

# Contribution of interleukin 27 serum level to pathogenesis and prognosis in children with immune thrombocytopenia

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## Abstract

Concepts surrounding the mechanisms of thrombocytopenia in ITP have shifted from the traditional view of autoantibody mediated platelet destruction to more complex mechanisms in which impaired platelet production, T-cell-mediated effects, and disturbed cytokine profiles play a role. Interleukin 27 (IL-27) plays pleiotropic roles in immunomodulation and autoimmune diseases.

We aimed to determine the level of IL-27 in patients with ITP and its relationship to patient and disease characteristics as well as disease chronicity and response to treatment.

Sixty children with primary immune thrombocytopenia were consecutively enrolled in this study as well as 20 age and sex matched healthy controls.

ITP patients had significantly higher levels of IL-27 than controls (770.6 and 373.8 pg/ml, respectively). Patients with acute ITP had the highest levels of IL-27 among patient groups, while patients in remission had the lowest IL-27 levels (860.1 and 622.9 pg/ml, respectively). Patients who received IVIG and combined steroids plus IVIG had significantly higher IL-27 levels than others. Patients who received Eltrombopag had significantly lower IL-27 levels than others.

IL-27 seems to play a role in pathogenesis of childhood ITP. IL-27 can be used as a predictor for disease occurrence as well as responsiveness to treatment.

**Abbreviations:** ANOVA = analysis of variance, CBC = complete blood count, CI = confidence interval, ELISA = enzyme linked immunosorbent assay, IL-27 = interleukin 27, ITP = immune thrombocytopenia, IVIG = intravenous immunoglobulin, OR = odds ratio, TNF = tumor necrosis factor, TPO-RA = thrombopoietin receptor agonists, WBCs = white blood cells.

**Keywords:** IL-27, ITP, pathogenesis, platelets

## 1. Introduction

Childhood immune thrombocytopenia (ITP) is usually a self limited disorder lasting for a few weeks or months, but in approximately 25%–30% of the children, the condition becomes chronic. Confronted with a child with newly diagnosed ITP the physician cannot determine if the child has a self-limited disease or will have long-term chronic disorder.<sup>[1]</sup>

ITP is a very complex immune disease and its pathogenesis remains unclear although both antibody-mediated and/or T cell-mediated platelet destruction are key processes. In addition, impairment of T cells, cytokine imbalances, and the contribution of the bone marrow niche have now been recognized to be important.<sup>[2]</sup>

Biomarkers are very important in the pathogenesis of disease, many abnormal immune biomarkers play crucial roles in ITP pathogenesis including the abnormal B, T cell and some new biomarkers that may help people know the complicated pathogenesis of ITP.<sup>[2]</sup>

Recent studies have demonstrated that IL-27 could suppress inflammatory responses in T-cell differentiation and in autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus.<sup>[3]</sup> Previous studies reported that the expression of IL-27 was decreased and Cytotoxic T-lymphocyte mediated platelet destruction was increased in patients with ITP.<sup>[4]</sup>

Some studies revealed that IL-27 can play a regulatory role by suppressing the acquired immunity, inducing the development of

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T helper cells, and expansion of inducible regulatory T cells to produce IL-10. IL-27 also suppresses inflammation through the inhibition of Th17 cells.<sup>[5]</sup>

We aimed to determine the level of IL-27 in patients with ITP and its relationship to patient and disease characteristics as well as disease chronicity and response to treatment.

## 2. Methods

A case control study was carried out in Pediatric Hematology outpatient clinic, Zagazig University Hospitals. 60 patients with immune thrombocytopenia were consecutively enrolled in this study (20 patients with de novo ITP, and 20 patients with chronic ITP and 20 patients in complete remission after 1<sup>st</sup> line therapy) and 20 healthy children as a control group.

Inclusion criteria:

1. Patients diagnosed with 1ry ITP.
2. Age >1 year and <18 years.
3. Platelet count less than 100,000 cells/mcl.
4. Patients newly diagnosed, chronic or in complete remission after 1st line therapy.

Exclusion criteria:

1. Patients with secondray immune thrombocytopenia.
2. Patients age less than 1 year or more than 18 years.
3. Platelet count more than 100,000 cells/mcl.

All patients were subjected to the following.

1. Full history taking.
2. Thorough general examination.
3. Routine investigations in the form of complete blood picture at diagnosis, follow up CBC and platelet trend
4. Measuerment of serum IL-27 by ELISA in patients and control group.

### 2.1. Principles of the test

A double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used to measure the level of human IL-27 in samples. Add IL-27 to monoclonal antibody enzyme well which is precoated with human IL-27 monoclonal antibody. After incubation, a biotin-conjugated anti-human IL-27 antibody is added and binds to human IL-27. After incubation, unbound biotin-conjugated anti-human IL-27 antibody is washed away during a washing step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human IL-27 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human IL-27. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

5. Measurement of serum Granzyme B by ELISA in patients and control group.

### 2.2. Statement of ethics

The present study was conducted in accordance with the ethical standards of the Helsinki Declaration of 1964 as revised in 2000, and was approved by the Institutional Review Board, faculty of medicine, Zagazig University. Informed consent and ascent forms were obtained from the study participants and/or their guardians.

## 2.3. Statistical analysis

Data were assessed, entered and analyzed using SPSS version 20 (IBM Corp., Armonk, NY). Data are expressed as the mean  $\pm$  standard deviation for quantitative variables, number and percentage for qualitative variables. Chi Square ( $\chi^2$ ) test, independent *t* test, ANOVA (*f* test) and correlation coefficient (*r*) were used when appropriate. *P* < .05 was considered to indicate statistically significant differences.

## 3. Results

### 3.1. Demographics and clinical characteristics in study population

The mean age of our patients was  $7.38 \pm 4.0$  years. They were 33 (55%) males and 27 (45%) females. There was no significant difference between patients and controls regarding age and sex distribution.

Patients with chronic ITP were significantly older than patients with acute ITP (10.8 vs 5.32 years respectively, *P* < .00001). 55% of patients with chronic ITP were females versus 45% in patients with acute ITP and 35% of patients with ITP in remission with no significant difference (*P* = .53).

The mean age at diagnosis was  $5.32 \pm 3.7$  years in acute ITP,  $5.9 \pm 2.7$  years in acute ITP in remission, versus  $8.6 \pm 2.1$  years in chronic ITP with a statistically significant difference (*P* = .002).

Seventy percentages of our patients presented with purpura. Ecchymosis was present in 36.7%, epistaxis in 38.3%, bleeding gums in 11.7%, and menorrhagia in 5% of patients. There was no significant difference among patients with ITP as regards initial clinical presentation (*P* = .7).

Details of demographic and clinical characteristics were listed in Table 1.

### 3.2. CBC values among groups of patients with ITP

There was significant difference among patients with ITP as regards WBCs (*P* < .00001) and platelets (*P* < .00001) where patients with ITP in remission had significantly higher platelet count and lower WBCs than other groups. There was no significant difference among patients with ITP as regards hemoglobin level (*P* > .05) (Table 2).

### 3.3. First- and second-line therapies among groups of patients with ITP

There was significant difference among groups of patients with ITP as regards first line therapy. Forty percentages of patients with acute ITP were treated conservatively while 60% received first line therapy (20% steroids, 20% IVIG and 20% combined steroids and IVIG). Eighty percentages of patients with ITP in remission received steroids as first line therapy and 20% received combined steroids and IVIG. Sixty percentage of patients with chronic ITP in remission received steroids as first line therapy and 40% received combined steroids and IVIG. 50% with chronic ITP received Eltrombopag as a second line therapy (Table 3).

### 3.4. Interleukin 27 serum levels in in study population

ITP patients had significantly higher levels of IL-27 than controls ( $770.6 \pm 236.5$  vs  $373.75 \pm 55.5$  pg/ml, respectively, *P* < .00001). Patients with acute ITP had the highest levels of IL-27 among

**Table 1****Demographics and clinical characteristics of the study groups.**

	Acute ITP N = 20	ITP in remission N = 20	Chronic ITP N = 20	Test	P
Age (years) Mean ± SD	5.32 ± 3.7	5.9 ± 2.7	10.8 ± 3.0	F = 17.7	<.00001
Sex (n, %)					
Male	11 (55%)	13 (65%)	9 (45%)	$\chi^2 = 2.2$	0.53
Females	9 (45%)	7 (35%)	11 (55%)		
Age at diagnosis Mean ± SD	5.32 ± 3.7	5.9 ± 2.7	8.6 ± 2.1	F = 7.2	0.002
Initial clinical presentations					
Purpura	16 (80%)	12 (60%)	14 (70%)	$\chi^2 = 5.4$	0.7
Ecchymosis	5 (25%)	10 (50%)	7 (35%)		
Epistaxis	9 (45%)	5 (25%)	9 (45%)		
Bleeding gums	2 (10%)	3 (15%)	2 (10%)		
Menorrhagia	1 (5%)	1 (5%)	1 (5%)		

**Table 2****Mean CBC values in patients with ITP.**

Variable	Acute ITP N = 20	ITP in remission N = 20	Chronic ITP N = 20	F	P
WBCs ( $10^9/\mu\text{l}$ )	10.3 ± 1.5	7.2 ± 1.4	9.4 ± 1.2	25.13	<.00001
Hb (g/dl)	10.3 ± 1.3	9.8 ± 0.6	10.1 ± 1.5	0.87	>.05
Platelets ( $10^9/\mu\text{l}$ )	23.8 ± 8.7	143.6 ± 29.1	37.6 ± 13	235.5	<.00001

patient groups. While, patients in remission had the lowest IL-27 levels among patient groups. The mean serum level in patients with acute ITP was  $860.1 \pm 308.8$  versus  $622.9 \pm 237.7$  pg/ml in patients with ITP in remission and  $725.8 \pm 181.1$  pg/ml in chronic ITP patients ( $P = .01$ ) (Table 4).

**3.5. Granzyme B serum levels in study population**

ITP patients had significantly higher levels of Granzyme B than controls ( $68.6 \pm 17.5$  vs  $25.8 \pm 5.5$  pg/ml, respectively,  $P < .00001$ ). Patients with acute ITP had the highest levels of Granzyme B among patient groups. While, patients in remission had the lowest Granzyme B levels among patient groups. The mean serum level in patients with acute ITP was  $75.1 \pm 18.2$  versus  $45.9 \pm 8.7$  in patients with ITP in remission and  $63.8 \pm 12.1$  pg/ml in chronic ITP patients ( $P = .01$ ) (Table 5).

**3.6. Relation between Interleukin 27 serum levels and demographic data in ITP patients.**

No significant relationship was found between serum IL-27 and each of age and gender in patients with ITP.

**3.7. Correlation between Interleukin 27 serum levels and CBC parameters in ITP patients.**

Our results revealed significant negative correlation between platelet count and serum IL-27 in patients with ITP ( $r = -0.52$ ,  $P = .0002$ ) (Fig. 1). Also, there was significant positive correlation between WBCs and serum IL-27 in patients with ITP ( $r = 0.45$ ,  $P = .004$ ) (Fig. 2). On the other hand, there was no significant correlation between hemoglobin level and serum IL-27 ( $r = -0.19$ ,  $P = .12$ ).

**Table 3****First- and second-line therapies in patients with ITP.**

Variable	Acute ITP N = 20	ITP in remission N = 20	Chronic ITP N = 20	Test	P value
Steroids (n = 32)	4 (20%)	16 (80%)	12 (60%)	$\chi^2 = 19.4$	0.003
IVIg (n = 4)	4 (20%)	0 (0%)	0 (0%)		
Steroids + IVIg (n = 16)	4 (20%)	4 (20%)	8 (40%)		
Conservative (n = 8)	8 (40%)	0 (0%)	0 (0%)		
Eltrombopag (n = 10)	0 (0%)	0 (0%)	10 (50%)		

**Table 4**  
Interleukin 27 levels among study groups.

Variable	Patients N = 60	Controls N = 20	Test	P
IL-27 (pg/ml) Mean ± SD	770.6 ± 236.5	373.75 ± 55.5	t = 7.4	<.00001

**Interleukin 27 levels among ITP patients**

IL-27 (pg/ml) Mean ± SD	Acute N = 20	Remission N = 20	Chronic N = 20	Test	P
	860.1 ± 308.8	622.9 ± 237.7	725.8 ± 181.1	F = 4.6	0.01

**Table 5**  
Granzyme B levels among study populations.

Variable	Patients N = 60	Controls N = 20	Test	P value
Granzyme B (pg/ml) Mean ± SD	68.6 ± 17.5	25.8 ± 5.5	t = 16.6	<.00001

**Granzyme B levels among ITP patients**

Granzyme B (pg/ml) Mean ± SD	Acute N = 20	Remission N = 20	Chronic N = 20	F = 23.8	<.00001
	75.1 ± 18.2	45.9 ± 8.7	63.8 ± 12.1		

### 3.8. Interleukin 27 levels in relation to first- and second-line therapies in patients with ITP

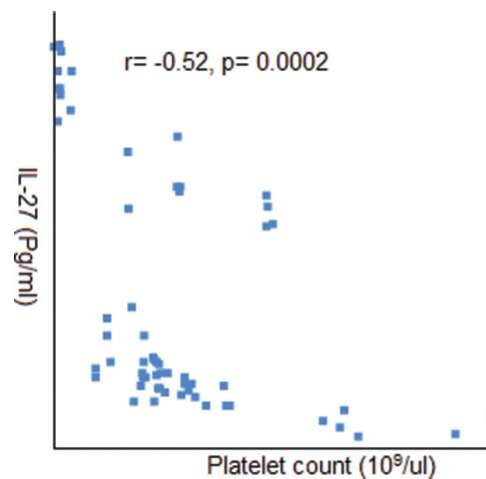
There was significant relationship between 1st line therapy and serum IL-27 levels in patients with ITP, where patients who received IVIG and combined steroids and IVIG had significantly higher IL-27 levels than other groups ( $924 \pm 335$  and  $886.6 \pm 323$  pg/ml compared to  $645.3 \pm 201$  in steroid group and  $705.1 \pm 141.9$  pg/ml in patients who were treated conservatively,  $P = .008$ ) (Table 6). Serum IL-27 levels was significantly lower in patients who received Eltrombopag than other second line therapies ( $655.1 \pm 80.5$  vs  $796.5 \pm 227.3$  pg/ml,  $P = .04$ ).

## 4. Discussion

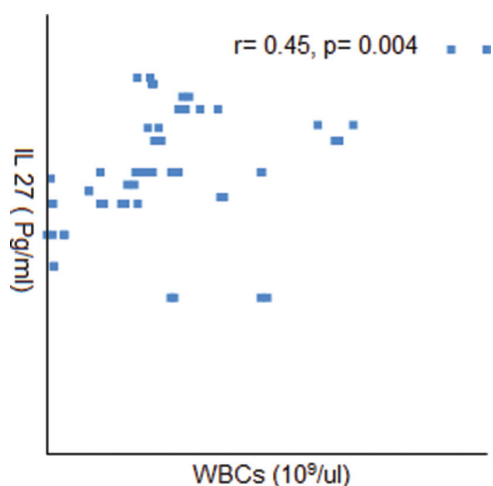
Interleukin-27 (IL-27) is a cytokine member of the IL-12 family with various immune-modulatory functions, showing both pro-inflammatory and anti-inflammatory effects. Numerous studies suggested a possible role of IL-27 in pathogenesis of ITP (4, 5). However, most of these studies were on adult populations.

In our study we found that patients with chronic ITP were significantly older than patients with acute ITP (10.8 vs 5.32 years, respectively,  $P < .00001$ ). Although percentage of females was higher in chronic ITP patients than other groups yet the difference did not reach a statistically significant level (55% in chronic ITP vs 45% in patients with acute ITP and 35% in patients with ITP in remission). This may be attributed to small sample size in our study.

In agreement with us, Evim et al<sup>[6]</sup> in their study on 201 pediatric patients reported that the risk of developing chronic ITP significantly increased in children older than 10 years of age (OR: 3.0, CI: 1.5–5.98), The other predictor for chronic ITP in their study was female sex, a significant increase of chronicity was noted in females (OR: 2.55, CI: 1.31–4.95).



**Figure 1.** Correlation between Interleukin 27 levels and platelet count in patients with ITP. This figure shows significant negative correlation between platelet count and serum IL-27 in patients with ITP ( $r = -0.52$ ,  $P = .0002$ ).



**Figure 2.** Correlation between Interleukin 27 levels and WBCs in patients with ITP. This figure shows significant positive correlation between WBCs and serum IL-27 in patients with ITP ( $r=0.45, P=.004$ ).

Also, Güngör et al<sup>[7]</sup> in their study on 211 children reported a mean age of 5.4 years at diagnosis and a female/male ratio of 1.03. The clinical courses were determined as acute or chronic in 72% and 28% of patients respectively. Mean age at diagnosis was significantly higher in chronic ITP ( $P < .01$ ). Chronic course was significantly higher in female patients ( $P < .05$ ).

On the contrary, Nazari et al<sup>[8]</sup> reported that there was a significant relationship between younger age and chronic ITP ( $P < .001$ ) where Chronic ITP was observed in 36 (20.9%) of their patients and their mean age was 15.8 months. Also, Hashemi et al<sup>[9]</sup> reported that there was no significant relationship between sex and disease progression towards the chronic phase ( $P = .554$ ).

The data reported by Nazari et al<sup>[8]</sup> is different from that was reported in literature. Nazari et al<sup>[8]</sup> did not explain their findings. However, it can be attributed to the different behavior of ITP in different ethnicities.

Our results showed that there was a significant difference among patients with ITP as regards first line therapy. Forty percentage of patients with acute ITP were treated conservatively while 60% received first line therapy (20% steroids, 20% IVIG, and 20% combined steroids and IVIG). Eighty percentage of patients with ITP in remission received steroids as first line therapy and 20% received combined steroids and IVIG. Sixty

percentage of patients with chronic ITP in remission received steroids as first line therapy and 40% received combined steroids and IVIG. 50% with chronic ITP received Eltrombopag as a second line therapy.

A final rationale for upfront therapy in an asymptomatic patient is the prevention of chronic or relapsing disease by way of exposure to immunomodulatory therapy. In children with ITP, this rationale is less applicable than adults, as children tend to have high spontaneous remission rates and low likelihood of disease recurrence or chronicity. The American Society of Hematology guidelines for the management of newly diagnosed ITP in adults and children recommend that children with no bleeding or mild bleeding (defined as skin manifestations only, such as bruising and petechiae) be managed with observation alone regardless of platelet count (Evidence grade 1B) and in pediatric patients requiring treatment, a single dose of IVIg (0.8–1.0) or a short course of steroids be used as first-line treatment (Evidence grade 1B). IVIg can be used if a more rapid increase in the platelet count is required (Evidence grade 1B).<sup>[10]</sup>

According to the American Society of Hematology guidelines for the management of newly diagnosed ITP in adults and children, oral corticosteroids is the preferred choice for first-line therapy unless there is a contraindication to corticosteroids or a need for more prompt increase in the platelet count such as life-threatening hemorrhage.<sup>[10]</sup>

This was the basis for choice of the 1st line therapy in our study where 53% of our patients received steroids as first line therapy. Approximately twenty seven percentage of our patients received steroids in combination with IVIG as those patients had marked thrombocytopenia (platelets  $< 10 \times 10^9/ul$ ) and severe bleeding. 6.7% of patients received IVIG alone and those patients were below 2 years with severe thrombocytopenia and we need prompt increase in the platelet count.

In 2008, the United States Food and Drug Administration authorized Thrombopoietin receptor agonists (TPO-RAs) use for adult chronic ITP patients who had relapsed after splenectomy and who were refractory to other treatments (i.e., corticosteroids, immunoglobulins), as well as for adult patients in whom splenectomy was contraindicated.<sup>[11]</sup> In 2015, the use of Eltrombopag was extended to any chronic ITP patient older than 1 year, as long as they did not respond to conventional medical management or splenectomy.<sup>[12]</sup> It is worth noting that, in real clinical practice, TPO-RAs seem to be replacing splenectomy in spite of the recommendations in the current published guidelines, although whether these drugs are really a better alternative still remains under discussion.<sup>[13]</sup>

**Table 6**  
Interleukin 27 levels in relation to 1st line and 2nd line therapy in patients with ITP.

Variable	1st line therapy					P
	Steroids N=32	IV IG N=4	Combined steroids + IVIG N=16	Conservative N=8	Test	
IL-27 (pg/ml) Mean ± SD	645.3 ± 201	924 ± 335	886.6 ± 323	705.1 ± 141.9	F=4.38	0.008

Variable	2nd line therapy			P
	Eltrombopag	Other 2nd line therapies	Test	
	655.1 ± 80.5	796.5 ± 227.3	t=-1.85	0.04

In our study, serum IL-27 levels were significantly higher in ITP patients than healthy controls ( $770.6 \pm 236.5$  vs  $373.75 \pm 55.5$  pg/ml respectively,  $P < .001$ ).

In agreement with our study, Gad Allah et al<sup>[14]</sup> in their study on 60 adult patients with ITP, found that the mean IL-27 for all patients was significantly higher than that for controls ( $113.4$  vs  $13.9$  pg/ml, respectively,  $P < .001$ ).

Similarly, Li et al<sup>[15]</sup> studied 59 adult patients with ITP and found that active ITP patients had higher IL-27 levels than controls ( $46.96$  vs  $25.54$  pg/mL, respectively,  $P = 0.023$ ).

Also, Li et al<sup>[16]</sup> found that IL-27 plasma level was significantly higher in patients with ITP compared with healthy controls ( $60.42$  vs  $32.91$  pg/ml, respectively,  $P = .001$ ).

On the contrary, Liu et al<sup>[5]</sup> studied 43 adults of ITP and found that IL-27 plasma levels were significantly lower in ITP patients with active disease than those of healthy controls ( $3562.3$  vs  $7010.9$  pg/ml, respectively,  $P < .001$ ).

This difference can be attributed to the different study populations (children in our study versus adults in Liu et al study<sup>[5]</sup>). Also, inclusion of patients who have received other modalities of treatment in Liu et al study<sup>[5]</sup>, including vincristine and danazol, raising the suspicion of the effect of these therapies on cytokine levels.

Our results showed that patients with acute ITP had the highest levels of IL-27 among patient groups. While, patients in remission had the lowest IL-27 levels among patient groups and the difference was statistically significant ( $P = .0048$ ). The mean serum level in patients with acute ITP was  $860.1 \pm 308.8$  versus  $622.9 \pm 237.7$  in patients with ITP in remission and  $725.8 \pm 181.1$  pg/ml in chronic ITP patients ( $P = .01$ ).

Our results are matched with Gad Allah et al<sup>[14]</sup> who found that There were significant differences in mean IL-27 levels between de novo and complete remission ( $P = .002$ ). Also, there was a nonsignificant difference in mean IL-27 between de novo and chronic ( $P = .452$ ). However, Gad Allah et al<sup>[14]</sup> found a significant difference in mean IL-27 between complete remission and chronic ( $P = .030$ ).

Also, our results are concordant with those of Li et al<sup>[15]</sup> where active ITP patients had higher IL-27 levels compared to patients in remission ( $P = .026$ ). Li et al<sup>[15]</sup> also found that the IL-27 levels in remission patients decreased to normal levels.

On the contrary, Liu et al<sup>[5]</sup> found significantly lower IL-27 plasma level was found in active ITP patients compared with ITP patients in remission ( $3562.3$  vs  $6663.7$  pg/ml, respectively,  $P = .000$ ). Moreover, they found no significant difference in plasma levels of IL-27 between ITP patients in remission and healthy controls ( $P = .334$ ).

This discrepancy again can be attributed to the different study populations (children in our study versus adults in Liu et al study).

In our study, to understand how IL-27 contribute to the pathogenesis of ITP. We measured serum granzyme B levels in patients and controls and we found that ITP patients had significantly higher levels of Granzyme B than controls ( $68.6 \pm 17.5$  vs  $25.8 \pm 5.5$  pg/ml, respectively,  $P < 0.00001$ ). Patients with acute ITP had the highest levels of Granzyme B among patient groups. While, patients in remission had the lowest Granzyme B levels among patient groups. The mean serum level in patients with acute ITP was  $75.1 \pm 18.2$  versus  $45.9 \pm 8.7$  in patients with ITP in remission and  $63.8 \pm 12.1$  pg/ml in chronic ITP patients ( $P = .01$ ).

The possible explanation is that higher IL-27 levels activate cytotoxic T cells and production of Granzyme B. This was supported by the findings of Li et al<sup>[15]</sup> in their large study to understand the pathogenesis of ITP where they found that elevated interleukin-27 enhances the polarization of Th1/Tc1 cells and the production of proinflammatory cytokines in primary immune thrombocytopenia including interferon  $\gamma$ , TNF  $\alpha$  and granzyme B and they found that IL-27 activates both T helper 1 and T cytotoxic cells.

Also in agreement of our study, Olsson et al<sup>[17]</sup> found higher levels of Granzyme B in ITP patients than healthy controls.

In our study, there is no significant correlation between age and serum IL-27 in patients with ITP. Also, there is no significant relationship between gender and serum IL-27 in patients with ITP.

In agreement with us, Gad Allah et al<sup>[14]</sup> found that there was no significant correlation between IL-27 level and age ( $r = -0.037$ ,  $P = .781$ ).

Our results showed that there was a significant negative correlation between platelet count and serum IL-27 in patients with ITP ( $r = -0.52$ ,  $P = .0002$ ).

These results are matched with those of Gad Allah et al<sup>[14]</sup> where there was a significant negative correlation between IL-27 level and platelet count ( $r = -0.375$ ,  $P = .003$ ).

On the contrary, Liu et al<sup>[5]</sup> reported in their study that there was no significant correlation between IL-27 levels and the platelet counts.

As regards other CBC parameters, in our study there was significant positive correlation between WBCs and serum IL-27 in patients with ITP ( $r = 0.45$ ,  $P = .004$ ) while there was no significant correlation between IL-27 level in patients with ITP and hemoglobin ( $r = -.19$ ,  $P = .12$ ).

Gad Allah et al<sup>[14]</sup> found that there was no significant correlation between IL-27 level and hemoglobin ( $r = -0.044$ ,  $P = .741$ ) which was similar to our data, however they found also no significant correlation between IL-27 level and WBC ( $r = 0.45$ ,  $P = .004$ ) which was not matched with ours.

In our study, there was significant relationship between 1st line therapy and serum IL-27 in patients with ITP. Where patients who received IVIG and combined steroids and IVIG had significantly higher IL-27 levels than others. These results can be attributed to lower platelet counts in patients who received IVIG alone or combined with steroids. Lower platelet counts were negatively correlated to IL-27 in our study. Our results showed that there was a significant relationship between 2nd line therapy and serum IL-27 in patients with chronic ITP. Where patients who received Eltrombopag had significantly lower IL-27 levels than others. This is can be explained based on the fact that Eltrombopag increased platelet counts in patients with chronic ITP and this increase was associated with decrease in IL-27 level.

## 5. Conclusions

We concluded that IL-27 was significantly higher in ITP patients than healthy controls and in patients with de novo ITP compared to those in remission. These findings potentiate previous suggestions about the role of IL-27 in pathogenesis of childhood ITP. Moreover, we suggest using IL-27 as a predictor for disease occurrence and to lower extent for responsiveness to treatment but this needs to be confirmed in larger studies. We promote

performing larger studies for longer follow-up periods including patients with relapsed ITP to detect the impact of IL-27 on relapse risk.

## 6. Limitation of the study

Small sample size was one of the limitations in this study and so larger multicenter studies are still needed to support these findings. Another limitation was that we need to start with patients with de novo ITP and to follow the changes of serum levels of IL-27 over time. However, many patients with de novo ITP lost follow up especially after improvement. We need also to include patients with persistent ITP.

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## Author contributions

All the authors contributed to designing and performing the research, manuscript writing, reviewing it critically, and approving the submitted version.

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