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Plasma Level of Interleukin 37 in Children with Primary Immune Thrombocytopenia

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ABSTRACT

Background: Interleukin (IL)-37, a novel anti-inflammatory cytokine previously known as interleukin-1 family member 7 before it was renamed, has a pivotal role in the suppression of immune responses. **Objective:** The present study aimed to investigate the expression of IL-37 and its potential role in the pathogenesis of immune thrombocytopenia (ITP). **Patients and Methods**: The study was carried out in Zagazig University Hospital, Pediatric Department. The total sample size was 60 participants divided into 4 groups. They were 45 patients (15 patients with newly diagnosed ITP, 15 patients with complete remission after 1st line therapy and 15 patients with chronic ITP) and 15 age - sex matched healthy children as a control group.

Results: Our study showed that the mean value of age among patients group was (6.89± 4.13) with female predominance than males (60%). The current work showed that regarding history of bleeding tendency it was present in 45 (100%). This study showed the bone marrow examination was done for 8 of our patients (17.8%) and revealed hypercellular megakaryopoiesis. In the current study there was no statistically significant difference between patients group and control group regarding hemoglobin (HB) and white blood cells (WBC). Our study showed that there was no statistically significant correlation between IL 37 level and platelets in patients group.

Conclusion: Accumulating evidence suggests that serum level of IL 37 has a pivotal role in autoimmune diseases. In the present study, the expression of IL-37 in ITP patients was evaluated for the first time in Egypt, but no significant abnormal expression of IL-37 was identified in these patients. It was therefore concluded that serum level of IL 37 may not have a pivotal role in the development of ITP.

Keywords: Children, Interleukin 37, Plasma Level, Primary Immune Thrombocytopenia

INTRODUCTION

Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by a low platelet count with mucocutaneous or other types of bleeding. The pathogenesis of ITP remains poorly understood, while immune disorders are thought to have an important implication. The presence of anti-platelet glycoprotein (GP) antibodies is also considered to be critically involved in ITP [(1)].

In addition, a complex dysregulation of cellular mechanisms has been reported, including the imbalance of the type 1 T-helper cell (Th1)/Th2 ratio [(2)], increased number of Th17 cells, decreased number or functional deficit of regulatory T cells [(3,4)] and increased cytotoxic T lymphocyte-mediated cytotoxicity [(5)]. Biomarkers are very important to the pathogenesis of disease. Many abnormal immune biomarkers play crucial roles in ITP pathogenesis. The abnormal B, T cell, such as transforming growth factor-beta1, Toll- like receptors helper 1 and T helper 2 and some new biomarkers in ITP were introduced. It may help people to know the complicated pathogenesis of ITP [(6)].

The major role of IL-37 is to decrease excessive inflammation in innate and adaptive immune diseases, mainly by inhibiting the expression, production and function of pro-inflammatory cytokines, including IL-1α, IL-6, tumor necrosis factor (TNF) and macrophage inflammatory protein-2. The abundance of these cytokines has been reported to increase with the silencing of endogenous IL-37 in human blood cells [(7)]. Interleukin (IL)-37, a novel anti-inflammatory cytokine previously known as interleukin-1 family member 7 before it was renamed, has a pivotal role in the suppression of immune responses [(8)].

Aberrant expression of IL-37 has been observed in several inflammatory and autoimmune diseases, including rheumatoid arthritis (RA) [(9,10)], systemic lupus erythematosus (SLE) [(11,12)], inflammatory bowel disease (IBD) [(13,12)], ankylosing spondylitis (AS) [(11)] and Graves' disease (GD) [(14)]. Interleukin (IL)-37 has an important role in autoimmune diseases by suppressing immunity and inflammation; however, the role of IL-37 in immune thrombocytopenia (ITP) has remained largely elusive. [(15)].

The present study aimed to investigate the expression of IL-37 and its potential role in the pathogenesis of ITP.

PATIENTS AND METHODS

Site of study: The study was carried out in Zagazig University Hospitals and Zagazig University outpatient clinic.

Sample size: Assuming that the mean ± SD of interlukin-37 in the control group is 75.61 ± 17.1 and that of ITP patients is 90.41 ±17.8. So, the total sample size is 60 participants divided into four groups. They were 45 patients who had ITP (15 patients with newly diagnosed ITP, 15 patients with complete remission after 1st line therapy and 15 patients with chronic ITP) and 15 age - sex matched healthy children as a control

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group using open EPI with power 80 % and CI 95 %.
Targets of population: Patients with ITP during their admission in the hospital and follow up after that in the outpatient clinic
Inclusion criteria: Patients with immune ITP, and age from 2 - 14 years.
Exclusion criteria: Secondary ITP, history of any autoimmune disease in the patient or family, and the presence of any inflammatory disorder.
Type of the study: Case control study.

All patients were subjected to the following:

1) Full history talking: regarding history of bleeding tendency and history of viral or bacterial upper respiratory tract infection in the previous 2-3 weeks
2) Thorough general examination
3) Thorough clinical examination of all systems and organs with special emphasis on the presence of skin rash (petechiae, purpura or ecchymosis), site and distribution of the rash , and the presence of bleeding from mucous membranes and other orifices.
4) Routine investigations in the form of complete blood picture at diagnosis, follow up CBC" platelet trend and blood smear
5) Bone marrow examination was done to exclude any secondary ITP
6) Patients and controls were subjected to: Measurements of serum IL-37 by ELISA

Test principle:
The kit used a double – antibody sandwich enzyme – linked immunosorbent assay (ELISA) to assay the level of human interleukin 37 in samples by adding interleukin 37 to monoclonal antibody enzyme well, which was precoated with human interleukin 37 monoclonal antibody.
Data collection: From each patient the following data were collected upon admission: Name, age, gender, medical and past history, physical examination.

Ethical consent:
An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Caregiver of every participant signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis
The collected data were coded, processed and analyzed using the SPSS (Statistical Package for the Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Wilk test. Qualitative data were represented as frequencies and relative percentages. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). P value < 0.05 was considered significant.

RESULTS

Table (1) shows the mean age and gender of the participants.

Table (1): Comparison between patients group and control group regarding demographic data

			Patients group	Control group
Age (days)	**Mean ± SD**		6.89± 4.13	8.60±2.26
Sex	**Female**	**No.**	27	7
		%	60.0%	46.7%
	Male	**No.**	18	8
		%	40.0%	53.3%

This table shows that in the patients group, there were no family history of ITP, while history of bleeding tendency was present in 45 (100%) **(Table 2).**

Table (2): History among patients group

		Patients group	
		No.	**%**
Family History of ITP	**No**	45	100.0%
History of Bleeding Tendency	**No**	0	0.0%
	Yes	45	100.0%
History of Upper Respiratory Tract Infection in the Previous 2-3 Week	**No**	24	53.3%
	Yes	21	46.7%

Table (3) shows that the mean value of age among patients group was (6.89± 4.13) and there was a female predominance than males (60%). Regarding the examination in patients group, petechiae and purpura were present in (33.3%) and ecchymosis in (66.6%), the rash was present all over the body in (66.6%)

Table (3): Examination among patients group

		Patients group	
		No.	%
Petichea	**No**	15	33.3%
	Yes	30	66.7%
Purpura	**No**	15	33.3%
	Yes	30	66.7%
Ecchynosis	**No**	15	33.3%
	Yes	30	66.7%
Site and Distribution of the Rash	**Allover the body**	30	66.7%
	Upper and lower limbs	15	33.3%
Presence of Bleeding from Mucous Membranes	**No**	22	48.9%
	Yes	23	51.1%
Presence of Bleeding from other Orifices	**No**	33	73.3%
	Yes	12	26.7%
Splenomegaly	**No**	45	100.0%
Lymphadenopathy	**No**	45	100.0%

There was no statistically significant difference between patients group and control group regarding serum level of IL 37 **(Table 4)**.

Table (4): Comparison between patients group and control group regarding serum level of IL 37

		Patients group	Control group	t. test	P. value
serum level of IL 37	**Mean ± SD**	51.60± 4.38	53.23±9.87	-.174	0.863

There was no statistically significant difference between patients group and control group regarding HB and WBC. Mean value of platelets was statistically lower among patients group than control group **(Table 5).**

Table (5): Comparison between patients group and control group regarding CBC

		Patients group	Control group	t.test	P. value
HB	**Mean ± SD**	10.99± 1.88	11.19±0.605	-.402	0.689
WBC	**Mean ± SD**	6.13± 1.74	6.12±1.53	.031	0.976
Platelets	**Mean ± SD**	49500.00± 659.69	338733.33± 958.30	-15.186	<0.001

Table (6) shows that there were statistically significant positive correlation between serum level of IL 37 and age. While there was no statistically significant correlation between serum level of IL 37 and other numerical data.

Table (6): Correlation between serum level of IL 37 and other data

Correlation	Pearson's correlation	
	r	p
Age * IL 37	0.312	0.015
HB * IL 37	0.127	0.334
WBC * IL 37	0.107	0.418
Platelets * IL 37	0.035	0.793

DISCUSSION

Our study showed that the mean value of age among patients group was (6.89± 4.13) and female predominance than males (60%). **El Faki Osman** [16] reported that ITP in children typically affects a previously healthy young child who is between two to seven years of age with equal male to female ratio but in infancy males are affected more frequently than females.

This comes in disagreement with **Kistangari and McCrae** [17] who found that analysis of the United Kingdom (UK) General Practice Research Database (GPRD) identified a higher incidence of ITP in boys between ages 2 and 5 (9.7 patients versus 4.7 patients in girls per 100,000 patient-years, respectively) compared to that in teenagers between ages 13-17 (2.4 patients per 100,000 patient-years, with equal sex distribution). **Zainal *et al.*** [18] found that ITP is more common in females and **Tsao *et al.*** [19] reported peak incidence of ITP at 6 years old. **Pietras and Pearson-Shaver** [20] found a slight predominance in males than females. This is in disagreement with **Moulis *et al.*** [21] who found that boys are more often affected than girls.

The current work showed that regarding history of bleeding tendency it was present in 45 (100%). Bleeding is the most common clinical manifestation of ITP, presenting as mucocutaneous bleeding involving the skin, oral cavity and gastrointestinal tract. Purpura, usually on the extremities ("dry purpura") may often appear without an obvious precipitating event. Mucosal bleeding include epistaxis, menorrhagia, and gingival and gastrointestinal bleeding [22].

Most children are well-appearing other than presenting with the classic petechial rash, which does not blanch when pressure is applied. In adults, about two-thirds may present with bleeding, ranging from a petechial rash, mucosal bleeding, or rarely hemorrhage. The priority of the physical exam is on signs of bleeding, specifically of the skin and oral mucosa, as well as the presence of lymphadenopathy or hepatosplenomegaly, which suggests an underlying condition causing secondary ITP. Mucocutaneous bleeding presents in the form of petechiae, purpura, or ecchymosis on the skin. It can also involve the nasal passages (epistaxis), buccal and gingival surfaces (gum bleeding), GI tract, genitourinary system, or vaginal bleeding. However, conjunctival or retinal hemorrhages are rarely seen in ITP [23].

The bleeding tendency in ITP patients is lower than in patients with a comparable thrombocytopenia from other causes (e.g., after chemotherapy or with myelodysplasia, leukemia). Some authors suggest that ITP platelets are 'younger' and more reactive than platelets in other diseases. The increased reactivity of ITP platelets not only explains the relatively low bleeding tendency, but also the increased risk of thrombosis [24].

In harmony with the present study, **Ebeid *et al.*** [25] who found that all studied patients presented with cutaneous bleeding. In our study history of bleeding was present in 100% of patients. **Grimaldi-Bensouda *et al.*** [26] found that of all pediatric patients, 10% and 20-30% of all adult patients with newly diagnosed ITP have no bleeding symptoms at all. In chronic ITP, the proportion of patients without any bleeding symptoms is 30-40%.

The present results showed that as regards to presence of bleeding from mucous membranes and presence of bleeding from other orifices, it was higher among group C than group A, group B and controls.

As regarding to examination in our patients group, petechiae, purpura and ecchymosis were (66.7%) of our patients, regarding site and distribution of the rash 30 (66.6%) were allover the body and 15 (33.3) had no rash, regarding. Presence of bleeding from mucous membranes was present in 23 (51.1%), regarding presence of bleeding from other orifices was present in 12 (26.7%). **Bohn and Steurer** [27] reported that common bleeding symptoms include petechiae, ecchymosis, epistaxis, genitourinary hemorrhage, gingival hemorrhage, and gastrointestinal hemorrhage.

In the current study there was no statistically significant difference between patients group and control group regarding HB and WBC**.** This comes in agreement with **Zainal *et al.*** [18] who found that in patients with ITP, isolated thrombocytopenia with normal hemoglobin and leukocyte count are evident, unless bleeding has occurred.

The present work showed that mean value of platelets was statistically lower among patients group than control group**. Vollenberg *et al.*** [28] found that platelet bound anti-glycoprotein V antibodies were present in the majority of samples tested (222 of 343). MAIPA identified 13.5% of patient sera containing free anti-GPV autoantibodies, however 39.6% were detected by surface plasmon resonance. These antibodies could mediate platelet phagocytosis in vitro and cleared human platelets in a NOD/SCID humanized mouse model.

According to **Tsao *et al.*** [19] risk factors include older age, insidious symptom onset, higher platelet count at time of diagnosis, and lack of preceding infection or vaccination**.**

Our study showed that there was no statistically significant correlation between serum level of IL 37 level and platelets in patients group. This comes in accordance with **Zhang *et al.*** [29] who found that no correlation was identified between serum level of IL 37 and anti-platelet autoantibodies. The correlation between serum level of IL 37 and the platelet count was also analyzed, with no statistical significance observed.

This result disagreed with **Zhao *et al.*** [30] who found that plasma IL-37 levels correlated with the platelet number of ITP patients.

This work showed that the percentage of history of bleeding tendency was (100%) in group A, group B and group C. This contradicts with **Zhan *et al.*** [15] who found that a significant positive correlation was found between serum level of IL 37 and platelet count in ITP patients.

Our results showed that there was no statistically significant difference between the patients group and the control group as regard IL 37 Level. This contradicts with **Zhang *et al.*** [29] who found that the results indicated no significant difference in serum level of IL 37 levels between ITP patients and controls. The mean value of plasma serum level of IL 37 in ITP, particularly active ITP, was much higher than that in the controls. However, the P-value for the comparison of plasma IL-37 between patients with active ITP and controls was 0.107, as determined by Bonferroni's post-hoc test. Further studies with a bigger sample size are required to confirm these results, which may obtain a higher statistical significance.

According to **Zhao *et al.*** [30] who aimed to identify that serum level of IL 37 expression was elevated in ITP patients, It was correlated with platelet count and the severity of bleeding in ITP, indicating that serum level of IL 37 could be a candidate in evaluating disease severity of ITP. They found a significant increase in serum level of IL 37 expression in ITP patients with platelet count below 30 × 109 /L or ITP Bleeding Scale (IBLS) bleeding scores higher than 5. In disagreement with **Su *et al.*** [31] who found the expression of serum level of IL 37 is increased in the plasma of ITP patients, which is correlated with platelet count and the severity of bleeding in ITP**.** This agrees with **Zhan *et al.*** [15] who aimed to investigate the role of IL cytokines in patients with ITP and SLE and the potential pathophysiologic mechanism to differentiate SLE-associated thrombocytopenia (SLE-TP) from ITP. Multiplex cytokine assay and real-time polymerase chain reaction (RT-PCR) were used to measure IL-1 cytokines in 17 newly diagnosed ITP patients, 17 SLE-TP patients, 19 SLE patients without thrombocytopenia (SLE-NTP), and 10 healthy controls. They observed a down-regulation of IL-1β, IL-18, IL-36α, IL-36β, IL-36γ, IL-33, IL-37, and IL-38 in ITP patients compared to healthy controls. In their study, IL-1α, IL-1β, IL-1Ra, IL-18, IL-36Ra, IL-36α, IL-36β, IL-37, and IL-38 mRNA expression showed no significant differences in ITP patients compared to healthy controls.

CONCLUSION

In the present study, the expression of serum level of IL 37 in ITP patients was evaluated for the first time in Egypt, but no significant abnormal expression of serum level of IL 37 was identified in these patients. It was therefore concluded that serum level of IL 37 may not have a pivotal role in the development of ITP. However, the lack of significant differences may be due to the limited number of patients in different groups. The correlation between serum level of IL 37 and the platelet count was also analyzed, with no statistical significance observed. Mean value of serum level of IL 37 was statistically higher among chronic than acute, after first remission and controls.

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