



## Chemokine 12 plasma level in pediatric patients with Immune Thrombocytopenic purpura and its relation to Disease Activity

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### Abstract:

The goal of this study was to investigate a possible association of chemokine 12 plasma level and its association to ITP, This was a prospective, observational, case-control study which was conducted on 60 patients with ITP with age ranging from 1 -18 years old together with 90 healthy controls. The plasma level of chemokine 12 in pediatric ITP patients and healthy controls were determined using enzyme linked immunosorbent assay (ELISA) The detection limit for this assay was 1.5 pg per ml. Patients were subjected also to lab investigation in the form of CBC and BM aspirate and There was a high plasma level of chemokine 12 in cases than controls (p value <0.05). Furthermore, cases with high plasma level of chemokine 12 were more susceptible for steroid dependency than controls (p<0.05) otherwise, no significant differences between plasma level of chemokine 12 among cases as regard clinical picture, history data, ITP course.

**Keywords:** ITP, chemokine 12:

### 1. Introduction:

Primary immune thrombocytopenia is one of the autoimmune disorders. It is characterized by isolated thrombocytopenia with absence of any abnormalities in the erythroid and myeloid/lymphoid lineages. It is conventionally believed that thrombocytopenia in ITP results from

enhanced amage of platelets opsonized by anti-platelet autoantibodies [1]. Additionally, abnormalities in megakaryocytopoiesis along with impaired production of platelets have been suggested as etiologic factors. The remarkable success of thrombopoietin receptor agonists

(TPO-RAs) for ITP patients has revealed that reduced platelet production has an essential role in ITP [1]. ITP can be classified into 3 types according to the disease duration; namely, acute, chronic, and persistent [2]. The International Working Group (IWG) also defines ITP as newly diagnosed (diagnosis to three months), persistent (3 -12 months from diagnosis), or chronic (lasting >12 months) [3]. Primary ITP is still diagnosed by exclusion and it is a must to be differentiated from non-autoimmune causes of thrombocytopenia along with secondary etiologies of ITP [4]. In the infectious cases, it is possible that the viral antigens were recognized as being the same as the platelet antigen, a process called molecular mimicry that leads to cross-reactive anti-platelet autoantibodies [5]. Chemokine 1 2 is produced by various cells including bone marrow cells and is incorporated in the proliferation and differentiation megakaryocytes (MKs) along with platelet production. Nearly 80% of patients suffering primary ITP, and 20% can be identified as secondary variety [6]. Following a measles, mumps, and rubella (MMR) vaccination, ITP occurs in ~ 2.6 cases/ 100,000 doses of vaccine within 6 weeks of vaccination [7]. In children, the typical presentation of ITP include sudden appearance of petechiae and purpura all over the body most prominent on the extensor surfaces of the skin and at

pressure sites on the extremities such as sites of measurement of blood pressure. Usually, the child will be totally well one day and then covered with petechiae and purpurae the next; despite some patients may suffer “wet purpurae” inside the mouth, epistaxis or frank bleeding occurs in < 1/3 of patients [8]. ITP was defined “severe” if the presence or recurrence of bleeding manifestations was sufficient to necessitate therapy irrespective the platelet count. “Mild” or “moderate” ITP terms are discouraged as they are vague [9]. The aim of the study was to investigate plasma level of chemokine 12 in ITP pediatric patient and its relation to incidence of ITP and disease activity.

## **2. Patients and Methods:**

This was a prospective, observational, case-control study which was conducted on 60 patients with ITP. They were 28 males (46.6%) and 32 females (53.3%) with age ranging from 1 -18 years old from Pediatric hematology Clinic, Beni-suef university Hospital, Health insurance hospital, Beni-suef together with 90 healthy controls.

### **2.1 Inclusion criteria:**

The Inclusion criteria were classified according to phases of the ITP disease as follows (*Neurent et al., 2011*):

- Newly diagnosed ITP: within 3 months from diagnosis.

- Persistent ITP: between 3- 12 months from diagnosis.
- Chronic ITP: lasting for >12 months.
- Age group from 1 - 18 years.

**Accordingly the studied children were divided into 4 groups:**

- Group I: 90 normal healthy subjects of comparable age and sex.
- Group II: 19 patients with newly diagnosed ITP.
- Group III: 19 patients with persistent ITP.
- Group IV: 22 patients with chronic ITP.

**According to response to treatment into:**

- No response ( platelet increase after treatment < 30000
- Partial response ( platelet increase after treatment > 30000 but < 100000
- Complete response ( platelet increase after treatment >100000

**2.2 All patients were subjected to: Full history:**

- Personal history including age, gender, and residence.
- Onset & duration of ITP.
- Bleeding symptoms with special emphasis on signs of thrombocytopenia bruises (purpura) and petechiae , epistaxis, gum bleeding, and menorrhagia .

- Medications, including heparin, and sulfonamides that might cause thrombocytopenia, and aspirin that might worsen bleeding.

- Family history of thrombocytopenia or hematologic disorders.

- Treatment received.

**Thorough clinical examination:**

Through clinical examination including cardiac, chest, abdominal examination with special emphasis on thrombocytopenia signs as petechiae and bruises.

**Laboratory investigations including:**

CBC.

Bone marrow aspirate.

**Methods :**

Detection of chemokine 12 plasma level by Enzyme linked immune sorbent assay (ELISA) .The minimum detectable concentration of IL 12 were 1.5 pg per ml.

**Statistical methodology**

Statistical analysis were performed with SPSS17.0 software (Chi-cago,III., USA).

Data were described in the form of (mean, SD) for quantitative data, and frequency and proportions for qualitative data. Differences were analyzed between the groups by student's test and ANOVA tests as regard normally distributed numerical data.

Otherwise Chi square test was used for comparison between ratios.

- P value > 0.05 insignificant
- P < 0.05 significant

### 3. Results:

The present study was carried out on 60 patients with ITP in Beni suef university hospital, and Health insurance hospital Beni suef and 90 age and sex matched healthy controls.

#### The objectives of this study were:

1-To investigate the association between chemokine 12 plasma level and susceptibility to ITP in the Egyptian population.

#### Baseline features of the participants enrolled:

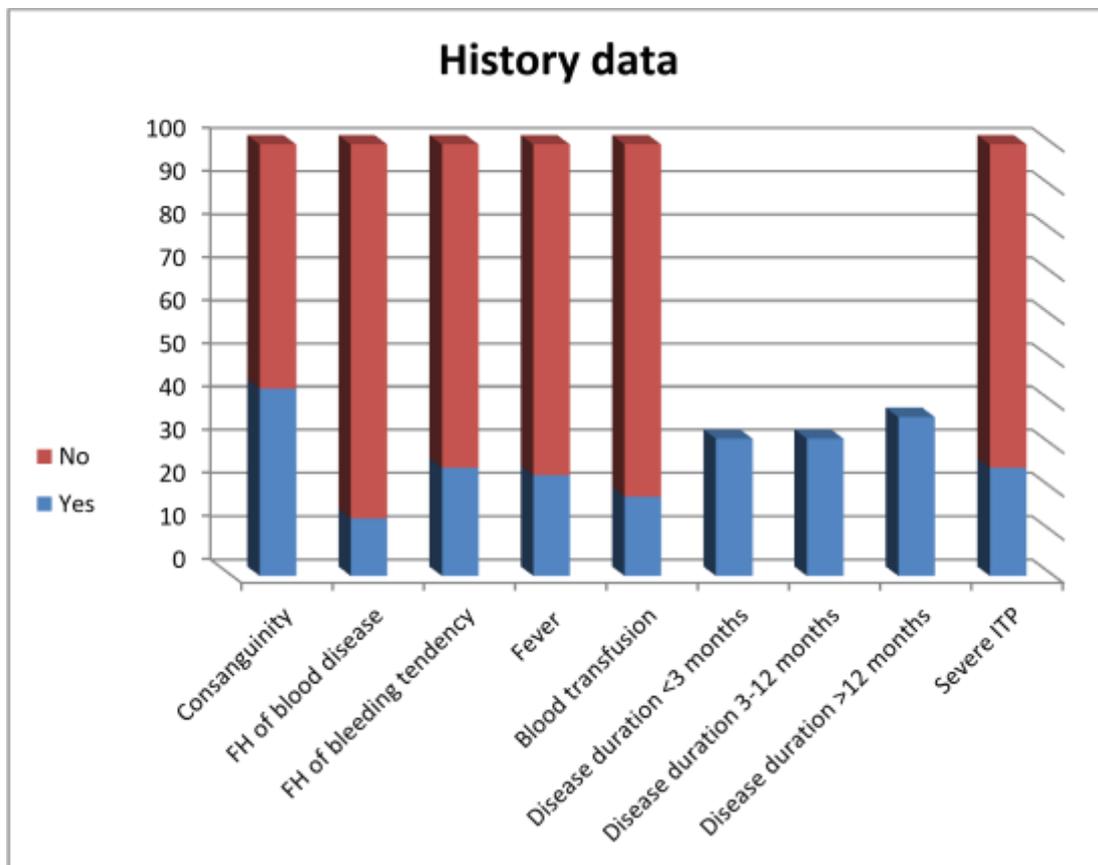
Table (1) demonstrated that cases group show positive consanguinity by (43.3%) , FH of blood disease by (13.3%) , FH of bleeding tendency by (25%) , fever by (23.3%) , infection by (23.3%) , blood transfusion by (18.3%) , and according to disease duration newly diagnosed cases were (31.7%) persistant cases were (31.7%) and chronic cases (36.7%)

**Table (1): Frequency of different history items in the patient group**

		Frequency	Percent
Consanguinity	Positive	26	43.3
	Negative	34	56.7
		60	100.0
FH of blood disease	Yes	8	13.3
	No	52	86.7
FH of bleeding tendency	Yes	15	25.0
	No	45	75.0
Fever	Yes	14	23.3
	No	46	76.7
Infection	Yes	14	23.3
	No	46	76.7
Blood transfusion	Yes	11	18.3

	No	49	81.7
Disease duration	<3 months	19	31.7
	3-12 months	19	31.7
	>12 months	22	36.7

**Fig 1 .Frequency of different history items in the patient group**



**Frequency of different clinical presentation in the patient group**

On demonstrating the clinical features of cases we find that (17.9%) presented by purpura , (42.3%) presented by ecchymosis , (41%) presented by pallor , (47.5%) presented by bleeding gums , (12.8%) presented by hematuria , (3.8%) presented by hematemesis . as demonstrated in( **Table 2**).

**Table 2 : Frequency of different clinical presentations in the patient group**

		Frequency	Percent
Purpura	no	64	82.1
	yes	14	17.9
Ecchymosis	no	45	57.7
	yes	33	42.3
Pallor	no	46	59.0
	yes	32	41.0
Bleeding gums	Yes	28	47.5
	No	31	52.5
Hematuria	No	68	87.2
	Yes	10	12.8
Hematemesis	No	75	96.2
	Yes	3	3.8

**Treatment option of the patient group**

Patients that were on full dose oral steroid at onset represent (98.3%) and those were on current full dose steroid treatment and methyl prednisolone (solumoderol) represent ( 47.5% , 39% ) respectively , patients were on Azathioprine (immuran) represent (3.3%) , and on IVIG by (22.5%) as demonstrated in **(Table 3)**

**Table 3: Treatment data of the patient group**

		Frequency	Percent
Full dose oral steroids at onset	No	1	1.7
	Yes	59	98.3
Current full dose steroid	No	31	52.5

therapy	Yes	28	47.5
Immuran	No	58	96.7
	Yes	2	3.3
IVIG	No	46	77.5
	Yes	14	22.5
SOIUMODEROL	No	36	61.0
	Yes	23	39.0

### Clinical course of the disease in ITP group

Patients were classified into newly diagnosed by (30%), persistent by (28.3%) and chronic by (41.7%)

And according to response to steroid therapy into: no response (6.7%) partial response (55%) and complete response (38.3%)

There was relapse in (21.7%) of cases and steroid dependence in (30%) as present in **(Table 4)**

**Table 4 : Clinical course of the disease in the ITP group.**

		Frequency	Percent
ITP classification	Newly diagnosed	18	30.0
	Chronic	25	41.7
	Persistent	17	28.3
Response to Steroids	no response	4	6.7
	partial response	33	55
	complete response	23	38.3

Relapse	No	47	78.3
	Yes	13	21.7
Steroid dependent ITP	No	42	70
	Yes	18	30

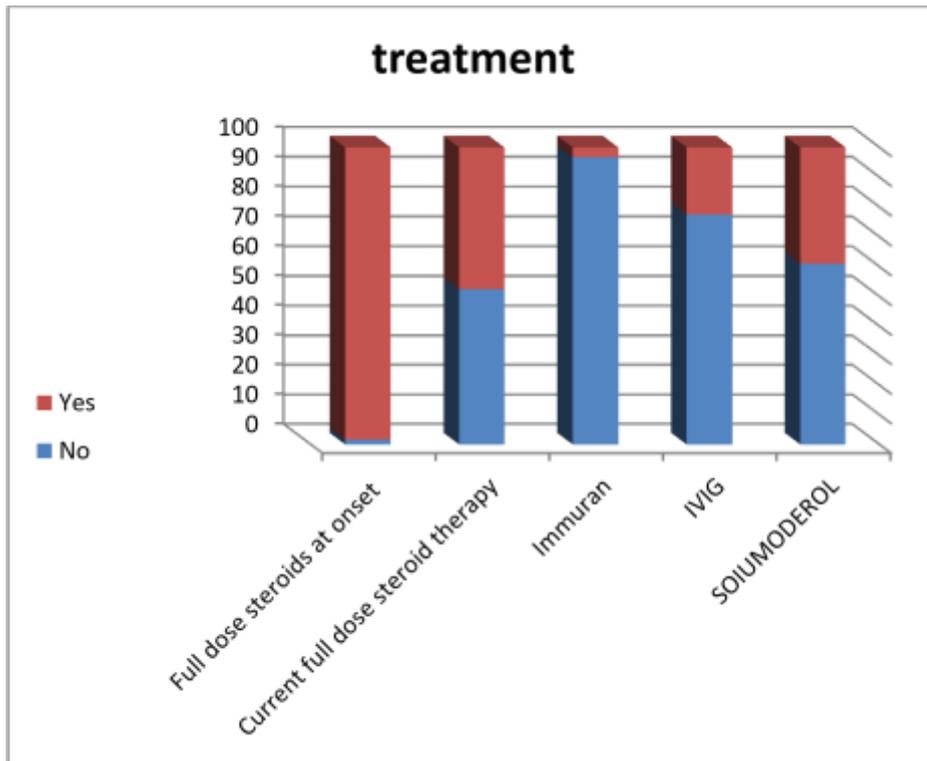


Fig 2 : Treatment data of the patient group

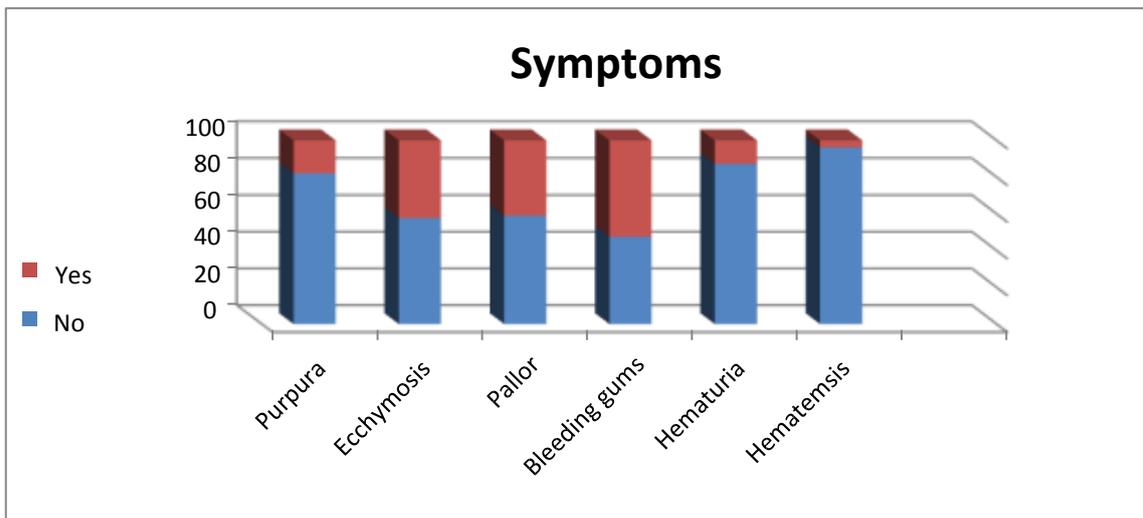
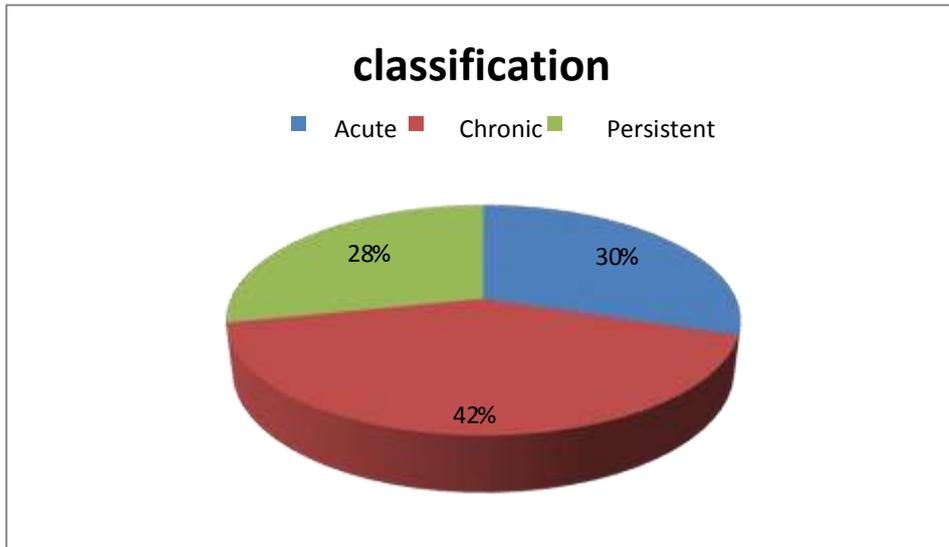
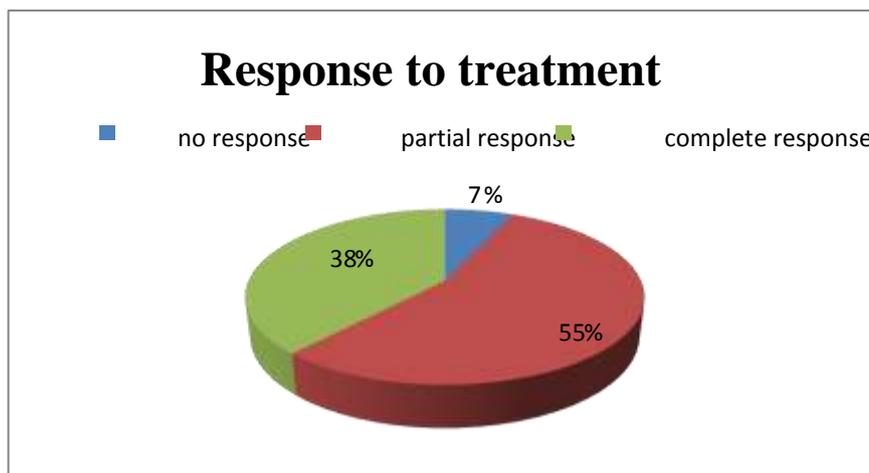


Fig 3 : Frequency of different clinical presentations in the patient group



**Fig 4 : ITP classes among the study population**



**Fig 5 : response to treatment among the ITP population**

We analysed the distribution of plasma level of chemokine 12 in ITP patients and controls and we found that ITP patients had a higher level of chemokine 12 in comparison to healthy controls (p value < 0.05 ) and this association was statistically significant .

The distribution of plasma level of chemokine 12 were significantly associated with steroid dependency (p value=0.01) .

#### **4. Discussion:**

Immune thrombocytopenia (ITP) is a complex autoimmune disorder characterized by

decreased platelet counts. The pathogenesis of ITP is still mystery

despite antibody-mediated and/or T cell-mediated platelet damage is key processes. In addition, impairment T cell functions, cytokine imbalances, and the contribution of the bone marrow niche have now been proved to play a role [10]. In ITP, the risk of bleeding is increased. The initial event(s) resulting in anti-platelet autoimmunity is still vague, but strong evidence supports the findings that autoantibodies and autoreactive CD8<sup>+</sup> cytotoxic T cells (Tc) stimulate platelet destruction and suppress platelet formation by megakaryocytes (MKs) in the bone marrow [11]. Primary ITP is a complex, chronic, cell-specific, autoimmune disease. currently It is refers to isolated thrombocytopenia (peripheral blood platelet count less than  $100 \times 10^9/l$ ) without any causes known to produce thrombocytopenia .Based on population-based studies, the overall incidence of ITP ranging 3.2 -12.1 / 105 adults annually, with prevalence ranging from 9.5 -23.6 / 105 subjects. The incidence of ITP rises with age [12]. ITP is accompanied by cytokine response and dysregulated cytokine network [13] The aim of the present study was to investigate the relation between plasma level of chemokine 12 in pediatric patient with ITP and its relation to the disease activity.

This is a case control study that were carried out on 60 patients with ITP in Beni -suef university hospital, and Health insurance

hospital Beni -suef and 90 age and sex matched healthy controls.

On analysis of participants data, the findings of the present study revealed that there is +ve consanguinity by (43.3%), family history of blood disease by (13.3%), family history of bleeding tendency by (25%), fever by (23.3%), infection by (23.3%), blood transfusion by (18.3%), and according to disease duration newly diagnosed cases were (31.7%) persistent cases were (31.7%) and chronic cases (36.7%).

In accordance to our result , the study of [14] that aimed to evaluate the effects of different treatment modalities on the clinical course, and long term outcome in ITP children and revealed that (50%) of patients have +ve consanguinity ,and (28%) patients were +ve family history of bleeding disorder. Similar to the study of [15] that aimed to evaluate the clinical features of ITP and the different treatment modalities found that only (20%) of patients have +ve family history of bleeding disorder .

In contrast to our results, the study of [16] revealed that in about 60% of patients, the onset of the disease was preceded by an infection in the previous few days to several weeks. The infection was mostly viral

infection of upper respiratory tract. In addition the interval between the infection and the

onset of ITP was in the range of 2 weeks, which might be due to old aged cases or because of seasonal variation.

The present study revealed that bleeding gums presented by (47.5%), ecchymosis presented by (42.3%), pallor presented by (41%) , (17.9%) presented by purpura , (12.8%) presented by hematuria , (3.8%) presented by hematemesis.

In contrast to our results, a study carried out by [14] revealed that the main complaint or the most presenting symptom in their study was multiple ecchymosis in about 33.1% and single ecchymosis in nearly 32.2%. Similar to [16] revealed that the disease onset was sudden appearance of bruises and petechial rashes that affect almost all patients. This discrepancy might be explained by genetic variation between Iraqi, American and Egyptian population.

The present study assessed the treatment modalities among the patients and reported that patients that were on full steroid dose at onset represent (98.3%) and that were on current full dose oral steroid therapy and Iv methyl prednisolone(solumoderol) represent by ( 47.5% , 39% ) respectively , patients were

on azathioprine (immuran) represent by (3.3%) , and on IVIG by (22.5%).

In accordance with the current study, the study of [14] showed that (69.5%) of the patients was treated with steroids only and in Turkish study [17] 2.5% of the patients only treated with IVIG.

In contrast to our results, the study of [18] revealed that IVIG was initiated as 1<sup>st</sup> line treatment in all patients treated for ITP. And this might be due to cost related factors as well as the availability of drug in American than Egyptian hospitals.

## **5. Conclusion and Recommendations:**

This study determined that high chemokine 12 plasma level is associated with susceptibility to develop ITP along with steroid dependency in patients.

Further studies of chemokine 12 plasma level and its relation to ITP on larger samples and long follow up period are needed to determine their role in etiology of ITP.

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