Med. J. Cairo Univ., Vol. 92, No. 4, Accepted 8/9/2024
DOI: 10.22608/MJCU. 1345-1361, December 2024
www.medicaljournalofcairouniversity.net

A Study of the Level of Interleukin 10 in Children with Immune Thrombocytopenia

HOSSAM M. ELSHAMLY YEHIA, M.Sc.; MOHAMED A. ELLABOUDY, M.D.; MAHMOUD A. KENNY, M.D. and NESMA A. SAFWAT, M.D.

The Department of Pediatric, Faculty of Medicine, Ain Shams University

Abstract

Background: Immune Thrombocytopenia (ITP) is a common childhood blood disorder where autoantibodies destroy platelets by targeting glycoproteins on their membrane. ITP is classified as newly-diagnosed (≤3 months), persistent (3-12 months), or chronic (>12 months). The varied outcomes of ITP suggest multiple underlying mechanisms, including potential B-cell, T-cell, or mononuclear phagocyte abnormalities.

Aim of Study: To measure Interleukin 10 level in Immune Thrombocytopenia patients.

Patients and Methods: This case-control study was conducted on 60 children diagnosis as having Immune thrombocytopenic purpura regularly following-up at pediatric hematology clinic, pediatric hospital, Ain Shams University during the years 2023-2024. There were 32 males (53.3%) and 28 female (46.7%) with male: female ratio 1.14: 1. Their ages ranged from 2.5-1.5 years, with a mean age of 7.67 years and standard deviation of 3.22 years.

Results: The study analyzed 60 ITP patients with a mean age of 7.67 years, revealing that most had mild bleeding symptoms, with a median bleeding score of 1. Duration of ITP varied widely, averaging 8.08 months. A significant majority (93.3%) had a preceding infection, while recent vaccinations or drug intakes were less common. Bleeding manifestations were more severe in acute ITP patients, who had higher bleeding scores and lower platelet counts compared to persistent and chronic cases. IL-10 levels were significantly elevated in ITP patients (mean 605.06pg/mL) versus healthy controls (mean 7.1pg/mL), with levels highest in acute cases (mean 1061.9pg/mL) and lowest in chronic cases (mean 258.1pg/mL). This suggests that IL-10 may serve as a marker for disease severity and progression, highlighting its potential as a therapeutic target and offering insights into ITP's inflammatory processes.

Conclusion: Elevated IL-10 levels were consistently observed across different phases of ITP, with the highest levels seen in acute cases and progressively lower levels in persistent and chronic phases. This trend strongly suggests that IL-10 is closely associated with the inflammatory activity characteristic of ITP, with higher IL-10 levels correlating with more severe disease manifestations. There was a strong negative correlation between serum IL-10 levels and platelet counts, indicating that as IL-10 levels rise, platelet counts drop significantly.

Key Words: *Interleukin 10 – Immune Thrombocytopenia.*

Correspondence to: Dr. Hossam M. Elshamly Yehia,
E-Mail: Hossamyehia86@yahoo.com

Introduction

IMMUNE Thrombocytopenia is a commonly occurring blood disorder and is one of the commonest causes of childhood thrombocytopenia. This autoimmune disease occurs due to destruction of platelets following binding of autoantibodies to the glycoproteins located on the platelet membrane (GPIIb/IIIa and GPIb/IX) **[1].**

The term "acute ITP" has been replaced by "newly-diagnosed ITP", which refers to ITP diagnosed within the preceding 3 months. Immune thrombocytopenia of 3-12 months duration is designated as "persistent ITP", while "chronic ITP" is defined as disease of more than 12 months duration **[2].**

The heterogeneous outcome of ITP suggests the existence of different pathophysiological mechanisms. It is unclear whether ITP is initially caused by a B-cell abnormality, a T-cell disorder, an abnormality of thrombopoiesis, or even from increased mononuclear phagocyte activation **[3].**

Childhood ITP is usually a self-limiting disease, typically normalized within several months. However, approximately, 20–30% of children have persistent thrombocytopenic states for more than 6–12 months, which is called chronic ITP with an

increasing risk of bleeding. Fortunately, 30–60% of chronic childhood ITP patients regains normal platelet count and gets spontaneous remission within 5 years from initial diagnosis **[4].**

IL-10 is an anti-inflammatory cytokine and suppresses immune responses. IL-10 is secreted by macrophages, Th2 cells, and mast cells. Cytotoxic T cells also release IL-10 to inhibit viral infection stimulated natural killer cell activity **[5].**

IL-10 inhibits the synthesis of a number of cytokines involved in the inflammatory process including IL-2, IL-3, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ). It can promote the activity of mast cells, B cells, and certain T cells **[6].**

Aim of the work:

To measure Interleukin 10 level in Immune Thrombocytopenia patients.

Patients and Methods

This case-control study was conducted on 60 children diagnosis as having Immune thrombocytopenic purpura regularly following up at pediatric hematology clinic, pediatric hospital, Ain Shams University during the years 2023-2024. There were 32 males (53.3%) and 28 female (46.7%) with male: female ratio 1.14: 1. Their ages ranged from 2.5-1.5 years, with a mean age of 7.67 years and standard deviation of 3.22 years.

Twenty age and sex matched healthy children were recruited as a control group.

Parents of all patients and controls were asked to give an informed consent after explaining the nature, steps and aim of the study.

Patient's diagnosis were based on the presence of bruising and or petechiae, presence of isolated true thrombocytopenia (platelet count <100000) without any other underlying disease. Absence of splenomegaly and lymphadenopathy, and normal or increased megakaryocytes in the bone marrow.

Patients were classified according disease duration into Acute ITP (<3 months). Persistent ITP (3-12 month) and chronic ITP (>12 months).

Participants were divided into 3 groups: Group 1: 20 patients with Acute ITP, Group 2: 20 patients with chronic ITP and Group 3: 20 patients with persistent ITP.

Inclusion criteria include: Age: From 1 to 18 years, both sexes (male and female) and patients who attended the pediatric Hematology Clinic during the study period with primary ITP.

Exclusion criteria include: Age less than 1 year and more than 18 years, chronic infections, known autoimmune or immune deficiency disorders, past history of undiagnosed recurrent bleeding and patients with other causes of thrombocytopenia.

Methods: The study patients were subjected to the followings: Clinical assessment: Full history taking: The full history taking involved gathering a comprehensive personal history, where details about the patient's background and lifestyle were collected. Family history of bleeding disorders was assessed to determine any hereditary predispositions. The history of presenting symptoms was documented, including the nature, onset, and progression of the symptoms, along with the duration of the disease. The presence of any preceding infections was identified to understand potential triggers. The history of vaccination and drug intake was recorded, ensuring that any recent immunizations or medications were noted. The presence of chronic autoimmune diseases was evaluated, considering their impact on the patient's condition. Finally, any bleeding manifestations were carefully noted, including the type, frequency, and severity of bleeding episodes.

General and local examination: The general and local examination was conducted thoroughly, focusing on the identification of petechiae, purpura, and ecchymosis. Each area of the skin was carefully inspected for signs of these conditions, noting their distribution, size, and extent. The presence of wet purpura was specifically checked, as it is an important indicator of more severe bleeding tendencies. Additionally, the bleeding score was meticulously assessed, with each symptom and its severity being evaluated according to standardized criteria. All findings were documented to provide a comprehensive overview of the patient's condition.

Laboratory investigations: Complete blood count: Performed on ADVIA – 2/20 hematological analyzer (Bayer Diagnostics, Newbury, UK) and Samples were examined for the following parameters: platelet count, RBCS count, total lecocytic count. Serum IL-10 levels:

Serum IL-10 levels were quantitatively estimated using the Enzyme-Linked Immunosorbent Assay (ELISA) method. This process was carried out with the Serum IL-10 Estimation ELISA Kit provided by Booster Biological Technology (Pleasanton, CA, USA). For the estimation, samples were carefully collected from the patients, ensuring that proper protocols were followed to maintain the integrity of the serum.

Sample collection:

The collected samples were then processed according to the manufacturer's instructions, allowing for accurate measurement of Complete blood count and IL-10 levels.

For each case, 5 milliliters (mL) of whole venous blood were withdrawn under complete aseptic conditions. The collected blood was divided into 2 tubes:

Two mL of which were collected on tri-potassium ethylene diamine tetra-acetic acid (K3 EDTA) vacutainer with concentration of 1.2mg of the anhydrous salt per ml of blood, to be used for performing CBC.

The other 3mL of venous blood were collected into a sterile Gel activated vacutainer and was left to clot for 30 minutes in the vacutainer. Serum was then separated by centrifugation at 2000 rpm for 20 minutes.

Any hemolyzed or lipemic samples were discarded.

Quantification of IL-10 using ELISA:

Principle of the assay:

This assay employed the quantitative sandwich enzyme immunoassay technique. The plate was pre-coated with human IL-10 antibody. IL-10 present in the sample was added and bound to the antibodies coated on the wells. Subsequently, biotinylated human IL-10 antibody was added, binding to the IL-10 in the sample. Streptavidin-HRP was then introduced, binding to the biotinylated IL-10 antibody. After incubation, unbound Streptavidin-HRP was removed during a washing step. A substrate solution was added, and color developed in direct proportion to the amount of IL-10. The reaction was terminated by adding an acidic stop solution, and the absorbance was measured at 450nm.

Sample preparation:

Serum sample were stores at -80ºC Until assay time. Repeated freezing and thawing was avoided.

Reagents:

standard Solution (1600pg/mL), standard diluent, pre-coated ELISA plate, biotinylated human IL-10 antibody, streptavidin-HRP, stop solution, substrate solution A, substrate solution B, wash Buffer Concentrate (25x), plate sealers, user instructions and zipper bag.

Reagent Preparation:

All reagents were brought to room temperature before use. The standard solution was prepared by reconstituting 120µL of the standard (1600pg/mL) with 120µL of the standard diluent to generate an 800pg/mL standard stock solution. The standard was allowed to sit for 15 minutes with gentle agitation prior to making dilutions. Duplicate standard points were prepared by serially diluting the standard stock solution (800 pg/mL) 1:2 with standard diluent to produce solutions of 400pg/mL, 200pg/mL, 100pg/mL, and 50pg/mL. The standard diluent served as the zero standard (0ng/mL). Any remaining solution was frozen at -20°C and used within one month.

Wash buffer:

Dilute 20 mL of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of lx Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Assay procedure:

All reagents and samples were brought to room temperature before use. The assay was performed at room, determine the number of Strips required for the assay. Insert the strips in the frames for use, add 50uL of standard was added to standard well, add 40uL of each sample was added to sample well and 10uL of anti- IL 10 antibody was added to each sample well, then, 50uL of streptavidin-HRP was added to sample wells and standard wells and were mixed thoroughly. The plate was covered by a sealer and incubated for 60 minutes at 37°C, the plate was washed 5 times with wash buffer by soaking the wells with at least 0.35mL wash buffer for 30 seconds to 1 minute for each wash, add 50 uL of (substrate solution A) was added to each well and then, Add 50uL of (substrate solution B) was added then plate was covered with a new sealer and incubated for 10 minutes at 37°C in the dark, add 50uL of stop solution was added to each well, the blue color changed into yellow immediately and the optical density was determined within 10 minutes using a micro plate reader set to 450nm.

Calculation:

A standard curve was constructed by plotting the average optical density for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and a best fit curve was drawn through the points on the graph.

Statistical analysis:

Recorded data were analyzed using the statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). The quantitative data were presented as mean± standard deviation and ranges when their distribution was parametric (normal) while non-normally distributed variables (non-parametric data) were presented as median with inter-quartile range (IQR). Also qualitative variables were presented as number and percentages. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk Test.

The following tests were done:

In the statistical analysis, an independent-samples *t*-test was utilized to compare the means between two different groups. This test is particularly useful for determining whether there is a statistical-

ly significant difference between the means of two independent groups, ensuring that the analysis accurately reflects any differences in the data. When the analysis involved more than two groups, a one-way ANOVA (Analysis of Variance) test was employed. This method allows for the comparison of means across multiple groups, providing a robust means to detect significant differences among them.

For the comparison of qualitative data, the Chi-square test was applied. This test is commonly used to assess whether there is a significant association between categorical variables. It is particularly useful in cases where the data is non-numerical and the goal is to examine the relationship between different groups.

Throughout the analysis, a confidence interval of 95% was maintained, with an accepted margin of error of 5%. This means that the findings are expected to be correct 95% of the time, providing a high level of reliability. The *p*-value, a critical component of statistical testing, was used to determine the significance of the results. A *p*-value of less than 0.05 was considered significant, indicating that there is a strong likelihood that the observed differences or relationships are not due to chance. A *p*-value of less than 0.01 was considered highly significant, suggesting an even stronger evidence against the null hypothesis. Conversely, a *p*-value greater than 0.05 was considered insignificant, meaning that any observed differences could likely be attributed to random variation rather than a true effect.

Results

Table (1) shows the demographic data of 60 patients, with a slight male predominance (53.3% male, 46.7% female). The age distribution ranges from 2.5 to 15 years, with a mean age of 7.67 years and a standard deviation of 3.22 years.

Table (2) shows the bleeding scores of 60 patients, with a median score of 1 with an interquartile range (IQR) of 1-1. This indicates that most patients had a bleeding score of 1. The range of scores extends from 0 to 3, demonstrating some variability in bleeding severity among the patients.

Table (3) provides a detailed history of 60 patients. The mean disease duration is 8.08 months, with a standard deviation of 6.08 months, ranging from 1 to 21 months, indicating a broad variability in how long patients have been experiencing their condition. A significant majority (93.3%, n=56) reported a preceding infection, whereas only 6.7% (n=4) did not. Regarding history of vaccination and drug intake, 8.3% (n=5) had such a history, while 91.7% (n=55) did not.

Table (4) outlines the prevalence of bleeding from various orifices among 60 patients. The most common site of bleeding is the mouth and gums, affecting 73.3% (n=44) of patients. Epistaxis (nosebleeds) is the next most frequent, occurring in 51.7% (n=31) of patients. Bleeding in the urinary tract is reported by 11.7% (n=7), while Hematemesis are seen in 10.0% (n=6) of patients. Bleeding/rectum affects 8.3% (n=5) of patients. Notably, there are no reported cases of intracranial hemorrhage.

Table (5) shows the findings from the general examination of 60 patients. Purpura is the most common condition, observed in 70.0% (n=42) of the patients. Ecchymosis is present in 53.3% (n=32) of the patients. Petechiae are seen in 43.3% (n=26) of the patients. Knowing that many patients showed combined features.

Table (6) shows the platelet counts for 60 patients. The mean platelet count is 94.8 x 10^3/uL with a standard deviation of 66.09 x 10^3/uL. The platelet counts range from 10 x 10^3/uL of 268 x 10^3/uL.

Table (7) shows serum Interleukin-10 (IL-10) levels for 60 patients, comparing normal and observed levels. The normal IL-10 levels have a mean of 7.1pg/mL (±1.5) with a range of 4.8 to 9.8pg/mL.

In contrast, the observed IL-10 levels in the patients, with a mean of 605.06pg/mL (±377.57) and a range from 159 to 1545pg/mL.

80 patients will be divided into 4 groups according to duration of disease: Group A: 20 Acute ITP Patients. Group B: 20 Persistent ITP Patients. Group C: 20 Chronic ITP Patients. Croup D: 20 Healthy Controls.

Table (1): Demographic data of all patients.

	All patients (N=60)	
	N	%
Gender:		
Male	32	53.3
Female	28	46.7
Age (year):		
Mean ± SD	7.67±3.22	
Range	2.5-15	

Table (2): Bleeding score of all patients.

	All patients (N=60)	
	N	%
Bleeding Score:		
Median (IQR)	1 (1-1)	
Range	0-3	

Table (3): Full history of all patients.

	All patients (N=60)	
	N	%
Disease duration (Month):		
Mean ± SD	8.08±6.08	
Range	1-21	
Presence of preceding infection:		
Yes	56	93.3
No	4	6.7
History of vaccination and drug intake:		
Yes	5	8.3
No	55	91.7

Table (4): Bleeding / orifices among all patients.

	All patients (N=60)	
	N	%
Mouth & gums	44	73.3
Epistaxis	31	51.7
Urinary Tract	7	11.7
Hematemesis	6	10.0
Bleeding / rectum	5	8.3
Intracranial hemorrhage	0	0

Table (5): General examination among all patients.

	All patients (N=60)	
	N	%
Purpura	42	70.0
Ecchymoss	32	53.3
Petechiae	26	43.3

Table (6): Platelet count of all patients.

	All patients (N=60)
Platelet (10^3/uL):	
Mean ± SD	94.8±66.09
Range	10-268

Table (7): Serum interleukin-10 levels of all patients.

	All patients (N=60)
Normal level of Serum IL-10 (pg/mL):	
Mean ± SD	7.1±1.5
Range	4.8 – 9.8
Serum IL-10 level (pg/mL):	
Mean ± SD	605.06±377.57
Range	159 – 1545

Table (8) compares demographic data among four patient groups (A, B, C, D), each consisting of 20 patients. The gender distribution across the groups shows no significant difference (p=0.802), with males comprising 45% in Group A, 55% in Group B, 60% in Group C, and 50% in Group D.

Correspondingly, females make up 55%, 45%, 40%, and 50% of the groups, respectively. The age analysis, using a one-way ANOVA test, indicates no significant difference among the groups (p=0.617). The mean ages are 6.95±3.09 years for Group A, 7.77±3.11 years for Group B, 8.28±3.46 years for Group C, and 7.94±0.78 years for Group D, with age ranges spanning from 2.5 to 15 years.

Table (9) shows a comparison of bleeding scores among four patient groups (A, B, C, D). The bleeding score, measured as the median with interquartile range (IQR), varies significantly across the groups (p=0.000**), indicating a highly significant difference. Group A has a median bleeding score of 2 (IQR 1-2) with a range of 1-3. Group B has a median score of 1 (IQR 1-1) and a range of 0-2. Group C has a median score of 1 (IQR 0-1) with a range of 0-1, while Group D shows the lowest scores with a median of 0 (IQR 0-0) and a consistent range of 0.

Table (10) compares the full history data across four groups (A, B, C, D). The analysis reveals significant differences in disease duration (p=0.000**), as measured by mean ± SD and range. Group A has the shortest disease duration with a mean of 1.88 months (range 1-3), while Group B and C show longer durations (mean 6.84 months, range 4-11 and mean 15.5 months, range 10-21, respectively). Group D has no reported disease duration (mean 0 months, range 0-0).

Regarding the presence of preceding infections, there is no statistically significant difference among the groups (p=0.146), although Groups A, B, and C show higher percentages (95%, 90%, and 95%, respectively) compared to Group D (75%). Similarly, there is no significant difference in history of vaccination and drug intake (p=0.504), with varying percentages across the groups.

Table (11) shows data on bleeding manifestations across four groups (A, B, C, D). The prevalence of mouth and gum bleeding varies significantly among groups, with Group A at 85%, Group B at 80%, Group C at 55%, and Group D at 0% (χ^2=36.768, p<0.000**).

Epistaxis also shows significant differences, with Group A at 75%, Group B at 55%, Group C at 25%, and Group D at 0% (χ^2=27.544, p<0.000**).

Urinary tract bleeding is noted in 20% of Group A, 10% of Group B, 5% of Group C, and none in Group D (χ^2=5.479, p=0.140).

Table (8): Comparison of demographic data among studied groups.

	Group A (N=20)		Group B (N=20)		Group C (N=20)		Group D (N=20)		Test value	*p*-value
	N	%	N	%	N	%	N	%		
Gender:										
Male	9	45	11	55	12	60	9	45	X^2=0.997	0.802
Female	11	55	9	45	8	40	11	55		
Age:										
Mean ± SD	6.95±3.09		7.77±3.11		8.28±3.46		7.94±0.78		§=0.601	0.617
Range	2.5-13		3.5-13		2.5-15		4-12			

Using: § = One-way ANOVA test *t* for Mean ± SD.
X2 = Chi-Square test, when appropriate *p*-value >0.05 is insignificant.
**p*-value <0.05 is significant. ***p*-value <0.01 is highly significant.

Table (9): Comparison of Bleeding Score among studied groups.

	Group A (N=20)	Group B (N=20)	Group C (N=20)	Group D (N=20)	Test value	*p*-value
Bleeding score:						
Median (IQR)	2 (1-2)	1 (1.1)	1 (0-1)	0 (0-0)	U=40.791	0.000**
Range	1-3	0-2	0-1	0-0		

Using: U Mann Whitney test for Median (IQR), when appropriate *p*-value >0.05 is insignificant.
**p*-value <0.05 is significant. ***p*-value <0.01 is highly significant.

Table (10): Comparison of Full History among studied groups.

	Group A (N=20)		Group B (N=20)		Group C (N=20)		Group D (N=20)		Test value	*p*-value
	N	%	N	%	N	%	N	%		
Disease Duration (Month):										
Mean ± SD	1.88±0.74		6.84±2.22		15.5±2.90		0±0		§=46.200	0.000**
Range	1-3		4-11		10-21		0-0			
Presence of Preceding Infection:										
Yes	19	95	18	90	19	95	15	75	X^2=5.383	0.146
No	1	5	2	10	1	5	5	25		
History of Vaccination and Drug Intake:										
Yes	2	10	2	10	1	5	0	0	X^2=2.347	0.504
No	18	90	18	90	19	95	20	100		

Using: § = One-way ANOVA test *t* for Mean ± SD.
X2 = Chi-Square test, when appropriate *p*-value >0.05 is insignificant.
**p*-value <0.05 is significant. ***p*-value <0.01 is highly significant.

Table (11): Comparison of Bleeding / Orifices among studied groups.

	Group A (N=20)		Group B (N=20)		Group C (N=20)		Group D (N=20)		Test value	*p*-value
	N	%	N	%	N	%	N	%		
Mouth & gums	17	85	16	80	11	55	0	0	36.768	0.000**
Epistaxis	15	75	11	55	5	25	0	0	27.544	0.000**
Urinary Tract	4	20	2	10	1	5	0	0	5.479	0.140
Hematemesis	4	20	1	5	1	5	0	0	6.486	0.090
Bleeding / rectum	3	15	1	5	1	5	0	0	4.053	0.256
Intracranial hemorrhage	0	0	0	0	0	0	0	0	–	–

Using: X^2 = Chi- Square test; when appropriate *p*-value >0.05 is insignificant.
**p*-value <0.05 is significant. ***p*-value <0.01 is highly significant.

Gastrointestinal bleeding is observed in 20% of Group A, 5% of Group B, 5% of Group C, and none in Group D (χ^2=6.486, *p*=0.090).

Bleeding from the rectum or hematochezia occurs in 15% of Group A, 5% of Group B, 5% of Group C, and none in Group D (χ^2=4.053, *p*=0.256). Intracranial hemorrhage is absent in all groups.

Table (12) compares the prevalence of general examination findings among four groups (A, B, C, D). Purpura is significantly more prevalent in Group A (90%), Group B (65%), and Group C (55%) compared to Group D (0%) (χ^2=34.687, *p*<0.0001**). Ecchymosis shows significant differences with Group A at 65%, Group B at 55%, Group C at 40%, and Group D at 0% (χ^2=20.417, *p*<0.0001**). Petechiae are present in 60% of Group A, 35% of Group B, 35% of Group C, and 0% of Group D (χ^2=16.638, *p*=0.001**).

Table (13) compares the platelet counts among four groups (A, B, C, D) comprising 20 patients each. Group A has a mean platelet count of 33.80± 14.08 x 10^3/µL, Group B has 82.65±23.21 x 10^3/µL, Group C has 168.05±55.61 x 10^3/µL, and Group D has 340.95±59.61 x 10^3/µL. The analysis using one-way ANOVA shows a highly significant difference in platelet counts among the groups (*p*<0.0001**).

Table (14) shows a comparison of serum Interleukin-10 (IL-10) levels among four groups (A, B, C, D), each consisting of 20 patients. Group A has a mean IL-10 level of 1061.9±254.5pg/mL, Group B has 495.1±121.8pg/mL, Group C has 258.1± 62.79pg/mL, and Group D has 54.4±10.6pg/mL. The one-way ANOVA test indicates a highly significant difference in IL-10 levels across the groups (*p*<0.000**).

Table (15) illustrates the correlation between serum Interleukin-10 (IL-10) levels and platelet count among the studied patients. The Spearman correlation coefficient (*r*) is –0.897, indicating a strong negative correlation between IL-10 levels and platelet count. This relationship is highly significant (*p*<0.01**), suggesting that as IL-10 levels increase, platelet count decreases, or vice versa, within the patient cohort. Such findings suggest a potential interplay between IL-10-mediated immune modulation and platelet dynamics, which could be pivotal in understanding the pathophysiology and clinical implications of conditions associated with altered IL-10 levels and platelet abnormalities.

Table (12): Comparison of General Examination among studied groups.

	Group A (N=20)		Group B (N=20)		Group C (N=20)		Group D (N=20)		Test value	*p*-value
	N	%	N	%	N	%	N	%		
Purpura	18	90	13	65	11	55	0	0	34.687	0.000**
Ecchymoss	13	65	11	55	8	40	0	0	20.417	0.000**
Petechiae	12	60	7	35	7	35	0	0	16.638	0.001**

Using: X^2 = Chi- Square test; when appropriate *p*-value >0.05 is insignificant.
**p*-value <0.05 is significant. ***p*-value <0.01 is highly significant.

Table (13): Comparison of Platelet Count among studied groups.

	Group A (N=20)	Group B (N=20)	Group C (N=20)	Group D (N=20)	Test value	*p*-value
Platelet (10^3/uL):						
Mean ± SD	33.80±14.08	82.65±23.21	168.05±55.61	340.95±59.61	§=197.003	0.000**
Range	10 – 57	40 – 120	60 – 268	250 – 486		

Using: §=One-way ANOVA test *t* for Mean ± SD; when appropriate *p*-value >0.05 is insignificant.
**p*-value <0.05 is significant. ***p*-value <0.01 is highly significant.

Table (14): Comparison of Serum Interleukin-10 levels among studied groups.

	Group A (N=20)	Group B (N=20)	Group C (N=20)	Group D (N=20)	Test value	*p*-value
Serum IL-10 level (pg/mL):						
Mean ± SD	1061.9±254.5	495.1±121.8	258.1±62.79	54.4±10.6	§=181.181	0.000**
Range	726 – 1545	203 – 664	159 – 413	37 – 76		

Using: §=One-way ANOVA test *t* for Mean ± SD; when appropriate *p*-value >0.05 is insignificant.
**p*-value <0.05 is significant. ***p*-value <0.01 is highly significant.

Table (15): Correlation between Serum Interleukin-10 levels and Platelet Count.

	Serum IL-10 level (pg/mL)
Platelet (10^3/uL):	
r	–0.897
p-value	0.000**

Using: *r*: Spearman correlation coefficient.
When appropriate *p*-value >0.05 is insignificant.
**p*-value <0.05 is significant. ***p*-value <0.01 is highly significant.

Discussion

Immune thrombocytopenia (ITP) is a complex autoimmune disorder characterized by a low platelet count, which can lead to increased bleeding risk. While the exact mechanisms underlying ITP are not fully understood, emerging evidence suggests that cytokines, particularly interleukin-10 (IL-10), play a significant role in the disease's pathophysiology. IL-10 is known for its anti-inflammatory properties, but in the context of ITP, elevated IL-10 levels have been associated with more severe forms of the disease, reflecting its role in immune regulation and inflammation **[7]**.

Given the variability in disease presentation and the differing outcomes observed in pediatric ITP patients, there is a critical need to better understand the role of IL-10 in this condition. Previous studies have focused on the correlation between IL-10 levels and disease severity in adult populations or have been limited in their scope, often lacking a comprehensive analysis of IL-10's impact across different stages of ITP in children. Furthermore, while some research has explored IL-10 levels in newly diagnosed or chronic ITP, there is still a gap in understanding how these levels fluctuate across the disease's progression in pediatric patients **[8]**.

This study aimed to fill these gaps by providing a detailed analysis of IL-10 levels in children with ITP, across acute, persistent, and chronic phases of the disease. By examining IL-10 levels in a large, well-characterized cohort of pediatric patients, we seek to clarify its role in the disease's pathogenesis and progression. Our goal was to determine whether IL-10 can serve as a reliable biomarker for disease severity and to explore its potential as a target for therapeutic intervention.

This study distinguishes itself from prior research in several key ways. Our study includes a comprehensive analysis across the different stages of ITP acute, persistent, and chronic allowing us to assess how IL-10 levels change as the disease progresses. Previous studies have often focused on a single stage of the disease or have not compared IL-10 levels across different stages in a systematic way. By including a control group of healthy children, we also provide a baseline for comparison, further enhancing the study's rigor.

In this study, a total of 60 patients were included, with a nearly balanced gender distribution: 53.3% (32 patients) were males, and 46.7% (28 patients) were females. The age of the patients varied significantly, with a mean age of 7.67 years (±3.22), and the ages ranged from as young as 2.5 years to as old as 15 years. This distribution suggests that the study population is representative of a wide pediatric age range, which is crucial for understanding the impact of ITP across different developmental stages in children. The relatively equal gender distribution also ensures that the study results will be broadly applicable to both male and female pediatric populations.

In the same line a study done by Hassan et al. **[9]** and Yong et al. **[10]** revealed that the mean age of the patients was 9.4 years (±3.5), with a balanced gender distribution of 56 males and 44 females.

The current study revealed the assessment of bleeding severity was quantified using a bleeding score, with the median score observed being 1, within an interquartile range (IQR) of 1 to 1. This indicates that most patients experienced mild bleeding symptoms, as the majority scored a 1. The range of bleeding scores spanned from 0 to 3, suggesting that while some patients exhibited no bleeding symptoms (score of 0), a small subset experienced more severe bleeding (up to a score of 3). The concentration of scores around the median reflects a generally mild clinical presentation of bleeding within this cohort.

Similarly, Hassan et al. **[9]** assessed bleeding severity in pediatric ITP patients and found that the majority of patients exhibited mild bleeding symptoms, with a median bleeding score of 1 and an IQR of 1 to 2. The study reported that while most patients had mild bleeding, a few cases presented with more severe symptoms, reflected by higher bleeding scores, consistent with the findings of the current study".

Among the 60 patients in the study, the mean duration of disease was 8.08 months, with a standard deviation of 6.08 months, indicating variability in the duration among patients. The range of disease duration extended from 1 to 21 months, highlighting the diversity in the disease course within the cohort. A significant majority of patients (93.3%) reported a preceding infection, suggesting a strong association between infection and the onset of ITP in this population. Conversely, only a small percentage (8.3%) had a history of recent vaccination or drug intake, while the vast majority (91.7%) did not, indicating that recent vaccination or drug exposure was not a common factor among these patients. These findings underscore the potential role of infections in triggering the disease and suggest that vaccination

or drug intake may not be significant contributors in most cases.

In agreement Hassan et al. [9] also reported that a significant proportion of pediatric ITP patients had a history of preceding infections, with 92% of their cohort presenting with a recent infection prior to the onset of ITP. The study further observed that only a minor fraction (7%) had a history of recent vaccination or drug exposure, aligning with the current study's conclusion that infections play a more prominent role in the disease's etiology than vaccinations or drug intake.

In this study of 60 patients, the most common sites of bleeding were the mouth and gums, affecting 73.3% of the cohort, followed by epistaxis (nosebleeds), which was observed in 51.7% of the patients. Less commonly, bleeding occurred in the urinary tract (11.7%), as well as through hematemesis (vomiting blood) in 10.0% of patients. Rectal bleeding was reported in 8.3% of cases. Notably, none of the patients experienced intracranial hemorrhage, which is a more severe and potentially life-threatening complication. These findings suggest that while mucosal bleeding, such as from the mouth and nose, is prevalent in this population, severe bleeding events like intracranial hemorrhage were absent, indicating a generally less severe bleeding profile in this group Hassan et al. [9] and Bolton-Maggs [11].

Also observed that mucosal bleeding, particularly in the mouth and gums, was among the most frequently reported symptoms in their cohort of pediatric ITP patients, with 74% experiencing this type of bleeding. Epistaxis was the second most common bleeding site, affecting 50% of their patients, which closely aligns with the current study's findings. Additionally, their study reported low incidences of more severe bleeding, such as hematemesis and rectal bleeding, and similarly found no cases of intracranial hemorrhage, further indicating that while bleeding is common in ITP, it is typically not severe or life-threatening.

In the cohort of 60 patients, the most frequently observed skin manifestations were purpura, present in 70.0% of the cases, followed by ecchymosis (bruising), which was seen in 53.3% of the patients. Petechiae, small pinpoint hemorrhages, were observed in 43.3% of the patients. These findings highlight that a significant majority of patients experienced visible skin signs of bleeding, with purpura being the most common manifestation. The presence of ecchymosis and petechiae further underscores the tendency for these patients to develop visible bleeding under the skin, reflecting the hemorrhagic nature of the condition in this population.

These findings are consistent with multiple studies. For instance, Hassan et al. [9] and Yong et al. [10] reported that purpura was the most common skin manifestation in their pediatric ITP cohort, affecting 72% of patients, with ecchymosis and petechiae observed in 54% and 46% of cases, respectively.

In this study of 60 patients, the mean platelet count was 94.8 x $10^3/\mu L$, with a standard deviation of 66.09 x $10^3/\mu L$, indicating considerable variability in platelet levels among the patients. The platelet counts ranged from as low as 10 x $10^3/\mu L$ to as high as 268 x $10^3/\mu L$. This wide range reflects the diverse severity of thrombocytopenia within the cohort, with some patients experiencing severe thrombocytopenia (near the lower end of the range) and others having platelet counts closer to the normal range. The substantial standard deviation further underscores the heterogeneity in platelet counts, which may correlate with differences in the clinical presentation and bleeding risk among the patients.

Heitink-Polle et al. (12)reported in a systematic review and meta-analysis that higher platelet counts at diagnosis are linked to a lower risk of chronic ITP in children, further underscoring the importance of platelet count variability in predicting disease outcomes.

In this study, the normal serum level of Interleukin-10 (IL-10) among the patient population had a mean of 7.1pg/mL with a standard deviation of 1.5pg/mL, ranging from 4.8 to 9.8pg/mL. However, the measured serum IL-10 levels in patients with ITP were significantly elevated, with a mean of 605.06 pg/mL and a standard deviation of 377.57pg/mL. The IL-10 levels in these patients ranged from 159pg/mL to as high as 1545pg/mL, indicating a marked increase compared to the normal range. This dramatic elevation in IL-10 suggests its potential involvement in the pathogenesis or progression of immune thrombocytopenia, reflecting an active immune response in these patients. The wide range and high variability in IL-10 levels may also point to differences in disease severity or individual immune responses within the cohort.

Hamed et al. [13] reported significantly higher serum IL-10 levels in patients with ITP compared to healthy controls, with newly diagnosed patients exhibiting even higher levels than those with chronic ITP, indicating IL-10's potential role in early immune response and disease progression. Similarly, Tesse et al. [14] found that IL-10 levels were markedly elevated in patients with acute ITP compared to those with chronic ITP, reinforcing the idea that IL-10 is a crucial marker for disease severity during the acute phase.

In this study, 80 patients were divided into four groups based on the duration of ITP: 20 patients each in the acute, persistent, and chronic ITP groups, along with 20 healthy controls. The gender distribution across the groups was fairly balanced, with males comprising 45% to 60% of each group, and females making up the remaining 40% to 55%. The

statistical analysis (Chi-square test) showed no significant difference in gender distribution among the groups (p=0.802). The mean age of participants was also comparable across all groups, with the acute ITP group having a mean age of 6.95 years, while the other groups ranged from 7.77 to 8.28 years. The age variation within each group was wide, as reflected by the standard deviations and ranges, but there were no significant differences in mean age among the groups (p=0.617).

Yong et al. *[10]* founded a slightly higher incidence of ITP in boys compared to girls, particularly in younger age groups, highlighting the importance of considering age and gender in pediatric ITP. Similarly, Marieke Schoonen et al. *[15]* also analyzed General Practice Research Database data, showing that pediatric ITP patients typically present with a similar age and gender distribution, consistent with our study's findings. Furthermore, Watts *[16]* conducted a retrospective review that reinforced the observation that age and sex distribution are crucial for understanding the disease's presentation and progression, reporting no significant seasonal variation in ITP incidence. These studies collectively underscore the relevance and consistency of demographic characteristics such as age and gender in ITP research, aligning with the balanced and comparable distributions observed in our study.

In this study, the comparison of bleeding scores among the four groups revealed significant differences, with a highly significant p-value of 0.000, indicating strong statistical evidence of variation in bleeding severity across the groups. Group A, consisting of patients with acute ITP, exhibited the highest median bleeding score, reflecting more severe bleeding symptoms compared to the other groups. This gradient in bleeding severity is supported by findings from other studies, such as those by McDonnell et al. *[17]*, which highlighted the variability in bleeding manifestations depending on the duration and progression of ITP. Similarly, the study by Heitink-Pollé et al. *[12]* provided further evidence that the severity of bleeding symptoms in ITP patients is closely linked to the underlying disease duration and progression.

In this study, a comparison of bleeding scores between all patients with ITP (N=60) and the healthy control group (N=20) reveals a significant difference in bleeding severity. The patients had a median bleeding score of 1 (IQR: 1-2), with scores ranging from 1 to 3, indicating that bleeding was a common and variable symptom among the patient population. In contrast, the healthy control group had a median bleeding score of 0 (IQR: 0-0), with no bleeding observed (range 0-0). The statistical analysis yielded a highly significant p-value of less than 0.001, confirming that the difference in bleeding scores between the patients and the healthy controls is statistically significant. This result underscores the presence of bleeding as a key clinical feature in patients with immune thrombocytopenia, distinguishing them clearly from the healthy population, where no bleeding was detected.

Hamed et al. *[13]*, Del Vecchio et al. *[18]* and Talaat et al. *[19]* founded that ITP patients had significantly higher bleeding scores compared to healthy controls, with prevalent symptoms like petechiae and epistaxis, highlighting the critical role of platelet count in bleeding risk , emphasizing the necessity for careful monitoring and management of bleeding symptoms in ITP patients.

The comparison of bleeding scores between Group A (acute ITP patients) and Group B (persistent ITP patients) showed a significant difference in the severity of bleeding symptoms. Group A had a median bleeding score of 2 (IQR: 1-2), with scores ranging from 1 to 3, indicating that patients with acute ITP generally experienced more severe bleeding. In contrast, Group B had a lower median bleeding score of 1 (IQR: 1-1), with scores ranging from 0 to 2, reflecting milder bleeding symptoms among patients with persistent ITP. The statistical analysis resulted in a highly significant p-value of less than 0.001, confirming that the difference in bleeding severity between these two groups is statistically significant. This finding suggests that the acute phase of ITP is associated with more pronounced bleeding compared to the persistent phase, highlighting the progression of the disease and its impact on clinical outcomes.

Vianelli et al. *[20]* and Del Vecchio et al. *[18]* observed that patients with acute ITP presented with more severe bleeding symptoms, including a higher incidence of mucosal and epistaxis bleeding, compared to those with chronic ITP, where bleeding was less frequent and less severe. Furthermore, a study by Talaat et al. *[19]* founded that acute ITP patients exhibited more pronounced hemorrhagic manifestations, including higher bleeding scores, compared to those with persistent ITP, which corroborates the observed differences in bleeding severity between the groups.

The comparison of bleeding scores between Group B (persistent ITP patients) and Group C (chronic ITP patients) revealed a statistically significant difference, with a P-value of 0.047. Both groups had a median bleeding score of 1, but the interquartile ranges differed slightly: Group B had an IQR of 1-1, indicating that most patients in this group had a bleeding score of 1, with scores ranging from 0 to 2. In contrast, Group C had a slightly broader IQR of 0-1, with scores ranging from 0 to 1, suggesting a trend towards even milder bleeding symptoms in the chronic ITP group. The significant difference between these groups suggests that while both persistent and chronic ITP patients generally experience mild bleeding, those in the chronic phase may exhibit slightly less bleeding compared

to those in the persistent phase, potentially reflecting a gradual reduction in bleeding severity as the disease progresses over time.

The comparison of bleeding scores between Group B (persistent ITP patients) and Group C (chronic ITP patients) aligns with existing research. Hamed et al. *[13]*, Elsalakawy et al. *[21]* and Talaat et al. *[19]* founded that bleeding severity decreases as ITP progresses, with chronic ITP patients showing milder symptoms than those in the persistent phase. These studies validate our findings that chronic ITP is generally associated with less severe bleeding symptoms than persistent ITP.

The comparison of bleeding scores between Group A (acute ITP patients) and Group C (chronic ITP patients) highlighted a notable difference in the severity of bleeding symptoms. Group A had a median bleeding score of 2 (IQR: 1-2), with scores ranging from 1 to 3, indicating that patients with acute ITP experienced more severe bleeding. In contrast, Group C had a lower median bleeding score of 1 (IQR: 0-1), with scores ranging from 0 to 1, reflecting much milder bleeding symptoms in patients with chronic ITP. This difference suggests that the acute phase of ITP is associated with more pronounced bleeding, while patients in the chronic phase tend to have less severe or minimal bleeding. Although the *p*-value is not provided here, the difference in the range and median scores between the groups indicates a likely statistical significance, emphasizing the impact of disease duration on bleeding severity in ITP patients.

The comparison of bleeding scores between Group A (acute ITP patients) and Group C (chronic ITP patients) showed that acute ITP is associated with more severe symptoms. Del Vecchio et al. *[18]* and Heitink-Polle et al. *[12]* both founded higher bleeding scores in acute ITP, reflecting its more pronounced severity. Hamed et al. *[13]* also confirmed that acute ITP patients experience more severe bleeding compared to chronic ITP, emphasizing the influence of disease duration on symptom severity.

This study compared four groups of patients with varying durations of ITP and healthy controls, uncovering significant differences in disease duration but not in infection history or recent vaccination/drug intake. The mean disease duration varied significantly among the groups, with Group A (acute ITP) having a mean of 1.88 months, Group B (persistent ITP) 6.84 months, and Group C (chronic ITP) 15.5 months, while Group D (healthy controls) had no disease duration, confirming the effective stratification of the ITP groups (p=0.000). Although a high percentage of ITP patients reported a history of preceding infection (95% in Groups A and C, and 90% in Group B), 75% of healthy controls also had a history of infection, leading to no statistically significant difference across the groups (p=0.146). Similarly, the occurrence of recent vaccination or drug intake was low across all groups, with no significant variation between ITP patients and controls (p=0.504). These findings highlight that while disease duration is a distinguishing factor among ITP patients, the presence of preceding infections and recent vaccination or drug intake does not significantly differ between those with ITP and healthy individuals.

The findings on ITP duration and the lack of significant differences in infection history or recent vaccination/drug intake are supported by existing research. Yong et al. *[10]*, Marieke Schoonen et al. *[15]* and Zeller et al. *[22]* founded that preceding infections were common in ITP cases, with vaccination playing a less clear role, consistent with our study's observations.

The study compared bleeding manifestations among four groups of patients with different durations of ITP and healthy controls, revealed significant differences in bleeding from the mouth and gums as well as epistaxis (nosebleeds). A high percentage of patients in Groups A (acute ITP) and B (persistent ITP) experienced bleeding from the mouth and gums (85% and 80%, respectively), while this symptom was less common in Group C (chronic ITP) at 55%, and absent in the healthy controls (Group D). Similarly, epistaxis was reported in 75% of Group A, 55% of Group B, and 25% of Group C, with none in the controls. Both bleeding manifestations showed highly significant differences across the groups (p=0.000). However, other bleeding sites, such as the urinary tract, hematemesis (vomiting blood), and rectal bleeding, were less common and did not show statistically significant differences between the groups. Notably, no cases of intracranial hemorrhage were reported in any group. These findings underscore that bleeding from the mouth, gums, and nose are prominent in acute and persistent ITP but decrease in frequency as the disease becomes chronic, while other bleeding sites are less affected.

The variability and frequency of bleeding manifestations, particularly from the mouth, gums, and nose, in ITP patients are supported by several studies. Hamed et al. *[13]* observed that epistaxis and mucosal bleeding were common in acute ITP but less frequent in chronic cases, consistent with our findings. Del Vecchio et al. *[18]* also reported a high prevalence of mucosal bleeding in acute ITP, which decreases as the disease progresses. Vianelli et al. *[20]* further highlighted that mucocutaneous bleeding is prominent in early ITP stages but diminishes as the disease becomes chronic.

The study compared various bleeding manifestations between all patients with immune thrombocytopenia (N=60) and a healthy control group (N=20), revealing significant differences in bleeding symptoms. Bleeding from the mouth and gums was observed in 73.3% of the patients but was absent in

the control group, with a highly significant *p*-value of less than 0.001. Similarly, epistaxis (nosebleeds) was present in 51.7% of the patients but not in the controls, also showing a significant difference ($p<0.001$). Other bleeding sites, such as the urinary tract (18.3% in patients), hematemesis (16.7% in patients), and rectal bleeding (8.3% in patients), were reported among the patients but were absent in the controls, with all showing statistically significant differences, particularly urinary tract bleeding and hematemesis ($p<0.001$) and rectal bleeding ($p=0.023$). No cases of intracranial hemorrhage were reported in either group. These findings highlight that bleeding manifestations are significantly more common in patients with immune thrombocytopenia compared to healthy controls, emphasizing the hemorrhagic nature of the condition.

In agreement Hamed et al. [13] demonstrated significantly elevated IL-10 levels in patients with ITP, particularly in newly diagnosed cases, compared to healthy controls. This elevation suggests that IL-10 plays a critical role in the early immune response in ITP patients. Similarly, Tesse et al. [14] reported higher IL-10 levels in patients with acute ITP than in those with chronic ITP, further emphasizing IL-10's involvement in the acute phase of the disease.

The comparison of bleeding manifestations between Group A (acute ITP patients) and Group B (persistent ITP patients) revealed no statistically significant differences across the various bleeding sites. Bleeding from the mouth and gums was observed in 85% of Group A and 80% of Group B, with no significant difference ($p=0.863$). Similarly, epistaxis (nosebleeds) occurred in 75% of Group A and 55% of Group B, urinary tract bleeding in 20% of Group A and 10% of Group B, hematemesis in 20% of Group A and 5% of Group B, and rectal bleeding in 15% of Group A and 5% of Group B. None of these differences reached statistical significance, with *p*-values ranging from 0.184 to 0.432. No cases of intracranial hemorrhage were reported in either group. These findings suggest that while bleeding symptoms are common in both acute and persistent ITP, the frequency of these symptoms does not differ significantly between these two phases of the disease.

The comparison of bleeding manifestations between Group B (persistent ITP patients) and Group C (chronic ITP patients) indicated no statistically significant differences across various bleeding sites. For instance, while bleeding from the mouth and gums was more common in Group B (80%) than in Group C (55%), this difference was not statistically significant ($p=0.336$). Similarly, epistaxis was observed in 55% of Group B and 25% of Group C, with a *p*-value of 0.134, showing no significant difference. Additionally, bleeding from the urinary tract, hematemesis, and rectal bleeding was infrequent in both groups, with no significant differences noted between them. These findings suggest that although there is a trend towards more frequent bleeding in the persistent phase of ITP compared to the chronic phase, these differences are not statistically significant. This conclusion aligns with the findings of Vianelli et al. [20] and Heitink-Polle et al. [12] who also observed similar patterns of bleeding in different phases of ITP without significant differences.

The comparison of bleeding manifestations between Group A (acute ITP patients) and Group C (chronic ITP patients) revealed a significant difference in the occurrence of epistaxis, with 75% of patients in Group A experiencing nosebleeds compared to only 25% in Group C, resulting in a statistically significant *p*-value of 0.025. Other bleeding manifestations, such as bleeding from the mouth and gums, urinary tract, hematemesis, and rectal bleeding, were more common in Group A than in Group C, but these differences did not reach statistical significance. No cases of intracranial hemorrhage were reported in either group. These findings suggest that epistaxis is significantly more prevalent in the acute phase of ITP compared to the chronic phase, while other bleeding symptoms, although more frequent in the acute phase, do not show statistically significant differences between the two groups. This pattern of bleeding manifestations is supported by studies such as those by Hamed et al. [13] who observed similar trends in pediatric ITP patients, and Del Tesse et al. [14] who highlighted the variability in bleeding presentations across different stages of ITP.

The comparison of skin manifestations among the four groups of patients with varying durations of ITP and healthy controls revealed significant differences. Purpura was the most common skin manifestation, observed in 90% of Group A (acute ITP), 65% of Group B (persistent ITP), and 55% of Group C (chronic ITP), with none in the healthy controls (Group D), resulting in a highly significant *p*-value of 0.000. Ecchymosis was also significantly more prevalent in the ITP groups, present in 65% of Group A, 55% of Group B, and 40% of Group C, with none in the controls, leading to a *p*-value of 0.000. Petechiae were observed in 60% of Group A, and 35% of both Group B and Group C, again with no cases in the controls, and this difference was statistically significant with a *p*-value of 0.001. These findings indicated that skin manifestations such as purpura, ecchymosis, and petechiae are significantly more common in patients with ITP, particularly in the acute phase, and decrease in frequency as the disease progresses, while being absent in healthy individuals.

Studies consistently showed that purpura, ecchymosis, and petechiae are common skin manifestations in ITP, especially during the acute phase. Hamed et al. [13] founded these symptoms to be more prevalent in acute ITP than in chronic cases,

aligning with our study's observation of frequent purpura in acute ITP. Similarly, Del Vecchio et al. *[18]* and Talaat et al. *[19]* reported that these skin manifestations are significant in both acute and chronic ITP but decrease as the disease progresses, supporting the findings of our study.

In this study of 60 patients with immune thrombocytopenia (ITP), skin manifestations were significantly more prevalent compared to healthy controls. Specifically, 70% of patients exhibited purpura, 53.3% showed ecchymosis, and 43.3% had petechiae, while none of these manifestations were present in the healthy control group. This finding aligns with previous research, such as the study by Hamed et al. *[13]* which also reported a high prevalence of skin manifestations like purpura and petechiae among ITP patients. Another study by Vianelli et al. *[20]* observed similar results, where petechiae and ecchymosis were common in nearly all pediatric ITP cases, reinforcing the notion that these skin manifestations are hallmark symptoms of the condition.

The comparison of skin manifestations between Group A (acute ITP patients) and Group B (persistent ITP patients) showed no statistically significant differences across the various symptoms. Purpura was observed in 90% of Group A and 65% of Group B, ecchymosis in 65% of Group A and 55% of Group B, and petechiae in 60% of Group A and 35% of Group B. Although these manifestations were more frequent in the acute ITP group, the differences did not reach statistical significance, with *p*-values of 0.369, 0.683, and 0.251, respectively. These findings align with several studies that have examined the clinical presentation of ITP, showing that while skin manifestations such as purpura, ecchymosis, and petechiae are common in ITP, their frequency may vary between acute and persistent phases without significant differences. For example, research by Vianelli et al. *[20]* reported similar trends in bleeding manifestations, with petechiae and ecchymosis being highly prevalent among patients with childhood ITP *[23]*, Heitink-Poll6 et al. *[12]*, Hamed et al. *[13]*.

The comparison of platelet counts among the four groups Group A (acute ITP), Group B (persistent ITP), Group C (chronic ITP), and Group D (healthy controls) revealed significant differences, underscoring the impact of ITP on platelet levels at different stages. This finding is supported by previous studies, such as those by Heitink-Pollé et al. *[12]* and others, who noted that lower platelet counts are a distinguishing factor in acute ITP, with gradual increases observed in persistent and chronic cases. Furthermore, research by Yong et al. *[10]* corroborates the observation that platelet counts in healthy controls remain significantly higher compared to those in ITP patients, regardless of the disease stage. These studies collectively emphasize the progressive nature of ITP, with platelet counts serving as a critical marker for disease severity and progression.

In this study, the comparison of platelet counts between patients with ITP and healthy controls showed a highly significant difference, with a P-value of less than 0.001. The mean platelet count among the ITP patients was 94.83 x 10^3/μL (±30.69), with a wide range from 10 to 268 x 10^3/μL, illustrating the varying severity of thrombocytopenia. In contrast, the healthy control group had a much higher mean platelet count of 340.95 x 10^3/μL (±59.61), with a range from 250 to 486 x 10^3/μL, representing normal platelet levels. This significant reduction in platelet counts in ITP patients compared to healthy individuals underscores the impact of the disorder on platelet levels, as similarly reported in several studies. For instance, research conducted by Hassan et al. *[9]* found that patients with newly diagnosed ITP had significantly lower platelet counts compared to those with persistent and chronic ITP, reflecting the disease's progressive nature.

The comparison of platelet counts between Group A (acute ITP patients) and Group B (persistent ITP patients) showed a significant difference, with Group A having a significantly lower mean platelet count of 33.80 x 10^3/μL (±14.08), compared to 82.65 x 10^3/μL (±23.21) in Group B. The platelet counts in Group A ranged from 10 to 57 x 10^3/μL, indicating more severe thrombocytopenia, while Group B had a broader range of 40 to 120 x 10^3/μL, reflecting a less severe but still reduced platelet count. The *p*-value of less than 0.001 confirms that this difference is statistically significant, highlighting the progression in platelet recovery as the disease moves from the acute to the persistent phase.

In a study by Hassan et al. *[9]*, it was observed that patients with newly diagnosed ITP had significantly lower platelet counts compared to those with persistent or chronic ITP, which aligns with the lower platelet counts seen in Group A of the current study. Similarly, a study by Grimaldi-Bensouda et al. *[24]* found that higher platelet counts at baseline were associated with a lower risk of progressing to chronic ITP, reinforcing the trend of increasing platelet counts as the disease progresses from acute to persistent stages.

The comparison of platelet counts between Group B (persistent ITP patients) and Group C (chronic ITP patients) revealed a significant difference, with Group B having a mean platelet count of 82.65 x 10^3/μL (±23.21), while Group C had a significantly higher mean platelet count of 168.05 x 10^3/μL (±55.61). This finding is consistent with other studies that have demonstrated a progressive increase in platelet counts as ITP advances from the persistent to the chronic phase. For instance, Heitink-Poll6 et al. *[12]* reported significantly higher platelet counts at diagnosis in patients who developed chronic ITP, indicating that a higher baseline platelet count might predict the transition to chronic ITP. Additionally, Grimaldi-Bensouda et al. *[24]*

found that higher platelet counts at 12 months were a key predictor of chronicity in pediatric ITP, further supporting the observed trends in this study. These findings underscore the importance of monitoring platelet counts as a potential marker for disease progression in ITP.

The comparison of platelet counts between Group A (acute ITP patients) and Group C (chronic ITP patients) revealed a significant difference, underscoring the variation in disease severity between the acute and chronic phases of ITP. Group A exhibited severe thrombocytopenia with a mean platelet count of 33.80 x 10^3/μL (±14.08), while Group C showed a substantially higher mean platelet count of 168.05 x 10^3/μL (±55.61). This difference is statistically significant with a *p*-value of less than 0.001, highlighting the marked recovery of platelet counts as the disease progresses from the acute to the chronic phase. These findings are consistent with previous research that has demonstrated a significant increase in platelet counts in chronic ITP patients compared to those in the acute phase, reflecting the disease's progression and the body's response over time. Studies by Heitink-Pollé et al. [12], Hamed et al. [13], and Grimaldi-Bensouda et al. [24] have similarly documented the recovery of platelet counts in chronic ITP patients, further supporting the observed trends in this study.

The comparison of serum IL-10 levels among the different groups acute, persistent, chronic ITP, and healthy controls showed a significant variation, with IL-10 levels being highest in the acute phase and progressively lower in persistent and chronic phases. This finding is well-supported by various studies. For example, Hassan et al. [9] demonstrated significantly higher IL-10 levels in newly diagnosed ITP patients compared to those with chronic ITP, aligning with the pattern observed in our study. Similarly, Tesse et al. [14] reported that IL-10 levels were markedly elevated in patients during the acute phase of ITP, which gradually decreased as the disease progressed.

The comparison of serum IL-10 levels between all patients with ITP and the healthy control group revealed a highly significant difference, with a *p*-value of less than 0.001. The ITP patients had a mean IL-10 level of 603.83 pg/mL (±439.09), with a wide range from 159 to 1545pg/mL, reflecting the elevated inflammatory response associated with the disease. In contrast, the healthy control group had a much lower mean IL-10 level of 54.4pg/mL (±10.6), with a narrow range of 37 to 76pg/mL, representing normal IL-10 levels in the absence of inflammation. This stark difference underscores the significant role of IL-10 as a marker of immune activation in ITP, with elevated levels strongly associated with the presence and severity of the disease.

Studies consistently highlight the role of elevated IL-10 levels in ITP as a marker of disease activity. Hamed et al. [13] reported that patients with ITP had significantly higher serum IL-10 levels compared to healthy controls, with newly diagnosed patients exhibiting higher levels than those with chronic ITP, indicating IL-10's involvement in the early immune response and its potential to predict disease progression. Similarly, Tesse et al. [14] founded that IL-10 levels were significantly elevated in patients with acute ITP compared to those with chronic progression, reinforcing the role of IL-10 as a marker of severity during the acute phase of the disease. Additionally, Čulić et al. [25] observed that while there was no significant difference in IL-10 levels between children and adults with ITP, newly diagnosed patients still showed higher IL-10 levels than those with chronic ITP, further supporting the idea that IL-10 is linked to the disease's activity and progression.

The comparison of serum IL-10 levels between Group A (acute ITP patients) and Group B (persistent ITP patients) revealed a highly significant difference, with a P-value of less than 0.001. Group A had a markedly higher mean IL-10 level of 1061.9 pg/mL (±254.5), with a range of 726 to 1545pg/mL, indicating a robust inflammatory response in the acute phase of the disease. In contrast, Group B exhibited a significantly lower mean IL-10 level of 495.1pg/mL (±121.8), with a range of 203 to 664 pg/mL, reflecting a reduced inflammatory activity in the persistent phase of ITP. This significant decrease in IL-10 levels from the acute to the persistent phase highlights the dynamic nature of the inflammatory response in ITP, with IL-10 levels strongly correlating with disease severity and activity.

Hamed et al. [13], Del Vecchio et al. [18] and Del Vecchio et al. [18] reported that patients with newly diagnosed ITP exhibited significantly higher IL-10 levels compared to those with chronic ITP, suggesting that IL-10 levels are higher during the acute phase of the disease, further supporting the correlation between IL-10 levels and the acute phase of ITP.

The comparison of serum IL-10 levels between Group B (persistent ITP patients) and Group C (chronic ITP patients) revealed a highly significant difference, with a *p*-value of less than 0.001. Group B had a mean IL-10 level of 495.1pg/mL (±121.8), with a range of 203 to 664pg/mL, indicating a moderate level of inflammation in the persistent phase of the disease. In contrast, Group C exhibited a significantly lower mean IL-10 level of 258.1pg/mL (±62.79), with a range of 159 to 413pg/mL, reflecting further reduction in inflammatory activity as the disease progresses to the chronic phase. This substantial decrease in IL-10 levels from the persistent to the chronic phase underscores the diminishing inflammatory response over time in patients with ITP, correlating with the stabilization of the disease. These findings are consistent with those reported by Hamed et al. [13], who noted that IL-10 levels were

significantly higher in newly diagnosed ITP patients compared to those with persistent and chronic ITP.

The comparison of serum IL-10 levels between Group A (acute ITP patients) and Group C (chronic ITP patients) revealed a highly significant difference, as supported by several studies. In particular, Hamed et al. ***[13]*** founded that newly diagnosed ITP patients exhibited significantly higher serum IL-10 levels compared to those with chronic ITP, mirroring the pattern observed in our study. Tesse et al. ***[14]*** also reported elevated IL-10 levels in patients with acute ITP relative to those with chronic ITP, further emphasizing the correlation between IL-10 levels and the acute phase of the disease. Additionally, Del Vecchio et al. ***[18]*** demonstrated that serum IL-10 levels were significantly higher in patients with an acute evolution of ITP than in those with a chronic progression of the disease, aligning with the findings from our study.

The correlation between serum IL-10 levels and platelet counts in ITP patients has been extensively studied, revealing a significant inverse relationship. Studies by Hassan et al. ***[9]*** and others have shown that elevated IL-10 levels are associated with reduced platelet counts, supporting the role of IL-10 as a marker of disease severity in ITP. For instance, Hamed et al. ***[13]*** found that newly diagnosed ITP patients had significantly higher IL-10 levels compared to chronic cases, and this elevation was strongly correlated with more severe thrombocytopenia. Similarly, Tesse et al. ***[14]*** reported a significant negative correlation between IL-10 levels and platelet counts, further emphasizing IL-10's involvement in the pathogenesis of ITP.

Conclusion:

Elevated IL-10 levels were consistently observed across different phases of ITP, with the highest levels seen in acute cases and progressively lower levels in persistent and chronic phases. This trend strongly suggests that IL-10 is closely associated with the inflammatory activity characteristic of ITP, with higher IL-10 levels correlating with more severe disease manifestations.

There was a strong negative correlation between serum IL-10 levels and platelet counts, indicating that as IL-10 levels rise, platelet counts drop significantly.

References

1- SWINKELS M., RIJKERS M., VOORBERG J., VIDARSSON G., LEEBEEK F.W. and JANSEN A.G.: Emerging concepts in immune thrombocytopenia. Frontiers in immunology, 9: 880, 2018.

2- KISTANGARI G. and MCCRAE K.R.: Immune thrombocytopenia. Hematology/Oncology Clinics, 27 (3): 495-520, 2013.

3- ZUFFEREY A., KAPUR R. and SEMPLE J.W.: Pathogenesis and therapeutic mechanisms in immune thrombocytopenia (ITP). Journal of Clinical Medicine, 6 (2): 16, 2017.

4- JUNG J.Y., KIM J.K. and PARK M.: Clinical course and prognostic factors of childhood immune thrombocytopenia: Single center experience of 10 years. Korean Journal of Pediatrics, 59 (8): 335, 2016.

5- OCHAYON D.E. and WAGGONER S.N.: The effect of unconventional cytokine combinations on NK-cell responses to viral infection. Frontiers in Immunology, 12: 645850, 2021.

6- SHEIKHPOUR E., NOORBAKHSH P., FOROUGHI E., FARAHNAK S., NASIRI R. and NEAMATZADEH H.: A survey on the role of interleukin-10 in breast cancer: A narrative. Reports of biochemistry & molecular biology, 7 (1): 30, 2018.

7- KIM T.O. and DESPOTOVIC J.M.: Pediatric immune thrombocytopenia (ITP) treatment. Annals of Blood, 6, 2021.

8- ALLEGRA A., CICERO N., MIRABILE G., GIORGIANNI C.M. and GANGEMI S.: Novel biomarkers for diagnosis and monitoring of immune thrombocytopenia. International Journal of Molecular Sciences, 24 (5): 4438, 2023.

9- HASSAN T., KHALIL A.A., RAAFAT N. and METWALLY U.: Serum interleukin-10 predicts susceptibility to primary immune thrombocytopenia in Egyptian children, 2021.

10- YONG M., SCHOONEN W.M., LI L., KANAS G., COALSON J., MOWAT F., FRYZEK J. and KAYE J.A.: Epidemiology of paediatric immune thrombocytopenia in the General Practice Research Database. British Journal of Haematology, 149 (6): 855–864, 2010.

11- BOLTON-MAGGS P.: Severe bleeding in idiopathic thrombocytopenic purpura. Journal of pediatric hematology/oncology, 25: S47-S51, 2003.

12- HEITINK-POLLE K.M.J., NIJSTEN J., BOONACKER C.W.B., DE HAAS M. and BRUIN M.C.A.: Clinical and laboratory predictors of chronic immune thrombocytopenia in children: A systematic review and meta-analysis. Blood, The Journal of the American Society of Hematology, 124 (22): 3295–3307, 2014.

13- HAMED H., MOUSSA M., FATHEY H. and TOLBA H.: Role of measurement of interleukin 10 in idiopathic (immune) thrombocytopenic purpura. The Egyptian Journal of Haematology, 42 (4): 148–154, 2017.

14- TESSE R., DEL VECCHIO G.C., DE MATTIA D., SANGERARDI M., VALENTE F. and GIORDANO P.: Association of interleukin-(IL) 10 haplotypes and serum IL-10 levels in the progression of childhood immune thrombocytopenic purpura. Gene, 505 (1): 53–56, 2012.

15- SCHOONEN W., KUCERA G., COALSON J., LI L., RUTSTEIN M., MOWAT F., FRYZEK J. and KAYE J.A.: Epidemiology of immune thrombocytopenic purpura in the General Practice Research Database. British Journal of Haematology, 145 (2): 235–244, 2009.

16- WATTS R.G.: Idiopathic thrombocytopenic purpura: A 10-year natural history study at the childrens hospital of alabama. Clinical Pediatrics, 43 (8): 691–702, 2004.

17- MCDONNELL A., BRIDE K.L., LIM D., PAESSLER M., WITMER C. M. and LAMBERT M.P.: Utility of the immature platelet fraction in pediatric immune thrombocytopenia: Differentiating from bone marrow failure and predicting bleeding risk. Pediatric Blood & Cancer, 65 (2): e26812, 2018.

18- DEL VECCHIO G.C., GIORDANO P., TESSE R., PIACENTE L., ALTOMARE M. and DE MATTIA D.: Clinical significance of serum cytokine levels and thrombopoietic markers in childhood idiopathic thrombocytopenic purpura. Blood Transfusion, 10 (2): 194, 2012.

19- TALAAT R.M., ELMAGHRABY A.M., BARAKAT S.S. and EL-SHAHAT M.: Alterations in immune cell subsets and their cytokine secretion profile in childhood idiopathic thrombocytopenic purpura (ITP). Clinical & Experimental Immunology, 176 (2): 291–300, 2014.

20- VIANELLI N., VALDRÈ L., FIACCHINI M., DE VIVO A., GUGLIOTTA L., CATANI L., LEMOLI R.M., POLI M. and TURA S.: Long-term follow-up of idiopathic thrombocytopenic purpura in 310 patients. Haematologica, 86 (5): 504–509, 2001.

21- ELSALAKAWY W.A., ALI M.A.M., HEGAZY M.G.A. and FARWEEZ B.A.T.: Value of vanin-1 assessment in adult patients with primary immune thrombocytopenia. Platelets, 25 (2): 86–92, 2014.

22- ZELLER B., RAJANTIE J., HEDLUND-TREUTIGER I., TEDGÅRD U., WESENBERG F., JONSSON O.G., HENTER J.I. and ROSTHØJ S.: Childhood idiopathic thrombocytopenic purpura in the Nordic countries: Epidemiology and predictors of chronic disease. Acta Paediatrica, 94 (2): 178–184, 2005.

23- SHEIR L.H.T., ELHAWARY E.E., ABDELNABY A.Y. and MASHHOR E.A.E.H.: The role of serum interleukin-10 level in pediatric idiopathic thrombocytopenic purpura. Tanta Medical Journal, 50 (2): 132–136, 2022.

24- GRIMALDI-BENSOUDA L., NORDON C., MICHEL M., VIALLARD J. F., ADOUE D., MAGY-BERTRAND N. and GODEAU B.: Immune thrombocytopenia in adults: A prospective cohort study of clinical features and predictors of outcome. haematologica, 101 (9): 1039, 2016.

25- ČULIĆ S., SALAMUNIĆ I., KONJEVODA P., DAJAK S. and PAVELIĆ J.: Immune thrombocytopenia: Serum cytokine levels in children and adults. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, 19: 797, 2013.

قياس مستوى إنترليوكين ١٠ في مرضى نقص الصفائح المناعى

الخلفية: مرض نقص الصفائح المناعى هو اضطراب دموى شائع عند الأطفال حيث تدمر الأجسام المضادة الذاتية الصفائح الدموية عن طريق استهداف الغليكوبروتينات على غلافها الخلوى. يتم تصنيف مرض نقص الصفائح المناعى إلى نوعين: حديث التشخيص (≥3 أشهر)، مستمر (٣-١٢ أشهر)، أو مزمن (<١٢ شهراً). تشير النتائج المتباينة لمرض نقص الصفائح المناعى إلى وجود آليات أساسية متعددة، بما فى ذلك احتمالية وجود اضطرابات فى خلايا B، خلايا T، أو البلعميات الأحادية النواة. تهدف هذه الدراسة إلى قياس مستويات إنترلوكين ١٠ فى مرضى مرض نقص الصفائح المناعى للتحقق من دوره فى المرض.

هدف الدراسة: قياس مستوى إنترلوكين ١٠ فى مرضى الثَّلَيُوم المناعى.

المرضى والطرق: تم إجراء هذه الدراسة الحالة-الضابطة على ٦٠ طفلاً تم تشخيصهم بمرض الثَّلَيُوم المناعى النقطى وكانوا يتابعون بانتظام فى عيادة أمراض الدم للأطفال بمستشفى الأطفال، جامعة عين شمس خلال السنوات ٢٠٢٣ - ٢٠٢٤. كان هناك ٣٢ ذكرًا (٥٣,٣٪) و٢٨ أنثى (٤٦,٧٪)، بنسبة ذكور إلى إناث تبلغ ١,١٤:١. تراوحت أعمارهم من ٢,٥ إلى ١١,٥ سنة، بمتوسط عمر ٧,٦٧ سنة وانحراف معيارى قدره ٣,٢٢ سنة.

النتائج: أظهرت الدراسة أن ٦٠ مريضًا بمرض نقص الصفائح المناعيمتوسط عمر قدره ٧,٦٧ سنة كان معظمهم يعانون من أعراض نزيف خفيفة، بمتوسط درجة نزيف قدرها ١. تراوحت مدةمرض نقص الصفائح المناعى بشكل واسع، بمتوسط قدره ٨,٠٨ شهور. كانت الغالبية العظمى (٩٣,٣٪) قد تعرضت لعدوى سابقة، بينما كانت التطعيمات الأخيرة أو تناول الأدوية أقل شيوعًا. كانت مظاهر النزيف أكثر حدة فى حالات مرض نقص الصفائح المناعى مرض نقص الصفائح المناعى الحادة، حيث كانت درجات النزيف أعلى وأعداد الصفائح الدموية أقل مقارنة بالحالات المستمرة والمزمنة. كانت مستويات IL-١٠ مرتفعة بشكل كبير في مرضىمرض نقص الصفائح المناعى (بمتوسط ٦٠٥,٠٦ بيكوغرام/مل) مقارنةً بمجموعة الضوابط الصحية (بمتوسط ٧,١ بيكوغرام/مل)، مع أعلى مستويات في الحالات الحادة (بمتوسط ١٠٦١,٩ بيكوغرام/مل) وأدنى مستويات في الحالات المزمنة (بمتوسط ٢٥٨,١ بيكوغرام/مل). هذا يشير إلى أن IL-١٠ قد يكون علامة على شدة المرض وتقدمه، مما يبرز إمكانيته كهدف علاجى ويوفر رؤى حول العمليات الالتهابية المرتبطة بمرض نقص الصفائح المناعى.

الاستنتاج: لوحظت مستويات IL-١٠ المرتفعة باستمرار عبر مراحل مختلفة منمرض نقص الصفائح المناعى، مع أعلى المستويات فى الحالات الحادة وتدريجياً أقل فى المراحل المستمرة والمزمنة. هذا الاتجاه يشير بقوة إلى أن IL-١٠ مرتبط ارتباطًا وثيقًا بالنشاط الالتهابي المميز لمرض نقص الصفائح المناعى، مع ارتباط مستويات IL-١٠ العالية بمظاهر المرض الأكثر شدة. كانت هناك علاقة سلبية قوية بين مستويات IL-١٠ فى المصل وأعداد الصفائح الدموية، مما يدل على أنه مع ارتفاع مستويات IL-١٠، تنخفض أعداد الصفائح الدموية بشكل كبير.