MJMR, Vol. 35, No. 3, 2024, pages (66-74)

Open Access
ISSN: 2682-4558

Research Article

Role of leptin in immune thrombocytopenic purpura in children

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DOI: 10.21608/mjmr.2024.310954.1775

Abstract

Purpose of study: Immune thrombocytopenia (ITP) is an acquired autoimmune disorder characterized by a low platelet count (platelet count of less than 100,000/µL) multiple humoral and cellular immune abnormalities resulting in accelerated platelet destruction and suppressed platelets production in ITP. **Basic procedures:** The study was conducted on eighty eight subjects divided into: 40 apparently healthy individuals as a control group (group II) and 48 patients of ITP(group I), they were subdivided into 2 subgroups (ITP children in acute state group Ia) and ITP children in chronic state group Ib). ITP children were diagnosed according to International Working Group (IWG) consensus report leptin was assessed for healthy and diseased subjects by EIA method. **Main findings**: results revealed that patients with ITP showed statistically significant higher Leptin level than control group and ITP **c**hildren in acute state had statistically significant higher Leptin level than ITP children in chronic state. There was a significant negative correlation between leptin and PLT in acute and chronic groups and there was significant correlation between leptin and Solumedrol and IVIG. **Principle conclusion:** The finding of this study showed the higher level of serum leptin in acute onset of ITP emphasize the role of leptin as a good marker for the assessment of ITP. So it can help to guide treatment.

Key words: ITP, Leptin , IWG.

Introduction

ITP is characterized by isolated thrombocytopenia, which refers to a platelet count below 100,000/µl, while maintaining normal levels of white blood cells and hemoglobin. The cause of ITP is still unclear in the majority of patients, but it can be initiated by several environmental variables such as viral infection and immunologic triggers [1].

Immune thrombocytopenic purpura (ITP) is characterized by symptoms and indicators of low platelet count, including bleeding gums, nosebleeds (epistaxis), easy bruising, purpura, and bleeding in the brain (intracranial hemorrhage)[2].

The pathophysiology of ITP is highly intricate and varied. Recent developments in ITP research indicate that the pathophysiology of the disease involves an intricate disruption of the immune system. Multiple studies have demonstrated that anomalies in T lymphocytes, natural killer cells, dendritic cells, cytokines, programmmed cell death, as well as oxidative stress, infection, and medications, are significant factors in the development of ITP [1].

Leptin, an extensive protein consisting of 167 amino acids, was initially characterized as a hormone-like cytokine. Adipose tissue

synthesizes it and it binds to a receptor belonging to the class I cytokine receptor family[3].

Studies have demonstrated that leptin has an impact on both the innate and adaptive immune responses. It serves as a chemical signal that attracts neutrophils, eosinophils, and basophils. Leptin signaling triggers phagocytosis and stimulates the generation of both pro- and anti-inflammatory cytokines by monocytes and macrophages. Leptin has the ability to activate basophils to release type 2 cytokines IL-4 and IL-13, but only in specific circumstances. [4]

An increased concentration of serum leptin has been linked to acute infections and autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and immune thrombocytopenic purpura (ITP). Therefore, it has been previously hypothesized that leptin plays a significant role in the development of ITP and could be a potential target for treatment. [3] .

Aim of the work

The aim of this study was to assess serum level of leptin in immune thrombocytopenic purpura among children and its clinical significance.

Subjects and methods

The study was conducted on eighty-eight cases: 40 apparently healthy individuals (group II) with matched age and sex who served as a control group. Also 48 ITP patients whom diagnosed according to International Working Group (IWG), they were further subdivided into 2 subgroups: group Ia (25 ITP children in acute state who newly diagnosed within three months) and group Ib (23 ITP children in chronic state who Lasting for more than 12 months). Patients were selected from pediatric hematological outpatient clinic at Minia University hospital. During the period from September 2022 to September 2023. The hospital ethics committee approved this study and a written consent was obtained from each patient (Approval number: 637 /2023, Date of approval: 20 February 2023). The patients are usually treated using Solubrid and IVIG drugs. Patients with other causes of thrombocytopenia and patients with diabetes mellitus were excluded from the study. Both patients and control groups were subjected to complete history taking, complete clinical examination and laboratory investigations.

Blood sampling protocol: about 8 ml of venous blood was withdrawn from each participant by using a disposable plastic syringe after disinfection of skin with isopropyl alcohol (70%) swaps, and this sample was divided as follows: (a) 1.8 ml of blood on a tube containing 0.2 ml trisodium citrate for detection of INR (dilution 9:1). (b) 1 ml in EDTA-containing tube for CBC. Then 5 ml of blood was transferred into two plain tubes, each tube was allowed to be clotted for 2 hrs at room temperature and then Centrifuged at 1000xg for 20 min, the expressed serum of first tube was used for determination of renal function tests, liver function tests and viral markers. the remaining serum of the other tube was stored refrigerated at -20°C for assay of leptin.

Methods

CBC was determined by (SYSMEX XN-1000, TAO Medical Incorporation, Japan), prothrombin time, concentration and INR (PT, PC and INR) determined by (Stago Compact CT Coagulation analyzer), renal function tests (urea and creatinine), liver function tests (ALT, AST, Albumin, total bilirubin and direct bilirubin) and random blood glucose determined by (**Selectra PROXL16.8361 ELTECH GROUP Clinical Systems, GERMANY)** and Viral markers (HCV antibody, HBsAg and HIV) determined by (**Cobas e411 Hitachi High -Technologies Corporation, Japan)**. Serum leptin was assessed by **enzyme–linked immunosorbent assay (EIA)**. The kit was supplied by **Bioassay Technology Laboratory (catalog no. DL-LEPHU)**, **China**. The analysis of the data was carried out using the IBM SPSS version 25 statistical package software (IBM; Armonk, New York, USA).

Data was tabulated and presented using various tests: frequency, calculation, and the mean, standard deviation, Chi-square test for qualitative data between control and study (acute and chronic variables). Spearman`s correlation was done for specific markers with others age, routine investigations, and lines of treatment in the control and study variables. A p value is less than 0.05 was considered significant. T-test for quantitative data between control and study variables and also ANOVA test was used to compare between control and study (acute and chronic variables).

Results

The age range in the patient's group was from 1 to 15 years with mean **8.96±3.75**, In control group was from 1 to 15 with mean of **9.80±2.20**. All studied groups showed that disease was more in females with the female percentage being (52%, 55%) in group I and group II respectively. There was no statistically significant difference in age and sex between all groups (as shown in table I).

Table (I): Distribution of patient`s socio-demographic data in the group I and group II (No = 88)

Variables	Group I (patients) (No=48)		Group II(control) (No=40)		P. value
	No		**No**		
Age					
Mean±SD (range)	8.96±3.75(1-15)		9.80±2.20(1-15)		0.216
Sex					
Male	23	48	18	45	0.153
Female	25	52	22	55	

Ecchymosis was the most common clinical presentation in the acute group and chronic group (64 %, 91%) respectively with statistical significance difference with p-value = **0.008*** (as shown in fig .1)

In the acute group, 28% received pulse steroids while in the chronic group it was received by 4 % with statistical significance difference between them with p .value = **0.020*.** also, 20.% of acute group was treated by solumedrol, while 100% of the chronic group was treated by solumedrol with a statistical significance difference between them with p-value =**0.005**,** also in the chronic group 17.4% were treated by revolade while no cases of the acute group treated by revolade with a statistical significance difference with p .value = **0.037***and 39.1% of the chronic group received IVIG but no cases of acute group received IVIG with a statistical significance difference with p-value = **0.001*** (as shown in table II)

Table II: Distribution of Treatment of ITP in both groups group Ia and group Ib (No=48)

	Group Ia (acute)		Group Ib (chronic)		P. value
	No=25	%	No=23	%	
Treatment of ITP					
Pulse Steroid	7	28	1	4	**0.020***
Solubrid	0	0.0	0	0.0	00.0
Solumedrol	5	20	23	100	**0.005****
Ritixumab	1	4	2	8.7	0.551
Folic acid	2	8	0	0.0	0.149
IVIG	0	0.0	9	39.1	**0.001****
Thrombopiotein receptor agonist (revolade)	0	0.0	4	17.4	**0.037***
Platelets transfusion	4	16	4	17.4	1.000
Splenectomy	0	0.0	0	0.0	00.0

The patients group had significantly lower platelet count than the control group (32.71±18.39, 233.45±66.68) respectively with p. value= **0.001****(**as shown in table III), patients with acute onset of ITP had significantly lower platelet count compared to chronic patients (17.83±7.6, 47.58 ±13.09)respectively with p. value =**0.001**** (as shown in fig. 2)

Table (III): Comparison between Group I and Group II related to platelet counts

laboratory Investigation	Group I (Patients)	Group II (control)	P. value
	Mean±SD	Mean±SD	
PLTs ($x10^3$/ µl)	32.71±18.39	233.45±66.68	**0.001****

It was observed that serum leptin level was higher in acute group and chronic group than the control group, also it was higher in the acute group than the chronic group with a statistically significant difference between all groups with p, p_1, p_2 and p_3 values = (0.001**, 0.001**, 0.01* and 0.001**) respectively. (as shown in fig. 3 and table IV)

Table (IV): Comparison between group Ia, Ib, and group II, related to serum leptin level for ITP (No = 88)

Specific markers for ITP	Group Ia (Acute) No=25	Group Ib (Chronic) No=23	Group II (Control) No=40	P.value	P1	P2	P3
	Mean±SD	Mean±SD	Mean±SD				
Serum Leptin (ng/ ml)	6.73±2.22	5.58±1.77	0.79±0.51	**0.001****	**0.001****	**0.01***	**0.001****

P. Value: Comparison Between All group
P1: Comparison between group II& group Ia
P2: Comparison between group II& group Ib
P3: Comparison between group Ia& group Ib

There was a significant positive correlation between serum leptin and IVIG in patients group with r = 0.**290*** (as shown in table V**)**

Table (V): Correlation between serum Leptin level with treatment in group I

Correlations	Serum Leptin level	
	Group I (patients)	
	r	P
IVIG	**.290***	.046

Also, There was a significant negative correlation between solumedrol and serum leptin in acute group with r = **-.408-*. (**as shown in table VI)

Table VI: Correlation Co-efficient between serum Leptin level with treatment in group Ia and Ib groups

Correlations / Variables	Serum Leptin level			
	Group Ia (acute)		Group Ib (chronic)	
	r	P	r	P
Solumedrol	**-.408-***	0.048	0.520	0.357

It was observed that there was a significant negative correlation between PLT and serum leptin in group Ia and group Ib patients with r = **-883-**** and **-830-**** (as shown in fig. 4 and table VII).

Table VII: Correlation between serum Leptin level with PLT in group Ia and Ib groups

Correlations / Variables	Serum Leptin level			
	Group Ia(acute)		Group Ib (chronic)	
	r	P	r	P
PLT (x10^3/ µl)	**-.883-****	0.001	**-.830-****	0.001

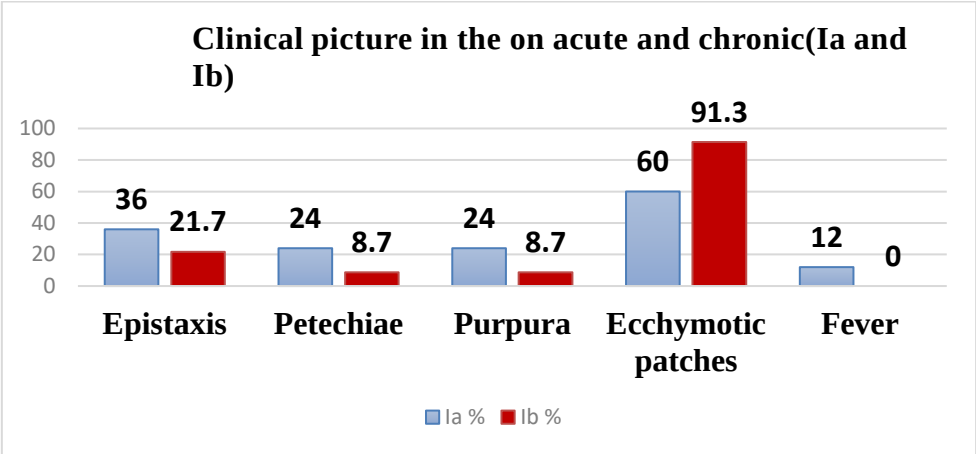

Figure (1): Distribution of clinical pictures of ITP related to patient group Ia and group

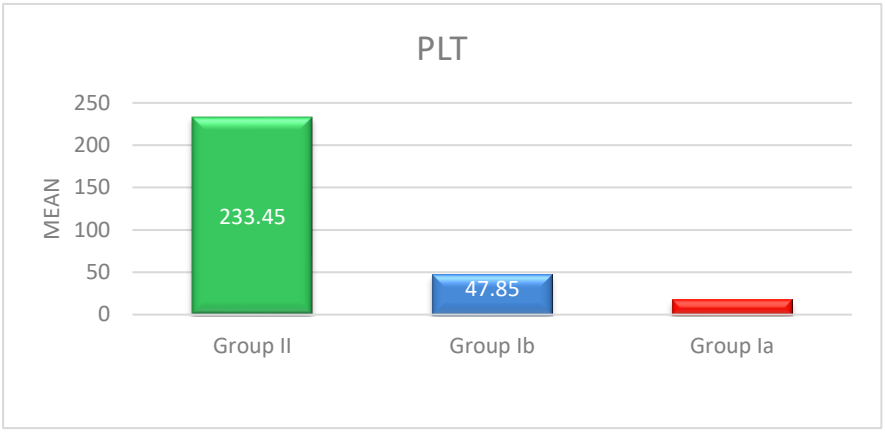

Figure (2): Comparison between group Ia, group Ib and group II related to PLTs ($\times 10^3$ / μL)

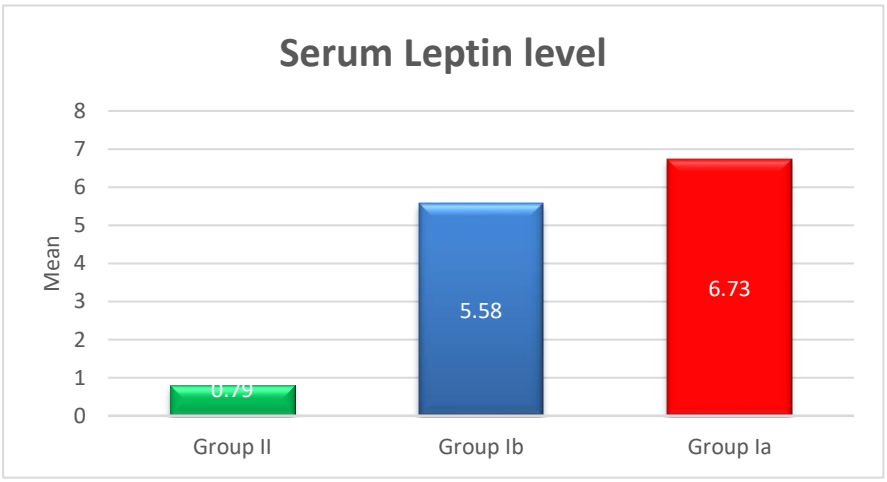

Figure (3): Comparison between group II, group Ia and Ib related to serum leptin level (ng /ml)

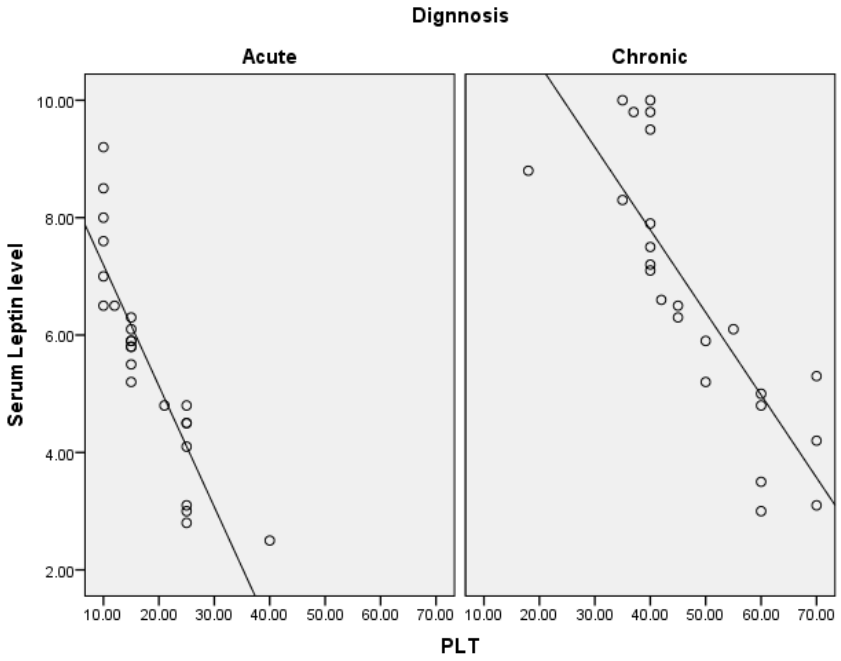

Figure (4): Correlation relationship between Serum Leptin level (ng /ml) and PLT ($\times 10^3$ / μL) related to group Ia and group Ib patients

Scatterplot illustrates a negative correlation (means increase serum leptin led to decrease PLT): hypothetical data for the relationship between serum Leptin level and PLT related to group Ia and group Ib patients

Discussion

ITP is a type of autoimmune condition that is acquired, meaning it is not present from birth. It is defined by a low platelet count, specifically less than 100,000 platelets per microliter of blood. This low platelet count is caused by an imbalance between immune cells that are successful at clearing platelets and immune cells that regulate this process. As a result, platelets are cleared from the blood at a higher rate and the production of new platelets is impaired. [5]

Leptin was initially identified as a hormone-like cytokine and has been demonstrated to impact both innate and adaptive immunity. The serum leptin level was elevated during the acute onset of ITP due to the interaction between leptin and the immune system. Leptin concentration increases during infection and inflammation. Proinflammatory cytokines elevate the levels of leptin in the bloodstream during an acute infection. In turn, leptin stimulates the production of cytokines from monocytes and macrophages. [6]

The present study showed that most of ITP cases was females in contrast to Liu & Liu. [7] who found that most of cases was males this result may be attributed to the small sample size, short period of observation and follow up.

Also we found that the majority of studied patients had ecchymotic patches on the same line with AbdalJabbar et al.,[8] who illustrated that the most of studied patients had ecchmotic patches, however Liu & Liu et al.,[7] who noticed the purpura was the most common complaint .

Most of acute patients received pulse steroid in a hormony with Liu et al.,[9] who found that the majority of acute patients received pulse steroid high dose dexamethasone as a first line of treatment, however majority of chronic patients received solumedrol in agreement with Aydn & Gürkan. [10] who documented that the majority of chronic patient take corticosteroid due to low cost and convenience of continuous use, this result related to the purpose of use of corticosteroid in ITP is to reduce antibody production by immunosuppression and prevent platelet destruction by macrophage / monocytes., also 39.1% of patients in chronic group received IVIG in contrast to Ozcelik et al.,[11] who documented that low percent of chronic ITP patients treated by IVIG due to life threatening side effect as renal failure and thrombosis.

Our study found that platelets count was lower in acute group than chronic group and control group on the same line with Hassan et al., [1] who documented that platelets count was lower in acute than chronic ITP patients furthermore, Karaman & Doğan.[12] who observed that platelet count at diagnosis is an important laboratory predictor of ITP course. And there were significantly lower platelet counts among the patients with acute onset of ITP and chronic ITP.

As shown in this work serum leptin level was higher in acute and chronic ITP than control group on the same line with Xu et al.,[13] who noticed significantly higher serum leptin in rapid onset of acute ITP patients and chronic child patients than control group which involved in patho-genesis in ITP, also serum leptin was higher in acute than chronic ITP patients in agreement with Thomas et al.,[3] who noticed that serum leptin increase in acute ITP patients compared with chronic group which involved in the pathogenesis and diagnosis of childhood ITP patients. Furthermore, leptin has a direct effect on immune function. [6]

The results of our study support the notion that leptin has an anti-inflammatory role in children with ITP. This is because high levels of leptin in the blood serve as a

soluble mediator during the acute phase of this autoimmune disease. [3]

Frasca et al.,[14] found that leptin plays a proinflammatory function in the development of autoimmune illnesses. This is because leptin in the blood interacts with the immune system and its levels rise during infection and inflammation. During the onset of inflammation, the levels of pro-inflammatory cytokines rise, leading to an increase in the amount of leptin in the bloodstream. Leptin, in turn, enhances the release of cytokines from monocytes and macrophages. The role of leptin in modulating immunological function in humans is highly supported by the higher occurrence of ITP pathogenesis. The results of our study demonstrated a statistically significant negative correlation between platelets and serum leptin in both the acute and chronic groups, which aligns with the findings of Thomas et al.,[3]. They also observed a statistically significant negative correlation between platelets and serum leptin in patients with acute and chronic conditions due to a positive correlation between serum leptin and platelet-associated antibodies (PA IgG and PA IgM) during the acute phase of ITP.

Furthermore, a statistically significant association was observed between serum leptin and IVIG in the patient group, which is in contrast to the findings of Thomas et al.,[3], who reported no link between serum leptin and therapy with IVIG in acute ITP.

There was a statistically significant link between serum leptin and solumedrol in the acute group, as indicated by Thomas et al.,[3]. They reported a favorable correlation between corticosteroid treatment and serum leptin in acute ITP.

Conclusions

The study's overall findings suggest that leptin is implicated in the development of children ITP, making it a potential target for treating autoimmune diseases. Furthermore, the elevated levels of leptin in acute patients highlight its significant role in immunological tolerance.

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