ORIGINAL ARTICLE

Interleukin 27 Serum Level and Its Prognostic Significance in Children with Immune Thrombocytopenia

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ABSTRACT

Aim of the work: was to determine the level of IL-27 in patients with immune thrombocytopenia and its relationship to demographic, clinical and laboratory characteristics of patients as well as disease chronicity and response to treatment. **Methods**: A case control study carried out in Pediatric Hematology outpatient clinic , Zagazig University Hospitals. It included 80 subjects were divided into 4 group 1 included 20 patients with denovo ITP, group 2 included 20 patients with chronic ITP, group 3 included 20 patients with complete remission after 1st line therapy and group 4 (control group) included 20 healthy children as a control group. **Results**: there is significant difference regarding WBCs and Platelets, there was no significant difference in patients with ITP as regards hemoglobin level. ITP patients had significantly higher levels of IL-27 than controls. 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. There was significant relationship between 1st line therapy and serum IL-27 in patients with ITP. Patients who received IVIG and combined steroids and IVIG had significantly higher IL-27 levels than others. There was 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. **Conclusion**: we propose that the using of IL-27 as a predictor for ITP occurrence and for responsiveness to treatment but this need to be confirmed in larger studies.

Key words: Immune thrombocytopenia, Interleukin 27, Interleukin 27 in immune thrombocytopenia

INTRODUCTION

Immune thrombocytopenia (ITP) is an acquired disease, due to immune destruction of platelets and characterized by platelet count $< 100 \times 10^9/L$. It occurs in children and adults, with a multimodal incidence, 1st peak in childhood , 2nd and 3rd peaks in young adults and the elderly , the process of the underlying disease in childhood ITP and adult ITP may be actually different. The adults ITP is usually a chronic disease while in most of children have self-limited disease [1].

Concepts around the mechanisms of decrease plateletes count in immune thrombocytopenia have changed from the classical view of increased platelet breakdown due to auto antibodies to more complicated mechanisms in which impaired platelet synthesis, disturbed cytokine profiles play a role and T-cell- mediated effects [2].

Biomarkers are one of the major factors of ITP pathogenesis , there are many abnormal immune biomarkers have a role in the pathogenesis of ITP ,The abnormal T , B cells and some new biomarkers in ITP were introduced, It should help us to know the pathogenesis of ITP [3].

Interleukin 27 (IL 27) ,a cytokine has a pro-inflammatory and an anti-inflammatory

effects, also plays a role in immunomodulation , Recent researches have revealed that interleukin 27 could inhibit inflammatory responses in Tcell differentiation and in autoimmune diseases , like with systemic lupus erythematosus . Previous studies showed that Cytotoxic T-lymphocyte mediated platelet breakdown was increased and the level of Interleukin 27 was decreased in patients with Immune thrombocytopenia [4]**.**

multiple studies showed that IL 27 can play an important regulatory role by suppressing the acquired immunity, which leading to the development of T helper cells ,and increase of inducible regulatory T cells to produce IL-10 . IL-27 also inhibition of Th17 cells which lead to suppression of inflammation [5]**.**

The American Society of Hematology guidelines for the treatment of newly diagnosed immune thrombocytopenia in children and adults recommend that children with mild bleeding or no bleeding, should be managed with observation alone regardless of platelet count (Evidence grade 1B) and in pediatric patients requiring treatment, a short course of steroids or a single dose of IVIg (0.8-1.0) 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) [6]**.**

Frederiksen et al. [7] reported a 1.5-fold higher mortality in ITP patients compared with the general population, with significant increased risk of bleeding and infection as well as hematologic malignancy. Thai et al. [8] have not confirmed the increased risk of malignancy.

Primary ITP is an acquired immune disorder where the thrombocytopenia results from pathologic antiplatelet antibodies, impaired megakaryocytopoiesis, and T-cell–mediated destruction of platelets, with each pathologic mechanism playing varying roles in each patient. Secondary ITP is associated with other underlying disorders, such as autoimmune disease (systemic lupus erythematosus or rheumatoid arthritis), Helicobacter pylori, or underlying immune dysregulation syndromes, such as common variable immunodeficiency [9]**.**

Aim of the work:

The aim of this work was to determine the level of IL-27 in patients with immune thrombocytopenia and its relationship to demographic , clinical and laboratory characteristics of patients as well as disease chronicity and response to treatment .

METHODS

A case control study was carried out in Pediatric Hematology outpatient clinic, Zagazig University Hospitals. It included 80 subjects. Written informed consent was obtained from all participants 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 4 groups:

Group 1: included 20 patients with denovo ITP

Group 2: included 20 patients with chronic ITP

Group 3: included 20 patients with complete remission after 1st line therapy

Group 4: (control group) included 20 healthy children as a Informed written consent and assent forms were obtained from parents and/or caregivers.

Patient group : 60 patients with immune thrombocytopenia were consequetively enrolled in this study.

Inclusion criteria :

1- Patients diagnosed with 1ry ITP. (Primary immune thrombocytopenia (ITP) is an immunemediated bleeding disorder in which platelets are opsonized by autoantibodies directed against platelet surface membrane glycoproteins (GPs), and prematurely cleared by phagocytic cells in the reticuloendothelial system [7].

2- Age between the age of 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 100,000cells /mcl.

4- Patients with hereditary thrombocytopenia

Control group: Age and sex matched 20 healthy children were included as a control group.

Methods: All patients were subjected to the following:

1- Full history taking.

2- Thorough general examination. 3- Routine investigations includes blood smear examination, complete blood picture at diagnosis, follow up CBC and platelet trend Patients and controls were subjected to: Measurement of serum IL-27 by ELISA.

Statistical analysis

Data were assessed, entered and analyzed using SPSS version 20 (IBM SPSS, Armonk, NY, USA). Data are expressed as the mean ± standard deviation for quantitative variables, number and percentage for qualitative variables. $\chi 2$ test , t-test and f test (ANOVA) were used when appropriate to compare between different groups. P<0.05 and P<0.001 were considered to indicate statistically significant differences.

First line therapy in patients with ITP

- **Steroids**, No = 32 (acute → 4, Remission → 16 and chronic → 12)
- **IVIG**, No = 4 (acute → 4, Remission → 0 and chronic → 0)
- **Steroids** + **IVIG** , No = 16 (acute → 4, Remission → 8 and chronic → 8)
- **Conservative**, No = 8 (acute → 8, Remission → 0 and chronic → 0)

Second line therapy in patients with ITP

- **Eltrombopag**, No = 10 (acute → 0, Remission → 0 and chronic → 10)

RESULTS

Our results showed that: **Table (1),** showed that patients with chronic ITP are significantly older than patients with acute ITP, where the mean age was 5.32 ± 3.7 for acute group, 5.9± 2.7 for remission group and 10.8± 3.0 for chronic group and regarding sex there was no significant differences among the studied groups. **Table (2),** showed that patients with chronic ITP are significantly older at time of

diagnosis than patients with acute ITP, where the mean age at diagnosis was 5.32 ± 3.7 for acute group and 8.6± 2.1 for chronic group**,** there was a significant difference among patients with ITP as regards WBCs and Platelets 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, there was significant difference among patients with ITP as regards first line therapy where most patients in remission and chronic groups received steroids as a first line therapy. Also none of patients in both groups (remission and chronic) were treated conservatively at their diagnosis. 10 patients (50%) with chronic ITP received Eltrombopag as a second line therapy**,** showed that ITP patients had significantly higher levels of IL-27 than controls, where the mean level of IL-27 was 770.6±236.5 in patients group and it was 373.75±55.5 in control group**,** 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, where the mean level was 860.1±308.8 for acute group, 622.9± 237.7 for remission group and 725.8±181.1 for chronic group (P = 0.01)**,** showed that there is significant relationship between 1ˢᵗ line therapy and serum IL-27 level in patients with ITP. Where patients who received IVIG (mean level was (924± 335) and who received combined steroids and IVIG (886.6± 323) had significantly higher IL 27 levels than other groups (in steroid group mean level was 645.3± 201 and 705.1± 141.9 in conservative group), there is significant relationship between 2nd line therapy and serum IL-27 in patients with chronic ITP, the mean level of IL 27 was 796.5± 227.3. Where patients who received Eltrombopag had significantly lower IL-27 levels (655.1±80.5) than other groups, there is no significant relationship between gender and serum IL-27 in patients with ITP where the mean level for males was 665.4±296.9 an fer females it was 623.8± 257.6.

Figure (1), showed that there is no significant correlation between age and serum IL-27 in patients with ITP. **Figure (2)**, showed that there is significant positive correlation between WBCs and serum IL-27 in patients with ITP. **Figure (3)**, showed that t there is no significant

correlation between hemoglobin level and serum IL-27 in patients with ITP.

Table 1. Age and sex among different groups of patients

Variable	Acute N= 20	Remission N= 20	Chronic N=20	Test	P value
Age (years) **Mean ± SD**	5.32 ± 3.7	5.9± 2.7	10.8± 3.0	F= 17.7	<0.00001
Sex (n, %) **Males** **Females**	11 (55%) 9 (45%)	13(65%) 7 (35%)	9 (45%) 11 (55%)	$X^2 = 2.2$	0.53

Table 2. Mean CBC values , first and second line therapies, Interleukin 27 levels among control and patients with ITP,

Variable	Acute N= 20	Remission N= 20	Chronic N=20	F	P value
WBCs ($10^{9/}$ul)	10.3± 1.5	7.2±1.4	9.4±1.2	25.13	< .00001
Hb(gm/dl)	10.3± 1.3	9.8± 0.6	10.1± 1.5	0.87	>0.05
Platelets ($10^{9/}$ul)	23.8± 8.7	143.6±29.1	37.6±13	235.5	< .00001
Variable	**Acute N= 20**	**Remission N= 20**	**Chronic N=20**	**Test**	**P value**
Steroids (n = 32)	4(20%)	16 (80%)	12(60%)	$X^2 = 19.4$	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(0.0)	10 (50%)		
Variable	**Acute N= 20**	**Remission N= 20**	**Chronic N=20**	**F**	**P value**
IL-27 (g/ml)	860.1±308.8	622.9± 237.7	725.8±181.1	.6	0.01

Variable	Patients N= 60	Controls N= 20	Test	P value
IL-27 (pg/ml)	770.6±236.5	373.75±55.5	t= 7.4	< .00001
Variable	**Eltrombopag**	**Other 2nd line therapies**	**t test**	**P value**
IL-27 (pg/ml) **Mean ± SD**	655.1±80.5	796.5± 227.3	-1.85	0.04
Variable	**Males N= 33**	**Females N= 27**	**t test**	**P value**
IL-27 (pg/ml)	665.4±296.9	623.8± 257.6	0.67	0.25

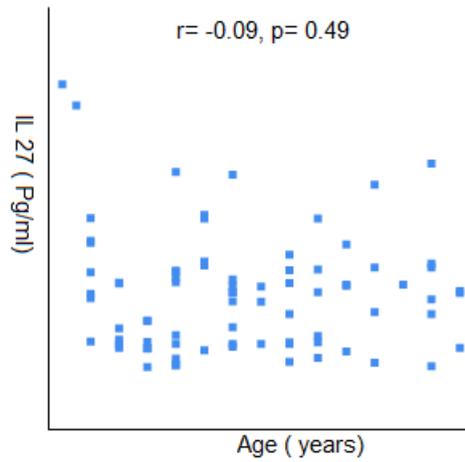| Variable	Steroids N= 32	IV IG N= 4	Combined steroids + IVIG N=16	Conservative N= 8	F test	P value
IL-27 (pg/ml) **Mean ± SD**	645.3± 201	924± 335	886.6± 323	705.1± 141.9	4.38	0.008

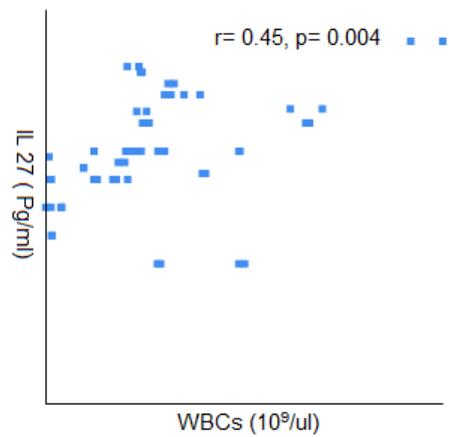**Figure 1: Correlation between Interleukin 27 levels and age of patients with ITP**

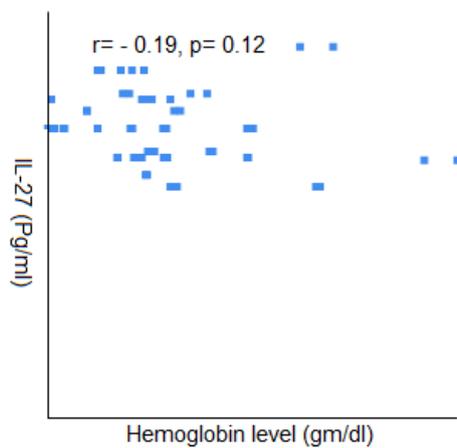**Figure 2: Correlation between Interleukin 27 levels and WBCs in patients with ITP**

Figure 3: Correlation between Interleukin 27 levels and hemoglobin level in patients with ITP

DISCUSSION

In our study we found that patients with chronic ITP are significantly older than patients with acute ITP (10.8 versus 5.32 years respectively, p<0.00001). Although percentage of females was higher in chronic patients than other groups yet the difference did not reach a statistical significant level (55% in chronic ITP versus 45% in patients with acute ITP and 35% in patients with ITP in remission).

In agreement with us, Evim et al., [10] in their study on 201 pediatric age group 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).

On the contrary, Nazari et al., [11] reported that there was a significant relationship between younger age and chronic ITP (P<0.001). Also, Hashemi et al., [12] reported that there was no significant relationship between sex and disease progression towards the chronic phase (P value: 0.554).

Our results showed that there was a significant difference among patients with ITP as regards first line therapy where most patients in remission and chronic groups received steroids as a first line therapy. Also none of patients in both groups (remission and chronic) were treated conservatively at their diagnosis. 50% with chronic ITP received Eltrombopag as a second line therapy.

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. 26.7% of our patients received steroids in combination with IV IG as those patients had marked thrombocytopenia (platelets <10 x 10^9/ ul) and 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.This results were in agreement with the study of Neunert et al. [6], who reported that 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).

In our study, serum IL-27 levels were significantly higher in ITP patients than normal controls (770.6±236.5 versus 373.75±55.5 pg/ml respectively, p< .00001).

In agreement with our study, Gad Allah et al., [2] 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 versus 13.9 pg/ml respectively, P<0.001).

On the contrary, Liu et al., [5] in their study on 43 adults of ITP, found that plasma concentrations of IL-27 were significantly lower in ITP patients with active disease than those of healthy controls (3562.3 versus 7010.9 pg/ml respectively, P = 0.000).

This difference can be attributed to the different study populations (children in our study versus adults in Liu et al study). Also, inclusion of patients who have received other modalities of treatment in Liu et al study, 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=0.0048). There was no significant difference between patients with chronic ITP and either that with acute ITP (P=0.051) or those in remission (P= 0.65).

Our results are matched with Gad Allah et al., [2] who found that There were significant differences in mean IL-27 levels between de novo and complete remission (P=0.002). Also, there was a non significant difference in mean IL-27 between de novo and chronic (P=0.452). However, Gad Allah et al., [2] found a significant difference in

mean IL-27 between complete remission and chronic (P=0.030).

On the contrary, Liu et al., [5] found significantly lower IL-27 plasma level was found in active ITP patients compared with ITP patients in remission (3562.3 versus 6663.7 pg/ml respectively, P = 0.000). Liu et al., [5] found no significant difference was found in plasma levels of IL-27 between ITP patients in remission and healthy controls (P = 0.334).

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, 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. This result was in agreement with the study of Gad Allah et al., [2]. who found that there was no significant correlation between IL-27 level and age (r=−0.037 , P=0.781)

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=0.0002). These results are matched with those of Gad Allah et al., [2]. where there was a significant negative correlation between IL-27 level and platelet count (r=−0.375 , P= 0.003). On the contrary , Liu et al., [5] 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=0.004) while there was no significant correlation between IL-27 level in patients with ITP and hemoglobin (r=-0.19, p=0.12). Gad Allah et al., [2] found that there was no significant correlation between IL-27 level and hemoglobin (r=−0.044 , P=0.741) which was similar to our results , however they found also no significant correlation between IL-27 level and WBC (r=0.45 , p=0.004) which was not matched with our study.

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 levels.

CONCLUSION

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. also, we propose using IL-27 as a predictor for ITP occurrence and for responsiveness to treatment but this need 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.

RECOMMENDATIONS

1- Serum IL-27 may be useful as a screening test for predicting the risk for ITP occurrence and response to treatment.

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

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