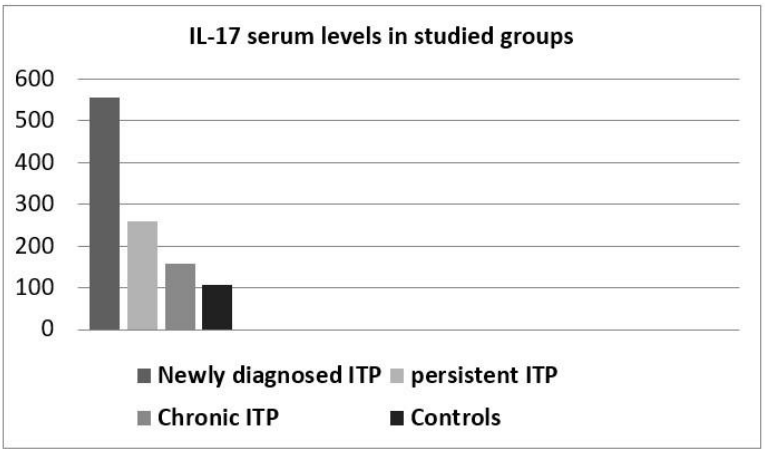

Fig.1: IL-17 serum levels in studied groups

Fig.1 There was significantly higher levels of serum IL-17 in patients compared to controls (331.4 pg/ml versus 106.7 pg/ml respectively, p < .00001). Moreover, Newly diagnosed ITP patients had notably higher serum IL-17 levels in comparison to persistent and chronic ITP patients.

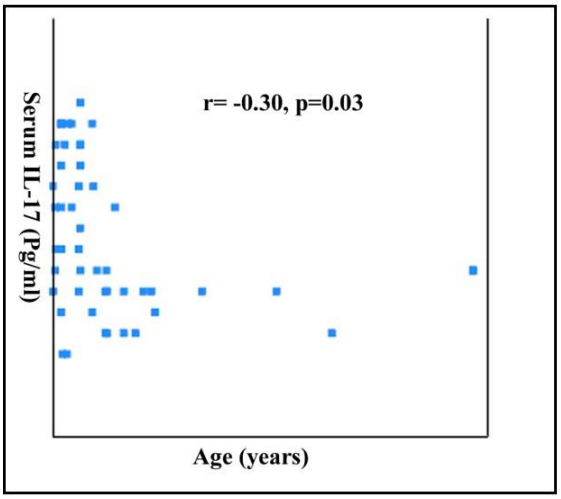

Fig.2 : Correlation between serum IL-17 level and age of patients

Fig.2 There was significant negative correlation between serum IL-17 levels and age of patients. (r = - 0.3, p = 0.03).

Table 1. Initial platelet counts in ITP patients based on disease classification and gender.

	Platelets count (10^3/ul) Mean± SD (Range)	Test	P
Newly diagnosed ITP	6.7 ± 4.2 (1-17)	f = 8.5	0.0007
Persistent ITP	13.2±7.8 (1-31)		
Chronic ITP	17.4±10.2 (2-33)		
Males	10.2 ± 6.8 (1-31)	t = 1.8	0.03
Females	14.64±10.1 (2-33)		

SD: Standard deviatio

Table 2. Percentage of Th-17 cells in our patients in relation to ITP classification.

Th 17 cells	Newly diagnosed N=36	Persistent N=30	Chronic N=34	F test	P
Mean ±SD (%)	2.05±0.35	1.75±0.21	1.53 ±0.15	36.7	< .00001
Range	(1.74- 2.35)	(1.55-2.15)	(1.25-.1.88)		

Th-17: T helper 17; SD: Standard deviation

Table 3. Serum IL-17 levels in our patients in relation to ITP classification.

Serum IL-17	Newly diagnosed N=36	Persistent N=30	Chronic N=34	F test	P
Mean ±SD (pg/ml)	554.3±247.2	259.9±209.0	158.6±156.3	17.0	< .00001
Range	(163.2-949.5)	(65.0-764.8)	(63.2-580.2)		

IL-17: Interleukin 17; SD: Standard deviation

DISCUSSION

The pathophysiology of ITP is extremely complex. Several studies support a central role of serum cytokines in the pathogenesis of this disease and provide evidence that T helper lymphocytes polarize into Th1 and Th2 immune responses. The Th1 response produces IL -2, INF -γ and TNF-α, while the Th2 response produces IL -4, IL -5, IL -6, IL -10 and IL -13 [2].

A new subset of CD4+ T cells distinct from Th1 and Th2 has recently been identified. It is characterized by the production of IL -17 and is therefore referred to as Th17 cells. IL -17 is a proinflammatory cytokine that recruits different cell types to the site of inflammation and thus plays a protective role in infections. However, overproduction of IL -17 has been found in many autoimmune diseases [5].

In our study, the percentage of Th 17 cells was significantly higher in patients than in controls (1.85% versus 0.82%, p < 0.0001). In addition, significantly higher serum levels of IL -17 were observed in patients compared to controls (331.4 pg/ml versus 106.7 pg/ml, p <.00001)

In 2009, Zhang and colleagues [9] first described an upregulation of Th17 cells along with Th1 cells in patients with ITP and suggested that Th17 cytokines promote an imbalance that favors a more Th1-like immune response in ITP

Our results are also in agreement with those of Ghallab et al [10]

where there was a statistically significant difference between untreated ITP patients and controls in terms of serum levels IL -17 (91.5 versus 59.9 pg/ml, p < 0.0001).

Similarly, Ye et al [11] reported increased Th17 cells and plasma levels IL -17and IL -23 in patients with ITP.

Zhou et al [12] investigated the role of interleukin-17-producing CD4-positive T cells in the pathogenesis of primary ITP and found that the percentage of Th17 and Th1 cells was significantly increased in ITP patients, especially in those with severe ITP compared with normal controls. Further ELISA analysis confirmed that ITP patients had

high levels of Th17-associated proinflammatory cytokines such as interleukin-17A/F, interleukin-6, and interleukin-23 and low levels of anti-inflammatory factors such as interleukin-10 and transforming growth factor-β compared with normal controls

In contrast, Ma et al [13] found that plasma levels IL -17 were not significantly different between patients with active ITP [median, 15.04 pg/ml (range, 8.15-66.78)] and the control group [median, 15.27 pg/ml (range, 10.25-40.36); P = 0.17]. There was also no significant difference in the other Th17-associated

cytokines (TGF-ß and IL -6) and Th1 cytokines (IFN-γ) were observed between ITP patients and controls. This discrepancy may be attributed to the difference in study population and sample size, because the study by Ma et al was conducted in only 29 adults with ITP, whereas our study was conducted in 100 children with ITP.

Our results showed that newly diagnosed ITP patients had a significantly higher proportion of Th 17 cells and IL -17 serum levels compared with persistent and chronic ITP patients (2.05, 1.75, and 1.53%, respectively, $p < 0.0001$ for T helper cells and 554.3, 259.9, and 158.6 pg/ml, respectively, $p < 0.001$ for IL -17).

Our results are in agreement with those of Huang et al [14], who found that the levels of IL -17 were lower in patients with chronic ITP than in patients with newly diagnosed ITP and comparable to the control group.

Ghallab et al [10] found that the level of IL -17 was increased in patients with untreated ITP compared to controls (p=0.0001). However, a statistically significant decrease in the level of IL -17 was observed in responder patients (p=0.0001), while the IL -17 level was insignificantly changed in non-responder patients (p=0.394).

Zhang and colleagues [9] found that there were no statistical differences between the three cell types studied (Th17, Th1 and Tc1) between primary and relapsing ITP patients (p=0.18 for Th17, p=0.36 for Th1, p=0.35 for Tc1).

Ye et al [11] observed no statistical difference in plasma levels IL -17 between the group with new diagnosis and the group with relapsed ITP.

El Husseiny et al [15] in their study of 45 patients with chronic and persistent ITP found significantly higher levels of IL -17 in their patients compared to the control group (0.42 versus 0.15, $p < 0.001$).

Our results can be explained by the clinical differences between newly diagnosed and chronic ITP, suggesting the existence of different pathophysiological mechanisms in the two forms

In our study, a significant negative correlation was observed between serum levels IL -17 and patient age ($r = - 0.3$, $p = 0.03$) (Figure 2). However, there was no significant correlation between serum levels IL -17 and age at diagnosis ($r = - 0.03$, $p = 0.8$) or platelet count ($r = 0.2$, $p = 0.1$). In addition, there was no significant association between serum levels IL -17 and any of the clinical presentations (p=0.07), first-line therapy (p=0.25), or second-line therapy (p=0.6).

Very few studies investigated the association between serum levels IL -17 and demographic, clinical or laboratory parameters in childhood ITP

Ma et al [13] found no significant association between plasma levels IL -17 and age, sex or platelet count.

El Husseiny et al [15] observed an insignificant correlation between IL -17 and platelet count

Okamoto et al [16] divided their patients into IL -17-low expression and IL -17-high expression group. The clinical information between the IL -17-low-expression group and the IL -17-high-expression group did not differ significantly in terms of age, sex, and platelet count.

We can explain the significant negative correlation between serum levels IL -17 and patient age based on the observation that patients with chronic ITP had lower serum levels IL -17 and were older than patients with newly diagnosed ITP, who had higher serum levels IL -17 and were younger in age.

CONCLUSION

We concluded that Th 17 cells and IL -17 appear to play an important role in the pathogenesis of ITP in Egyptian children. Larger multicenter studies are still needed to support our findings.

LIMITATION OF THE STUDY

Small sample size was one of the limitations of this study, and larger multicenter studies are still needed to confirm these findings. Another limitation was that we need to start with patients with de novo ITP and follow the changes in the percentage of T helper 17 cells and serum levels of IL -17 over time. However, in many patients with de novo ITP, follow-up has been neglected, especially after improvement.

Funding

No funding was provided for this study.

Ethical Statement

This study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki, as revised in 2000, and approved by the institutional review board of the Zagazig College of Medicine. Written informed consent was obtained from all study participants and/or their caregivers.

Patient Consent for Publication

All patients consented to the publication of this research. Informed consent for publication was obtained.

Competing interests

All authors declare that they have no competing interests with regard to publication of the study

List of abbreviations

ITP	**Immune thrombocytopenia**
IL	**Interleukin**
IL-17	**Interleukin 27**
Th-17	**T helper-17**
Th-1	**T helper-1**
Th-2	**T helper-2**
INF-γ	**Interferon gamma**
TNF-α	**Tumor necrosis factor alpha**
TGF-ß	**Transforming growth factor beta**
ELISA	**Enzyme linked Immunosorbent assay**
ANOVA	**Analysis of variance**
TPO-RAs	**Thrombopoietin receptor agonists**

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