Contribution of Serum Interleukin-10 to the Pathogenesis of Primary Immune Thrombocytopenia in Egyptian Children: A Single Center Experience

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ABSTRACT

Background: Research efforts have been directed to the role of different cytokines in the pathogenesis of primary immune thrombocytopenia (ITP) in children. Interleukin-10 (IL-10) is anti-inflammatory cytokine which may play a role in the pathogenesis of this disease and prediction of its chronicity.

Objectives: We aimed to determine the serum level of interleukin 10 in children with ITP and to evaluate its relationship with disease chronicity and other clinical and laboratory variables in these patients.

Patients and Methods: This study was carried out on 100 children with ITP and 100 age- and sex-matched healthy children as a control group. Patients were subjected to full history taking, thorough clinical examination and routine investigations according to our local standards. Serum IL-10 was measured in patients and controls by ELISA method.

Results: Patients had significantly higher levels of serum IL-10 than controls. Newly diagnosed patients had significantly higher levels than patients with persistent and chronic ITP. There was a significant negative correlation between serum IL-10 levels and age of patients. Females had significantly higher IL-10 levels than males.

Conclusion: We concluded that serum IL-10 seems to predict susceptibility to primary immune thrombocytopenia in Egyptian children.

Keywords: Interleukin-10, Immune thrombocytopenia, Egyptian children, Chronicity.

INTRODUCTION

ITP is defined as isolated thrombocytopenia (platelet count < 100,000/µl with normal white blood cell count and hemoglobin). The etiology of ITP remains unknown in most cases, but it can be triggered by many environmental factors including viral infection and immunologic triggers [(1-4)].

The hallmark of autoimmune diseases is the breakdown in self-tolerance which is caused by several factors and is characterized by the inability of the immune system to effectively distinguish self from non-self-antigens and to refrain from attacking self [(1)].

The pathophysiology of ITP is extremely complex and heterogeneous. Advances in ITP research suggest that a complex dysregulation of the immune system is involved in its pathogenesis [(5)]. Numerous studies showed that abnormalities in T lymphocyte, natural killer cell, dendritic cell, cytokines and programmed cell death in addition to oxidative stress, infection and drugs play a key role in the pathogenesis of ITP [(6)]. Among these abnormalities, the increased number of T helper 1 cells, the decreased number or dysfunction of regulatory T cells as well as the destruction of platelets by cytotoxic T cells [(7, 8)]. Moreover, dysregulated T cells in ITP patients may enable the formation of antiplatelet antibodies and impair platelet production by megakaryocytes [(9)]. Cytokines are signaling molecules responsible for controlling the intracellular communication that mediate and regulate the immunological reaction [(10)]. IL-10 is one of the anti-inflammatory cytokines that suppress the immune responses. IL-10 is secreted by macrophages, T helper 2 cells and mast cells. IL-10 is also released by cytotoxic T cells to inhibit viral infection-stimulated natural killer cell activity. As an anti-inflammatory cytokine, IL-10 inhibits the synthesis of a wide number of cytokines involved in the inflammatory process including IL-2, IL-3, granulocyte-macrophage colony-stimulating factor (G-M CSF), interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α). IL-10 can promote the activity of mast cells, B cells, and certain T cells [(11-13)].

We aimed to determine serum level of interleukin 10 in children with ITP and to evaluate its relationship with disease chronicity and other clinical and laboratory variables in these patients.

PATIENTS AND METHODS

A case-control study was carried out in Pediatric Hematology Outpatient Clinic of Zagazig University Hospitals during the period from July 2019 to May 2020. The study included 100 children with ITP who were recruited from Pediatric Hematology Outpatient Clinic, Zagazig University Hospitals during their regular follow up visits. Also, 100 age- and sex-matched healthy children was included as a control group.

Eligibility criteria: Both sex, patients with primary immune thrombocytopenia, and age from 1-15 years.

Exclusion criteria: Patients with secondary immune thrombocytopenia, patients with other causes of thrombocytopenia, and age < 1year or > 15 years.

Diagnosis of ITP and classification:

Diagnosis of ITP will be based on ASH clinical practice guidelines in 2011 as a platelet count less than 100,000/µl in the absence of other causes or disorders that may be associated with thrombocytopenia. ITP was classified as newly diagnosed (3 months to diagnosis), persistent (3 to 12 months from diagnosis), or chronic (lasting for more than 12 months) [(14)].

All patients were subjected to full history taking, thorough clinical examination and routine laboratory

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investigations for diagnosis and follow up of ITP according to our local standards including CBC and platelet trend. Measurement of serum level of IL-10 was performed for all patients and control using Enzyme Linked Immunosorbent Assay (ELISA) catalogue number 201- 12-0103 (48T) (Shanghai Sunder Biological Technology Co., Ltd)

Test principle:

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay to measure the level of human IL-l0 in samples. Add IL-10 to monoclonal antibody enzyme well, which is pre-coated with human IL-l0 monoclonal antibody, incubation. Then, add IL-l0 antibodies labeled with biotin, and combined with streptavidin-HRP to form immune complex. Then, carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue. And at the effect of acid, the color finally becomes yellow. The Chroma of color and the concentration of the human IL-10 of sample were positively correlated [(15)].

Summary procedures:

1- Preparing reagents, samples and standards.
2- Add prepared samples and standards, antibodies labeled with enzyme, reacting 60 minutes at 37 ℃.
3- Plate washed five times, adding Chromogen Solution A, B, reacting 10 minutes at 37 ℃.
4- Add stop solution.
5- Measure the OD within 10min. 6- Calculation.

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Every patient 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 data were checked, 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 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

The mean age of our patients was 9.4 ± 3.5 years (range: 3-15 years). The mean age at diagnosis was 6.9 ± 2.1. They were 56 males and 44 females. 36 patients had newly diagnosed ITP, 30 patients had persistent ITP and 34 patients had chronic ITP. No significant difference among different groups of patients (newly diagnosed, persistent or chronic) in relation to age at diagnosis (p = 0.25). However, patients with chronic ITP were significantly older than patients with newly diagnosed or persistent ITP (12.1 ± 3.1, 7.0 ± 2.0 and 9.3 ± 3.3 respectively, p < 0.001**).** Though female gender was higher in chronic ITP compared to persistent and newly diagnosed patients, yet the difference didn't reach a statistically significant level (53 %, 33.3% and 39% respectively, p = 0.5) (Figure 1). The mean age of controls was 9.6 ± 3.5 (range: 3-15 years). They were 50 females and 50 males. Patients and controls were matched as regards age and sex (p =0.38 and p = 0.55 respectively) (Table 1).

Purpura was the most common clinical presentation (92%) followed by ecchymosis (86%) and external bleeding (64%). The mean initial platelet count was 12,000/µl (1000-33000/µl). It was significantly higher in chronic ITP patients compared to persistent and newly diagnosed patients (17400, 13200 and 6700/µl respectively, p<0.001). Also, mean initial platelet count was significantly higher in females compared to males (14640 versus 10200 /µL respectively, p = 0.03) (Table 2). As regards 1st line therapy, 14% were managed conservatively, 66% received steroids, 10% received intravenous immunoglobulin (IVIG) and 10% received combined steroids and IVIG. As regards 2nd line therapy, 84% received thrombopoietin receptor agonists (TPO-RA), 14% received azathioprine and 4% performed splenectomy (Table 2).

Patients had significantly higher levels of serum IL-10 than controls (491 ± 623.5 versus 183.4 ± 72.4, p <0.0001) (Figure 1). Patients with newly diagnosed ITP had significantly higher levels compared to persistent and chronic patients with ITP (Table 3).

There was significant negative correlation between serum IL-10 levels and age of patients (r = - 0.3, p = 0.03) (Figure 2). No significant correlation between serum IL-10 levels and any of age at diagnosis (r = - 0.03, p = 0.8) or platelet count (r = 0.2, p = 0.1).

There was no significant association between serum IL-10 levels and any of clinical presentations (p=0.07). There was no significant relationship between serum IL-10 levels and any of 1st or 2nd line therapies (p=0.25 and p=0.6 respectively).

Table (1): Demographic characteristics of studied groups

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

Table (2) showed that TPO agonists were the most commonly used second line therapy.

Table (2): Second line therapy in patients

2nd line therapy	Patients N (%)
TPO	**26 (52%)**
Cytotoxic drugs	**6 (12%)**
splenectomy	**2 (4%)**

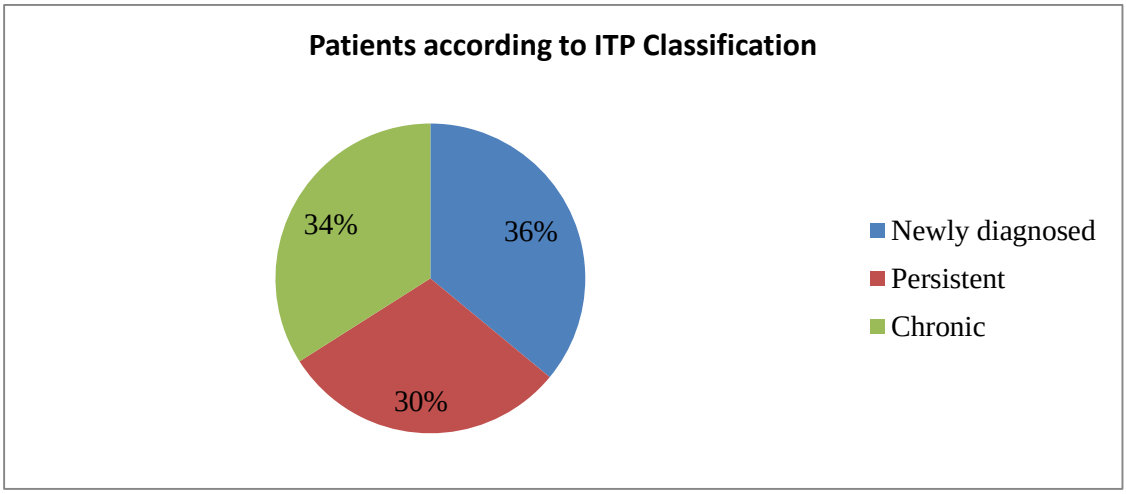

Figure (1): Classifications of ITP patients.

Table (3) showed that patients had significantly higher levels of serum IL-10 than controls.

Table (3): Serum IL-10 levels in studied groups

Serum IL10	Patients	Controls	T test	P
Mean ± SD (ng/ml)	491 ± 23.5	183.4 ± 7.4	3.47	0.00004

Table (4) showed that newly diagnosed patients with ITP had significantly higher levels of IL-10 compared to persistent and chronic patients with ITP.

Table (4): Serum IL-10 levels in our patients in relation to ITP classification

Serum IL-10	Newly diagnosed N=18	Persistent N=15	Chronic N=17	F test	P
Mean ±SD (ng/ml)	906.7±70.4	295.2±22.6	223.5±19.7	8.14	0.0009

This figure showed that there was significant negative correlation between serum IL-10 levels and age of patients at diagnosis (Figure 2).

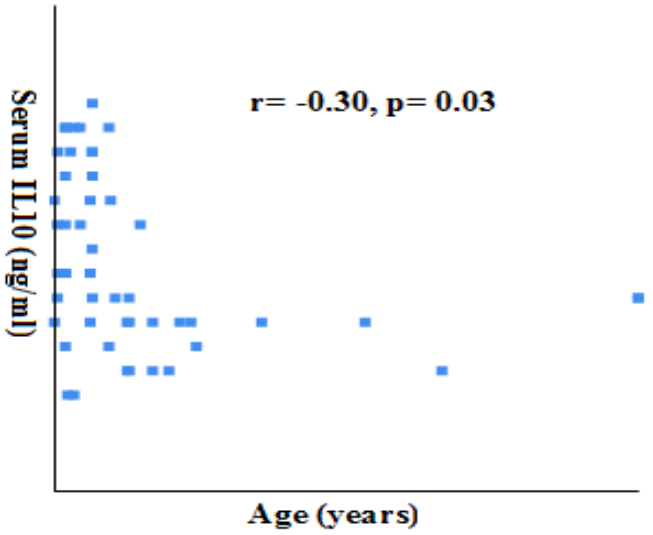

Figure (2): Correlation between serum IL10 level and age of patients.

This figure showed that there was no significant correlation between serum IL-10 levels and initial platelet count (Figure 3).

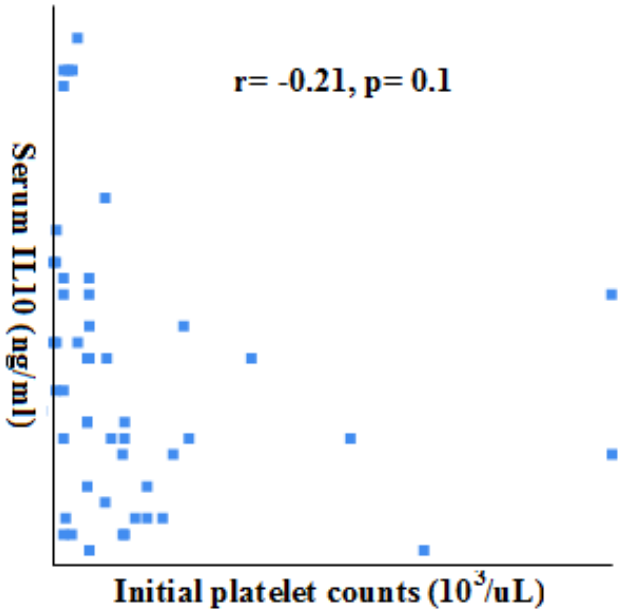

Figure (3): Correlation between serum IL10 level and initial platelet counts (10^3/ul).

DISCUSSION

Understanding the complex pathophysiology of ITP is very crucial to help design suitable preventive and therapeutic strategies. Derangement of immunological and genetic factors seems to have a pivotal role in ITP pathogenesis [(2)]. Cytokine imbalance was documented in many autoimmune disorders. Several studies provide evidence for a role of serum cytokines including IL-10 in ITP pathogenesis [(16-18)].

The mean age of our patients was 9.4 years. They were 56 males and 44 females. The mean age at diagnosis was 6.9 years. Purpura, ecchymosis and external bleeding were present in 92%, 86% and 64% of our patients respectively. We did not find any severe or life-threatening bleeding in our patients. Our findings were consistent with that reported in the literature where ITP in children typically affects children between 2-7 years of age without gender preference. However, a higher male/female ratio during infancy was reported in recent studies with a decreasing trend toward older age. The onset of ITP is abrupt. Bruises and petechiae represent the most common initial clinical presentation and affecting almost all patients [(19, 20)]. Severe life-threatening bleeding is rare (0.2–0.9%) in children with ITP [(21, 22)]. On the contrary, **Hamed *et al.*** [(23)] reported a female predominance in patients with ITP where 15.6% of their patients were males and 84.4% were females. Also, **Del Vecchio *et al.*** [(24)] and **Talaat *et al.*** [(25)] studies were performed on patients with ITP and showed that ITP among adults affects females more than males. This can be attributed to difference in the study population where the previous studies were performed on adults with ITP while 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.*** [(26)] 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.*** [(27)] 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 lead a statistically significantly level. Female gender was one of predictors of chronicity in a large meta-analysis conducted by **Heitink-Pollé *et al.*** [(27)] (odds ratio (OR) 1.17, 95% confidence interval (CI) 1.04-1.31).

In our study, we did not find any significant association between age of patients and disease chronicity. This finding was not matched with many previous reports where older age is considered a risk factor for chronic disease in children with ITP. **Heitink-Pollé *et al.*** [(27)] in their large meta-analysis found older age at presentation (age ≥11 years; OR 2.47, 95% CI 1.94-3.15) is an important predictor of chronicity. This can be explained based on small sample size in our study.

In our study, patients had significantly higher levels of serum IL-10 than controls (491 versus 183.4 ng/ml

respectively, p<0.001). Our results are consistent with **Hamed *et al.*[23]** where there was a statistically significant difference between ITP patients and controls as regards serum IL-10 levels (70.1, 39.4 and 29.0 ng/ml in newly diagnosed ITP, chronic ITP and controls respectively, p<0.001). Similarly, **Del Vecchio *et al.* [24]** reported that serum IL10 levels were significantly higher in patients with an acute evolution of ITP than in either healthy controls (p<0.001) or patients with chronic progression of ITP (17, 8 and 3 pg/ml in patients with acute ITP, chronic ITP and controls respectively, p<0.05). Our results were also are supported by **Tesse *et al.* [28]** where they found that serum concentration of IL-10 was significantly higher in patients with an acute course of their disease than controls (17 versus 3 pg/mL respectively, p < 0.01). **Talaat *et al.* [25]** also studied 35 children with ITP and 10 healthy controls. Plasma levels of Th1 cytokines (IFN-γ and TNF-α) and Th2 (IL-4, IL-6, and IL-10) cytokines were measured using ELISA. All evaluated cytokines (IFN-γ, TNF-α, IL-4, IL-6, and IL-10) were elevated significantly in patients with ITP (P<0.001, <0.05, <0.05, <0.05, and <0.001, respectively) compared to controls. On the contrary, **Culić *et al.* [29]** found no significant difference between patients and controls as regards serum IL-10 levels (p= 0.59). This discrepancy can be attributed to the difference in the study population where **Culić *et al.* [29]** investigated both adults and children with ITP where our study investigated only children with ITP.

In our study, newly diagnosed patients with ITP had significantly higher levels of IL-10 compared to persistent and chronic patients with ITP (906.7, 295.2 and 223.5 ng/ml in in newly diagnosed ITP, persistent ITP and chronic ITP respectively, p<0.001). These results are matched with **Hamed *et al.* [23]** where there was a statistically significant difference between patients with newly diagnosed ITP and patients with chronic ITP as regards serum IL-10 levels (70.1 and 39.4 ng/ml respectively, p<0.001). The same is also reported by **Del Vecchio *et al.* [24]** where serum IL10 levels were significantly higher in patients with an acute evolution of ITP than in patients with chronic progression of ITP (17 and 8 pg/ml respectively, p<0.05). Similarly, **Tesse *et al.* [28]** found that the serum concentration of IL-10 was significantly higher in patients with an acute course of their disease compared to chronic subjects (17 versus 3.5 pg/mL, p <0.01). Our results can be explained based on the clinical differences between newly diagnosed and chronic ITP which suggest the existence of different pathophysiological mechanisms in the two forms [30].

Our results showed that there was significant negative correlation between serum IL-10 levels and age of patient. No significant correlation between serum IL-10 levels and any of age at diagnosis or platelet count. Also, no significant association between serum IL-10 levels and any of clinical characteristics, first or second treatment lines.

There are very few studies that investigated the relationship between serum IL-10 levels and demographic, clinical or laboratory parameters in childhood ITP. In agreement with our study, **Hamed *et al.* [23]** did not find any significant association between serum IL-10 level and any CBC parameters including platelet counts. On the contrary, **Culić *et al.* [29]** found no significant difference between children and adults with ITP as regards serum IL-10 levels (17.2 versus 16.6 pg/ml respectively, p= 0.12).

Looking at the results of our study, we can explain that the significant negative correlation between serum IL-10 levels and age of patients are based on the observation that patients with chronic ITP had lower serum IL-10 levels and they were older in age than patients with newly diagnosed ITP who had higher serum IL-10 levels and were of younger age.

CONCLUSIONS

Serum level of IL-10 predicts susceptibility to primary immune thrombocytopenia in Egyptian children. Measurement of serum IL-10 level offers new insights into the pathogenesis of childhood ITP. Larger multicenter studies are still needed to support our findings.

Limitations:

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-10 over time. However, many patients with de novo ITP lost follow up especially after improvement.

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