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Thrombopoietin, Interleukin-11 And Soluble P-Selectin as Biomarkers in Children with Idiopathic Thrombocytopenic Purpura: Post-Steroid Therapy

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ABSTRACT

Idiopathic thrombocytopenic purpura (ITP) is a primary autoimmune disorder with a decreased platelet count due to the platelet destruction mediated mainly by the platelet antibodies. Although the disease has been well defined, the exact pathogenetic mechanisms, including the mechanisms of thrombocytopenia and the role of steroids in the treatment have been still far from complete elucidation. The aim of this study was to evaluate the plasma concentrations of thrombopioetin (TPO), interleukin 11(IL-11) and soluble P-selectin (sP-selectin) at diagnosis, and post-steroid recovery period of Idiopathic thrombocytopenic purpura (ITP) which has been a primary autoimmune disorder with a decreased platelet count. This was an analytical cross sectional study which was carried out on thirty children with ITP. All patients and controls were subjected to medical history, Physical examination, Laboratory investigations and biochemical markers assays including TPO, IL-11 and sP-selectin, and their concentrations were evaluated in children with ITP at diagnosis and after 3 weeks of the conventional steroid treatment. There was a significant increase in mean plasma levels of TPO, sP-selectin and IL-11 at diagnosis of patients as compared to the control group ($p<0.001$, $p<0.001$ and $p<0.0001$ respectively). However, there was a significant decrease in their mean plasma levels following steroid treatment as compared to their levels before the treatment ($p<0.001$, $p<0.001$ and $p<0.0001$ respectively). There was a significant difference between mean levels of sP-selectin post treatment as compared to the control. Determining the status and kinetics of TPO, IL-11, and sP-selectin, in ITP was helpful for understanding the pathophysiology of the disease.

Key words: *Idiopathic Immune Thrombocytopenic Purpura, Thrombopoietin, Interleukin-11, Soluble P-Selectin.*

INTRODUCTION

Idiopathic immune thrombocytopenic purpura (ITP) is a primary autoimmune disease with a diminished platelet count caused by the platelet destruction mediated mainly by the antibodies against it [1], and it has been possibly associated with ineffective megakaryocytopoiesis/thrombopoiesis [2]. Idiopathic thrombocytopenic purpura (ITP), characterized by a decrease of the peripheral platelet count ($<100\times10^9/L$) has been resulted from the platelet destruction with an increased risk of mucocutaneous bleeding [3]. Thrombopoietin (TPO) is the primary regulator of the platelet production, supporting the survival, proliferation and differentiation of the platelet precursors, and bone marrow megakaryocytes [4]. TPO levels are 10-fold elevated in patients with aplastic anemia (median = 1002 pg/ml, range: 37-161,538 pg/mL) but are virtually normal (median = 64 pg/mL, range: 22-256 pg/mL) in patients with ITP (median = 98 pg/mL, range: 17-313 pg/mL). In the ITP patients, the platelets are still being made at an approximately normal rate (see below), but are being cleared at an increased rate. Despite their shortened survival, the platelets can still bind and clear TPO at a normal rate, and TPO levels would not significantly increase [5]. P-selectin is stored in α-granules of platelets and in the Weibel-Palade bodies of endothelial cells; it is expressed on their surface after the activation,

and shed into the plasma in the soluble form. P-selectin plays an important role in thrombosis and prothrombotic states [6]. IL-11 is a critical modulator of megakaryocyte maturation. Clinically, recombinant human IL-11 has been used to treat thrombocytopenia [7]. The aim of this study was to evaluate thrombopoietin, interleukin-11 and soluble P-selectin as biomarkers in children with idiopathic thrombocytopenic purpura using post-steroid therapy.

MATERIAL AND METHODS:

This was an analytical cross section study which was carried out on thirty children with ITP, their ages ranging from 1 - 14, and they were under the conventional steroid therapy (2mg/kg/24hs), for 3 weeks, and then the treatment was stopped. Those patients were recruited from Pediatrics hospital in Khartoum state, Sudan. In addition, 30 apparently healthy children were included in this study as a control group. An informed written consent was obtained from the parents of the children participants, and an ethical permission was obtained from Karary University ethical committee.

The diagnosis of ITP was based on the following criteria: Isolated true thrombocytopenia (platelets count of $\leq 50 \times 10^9$/Lwith normal red and white blood cell indices) without any other underlying disease, the presence of clinical signs and symptoms of spontaneous bleeding, no history of recent infection, drugs or agents known to cause thrombocytopenia, the absence of splenomegaly, and the normal megakaryocytes in bone marrow [8].

The following patients were excluded: patients who had exposed to platelet-depleting medication (including phenytoin, valproic acid, sulfonamide antibiotics, the presence of a specified condition that may account for thrombocytopenia); patients with Down's syndrome, radial aplasia thrombo-cytopenia syndrome, or congenital heart disease; and patients with splenomegaly, hepatomegaly, adenopathy, and severe pallor.

All the enrolled participants were subjected to medical history for being focused on, and excluding factors that suggest another disease in which thrombocytopenia is a complication; and also, the participants were subjected to physical examination and laboratory investigations including complete blood cell count and peripheral blood smear.

In addition, biochemical markers assays including TPO, IL-11 and sP-selectin concentrations were evaluated in ITP children at diagnosis and after 3 weeks of the steroid treatment; the assays were done in the control group as well.

A 5 ml sample of venous blood was collected from both patients (at diagnosis and post steroid treatment) and control group by venipuncture under completely aseptic conditions. The samples were collected in 3.8% trisodium citrate (1: 9 dilution-containing tubes), then centrifuged at 3000g for 20 minutes. The plasma was divided into aliquots using a sterile plastic transfer pipette and frozen in -80 until being used.

Plasma TPO was determined by using a thrombopoietin Human ELISA Kit, supplied by Quantikine, USA & Canada | R&D Systems, Inc., (Cat# : DTP00B) [9].

Plasma IL-11 was determined by using IL-11 Human ELISA Kit, supplied by Quantikine, USA& Canada | R&D Systems, Inc., (Cat#: D1100) according to the method [10]. Plasma sP-selectin was determined by using sP-selectin Human ELISA Kit, supplied by (Bender Medsystems, Vienna, Austria) [9].

Statistical Analysis

The data were performed using SPSS statistical package17, and were expressed as mean ±SD for the quantitative characteristics and the number and percentage for the qualitative characteristics. The Mann-Whitney U test or unpaired Student's t test were used to compare the data at diagnosis between ITP patients and controls. The comparison between the basal (at diagnosis) data versus the post-steroid recovery data was done by Wicoxon's test. The correlation between the study parameters was done by Pearson's linear correlation. $P < 0.05$ was considered statistically significant.

RESULTS:

The demographic data have been shown in table 1. Hematologic laboratory data of the study groups at baseline diagnosis have been shown in table 2. There was a significant increase in the mean plasma levels of TPO, sP-selectin and IL-11 at the stage of diagnosis of patients as compared to the control group ($p<0.001$, $p<0.001$ and $p<0.0001$ respectively). However, there was a significant decrease in their mean plasma levels following steroid treatment as compared to their levels before the treatment ($p<0.001$, $p<0.001$ and $p<0.0001$respectively) as

shown in tables 3, also table 3 shows no significant differences in mean levels of platelet count, TPO, and IL-11 (post steroid treatment) as compared to the controls. On the other hand, there was a significant difference between the mean levels of sP-selectin post treatment as compared to the controls. The results of TPO and sP-selectin levels revealed significant negative correlations between their plasma levels and platelet count at baseline ($r = -0.632$, $P<001$; $r = -0.715$, $P<0.001$ respectively), and also a significant negative correlation was detected between their plasma levels and platelet count post steroid treatment ($r = -0.607$, $P<0.001$; $r = -0.715$, $P<0.001$ respectively). Throughout the study, there were no correlations between IL-11 levels and platelet count. Also, there were no correlations between sP-selectin and IL-11 or TPO.

Table 1: Demographic and clinical data of the study groups at baseline diagnosis

Demographic and clinical data	ITP Group (n = 30)	Control Group (n = 30)
Sex: Males Females	 8 (26.7%) 22 (73.3%)	 10 (33.3%) 20 (66.7%)
Age (years): Mean ± SD	 6.4±1.5	 8.1±2.6
The mean duration of illness (days): Mean ± SD	 25.5±6.3	-
Presenting symptoms: Skin bleeding (petechiae and/or bruising) Purpuric rash Mucosal bleeding (nose, mouth, rectum)	 12 (40%) 10 (33.33%) 8 (26.67%)	-
Acute illness within 6 weeks before initial diagnosis	21 (70%)	-
n – number, SD – standard deviation		

Table 2: Hematological data of the study groups at baseline diagnosis

Hematological data	ITP Group Mean ± SD (n=30)	Control Group Mean ± SD (n=30)
Hemoglobin level (gm/L)	11.43 ± 0.9	12.2 ± 1.9
Red blood cell count (1012/L)	4.24 ± 0.1	4.55 ± 0.07
White blood cell count (x109/L)	7.19 ± 3.0	7.39 ± 3.66
Neutrophils (%)	38.2 ± 12.1	45.5 ± 12.6
Lymphocytes (%)	37.3 ± 12.2	35.27 ± 11.33
Monocytes (%)	4.1 ± 0.22	4.5 ± 0.65
Esinophils (%)	2.8 ± 0.02	2.5 ± 0.07
Basophile (%)	1.01 ± 0.01	1.03 ± 0.04
Platelet count (x109/L)	22.65 ± 13.12	284.15± 69.5**
Bleeding time (minutes)	11.7 ± 1.4	4.7 ± 1.3**
*p value (>0.05). **p value (0.001), SD – standard deviation		

Table 3: The mean of plasma levels of platelets count, TPO, IL-11, and sP-selectin in ITP group before and after the steroid treatment compared with the controls

Variables	Control group (n=30) (I)	ITP Group at base line (n=30) (II)	ITP Group post steroid treatment (n=30) (III)	Significance of difference P value		
				I VS II	I VS III	II VS III
Platelets count ($x10^9$/L)	284.15± 69.5	22.65±13.12	227.7±180.17	p<0.0001	NS	p<0.0001
Thrombopoietin Concentrations (pg/ml)	24.2 ± 28.07	194.5±32.62	22.13±24.01	p<0.001	NS	p<0.001
Interleukin (IL-11) concentrations (pg/ml)	23.62 ± 4.1	230±112.2	27.9±11.3	p<0.0001	NS	p<0.0001
sP-selectin concentrations (ng/ml)	165.51±21.38	460.02±30.06	249.42±44.17	p<0.001	p<0.0001	p<0.001
ITP - Idiopathic thrombocytopenic purpura , n – number, pg – picogam, ng – nanogram, NS - non-significant, p value						

(>0.05) significant, p value (>0.001) highly significant, VS= versus.

DISCUSSION AND CONLUSION:

Childhood idiopathic immune thrombocytopenic purpura (ITP) is a hemorrhagic disorder characterized by the low platelet counts, spontaneous bruising, purpuric or petechial rash, and mucosal bleeding. It occurs at a rate of 2 to 5 cases per 100000 person- in healthy children younger than 15 years. Between 70% and 80% of the cases are acute, and spontaneously resolve within 6 months of the presentation. The remaining 20% to 30% of the cases have been classified as chronic ITP, which has been formally defined as having low platelet counts for >6 months after diagnosis. The most serious complication of ITP has been intracranial hemorrhage, which occurs in <0.5% of the cases [11].

The present study included thirty children with ITP who received conventional steroid therapy. The mean age of the ITP patients was 6.4±1.5 years. This agreed with Kuhne et al [12] who reported that childhood ITP is typically an acute, self-limited illness and mostly affects the children who are young (peak age 5 years) and in previous good health. There has been an equal incidence of ITP in both males and females in the 1-to 7-years-old age group in acute ITP of childhood. These features have been distinctly different from the adult form of the disease, which has been more likely to be chronic, with a much greater incidence in females [13].

In the present study, female ITP children were more than males (73.3% versus 26.7%, respectively). However, this was not statistically significant when compared to the female/male ratio in the control group. These results were in disagreement with previous studies on the natural history of childhood ITP [14] which demonstrated that chronic ITP tends to occur with the equal frequency in boys and girls, although one study found significantly more chronic cases among girls aged 6 to 14 years [15].

In the current study, the mean plasma levels of TPO were significantly higher in ITP group before the treatment as compared to the control group. However, platelets count showed a significant decrease in this group as compared to the control. After steroid treatment, the mean plasma levels of TPO showed a significant decrease, but platelet count was significantly increased as compared to ITP group before the treatment, this was possibly due to a reduction in megakaryocyte mass, increased platelets mass and/or TPO sensitivity and accordingly to a decrease of megakaryocyte-related TPO catabolism.

However, these results disagreed with those who found that basal TPO concentrations slightly elevated, and no significant change was observed after the platelet recovery, in [16]. TPO was bound and internalized by platelets, it was destroyed together with the platelets at an accelerated rate in the macrophage system, because the spleen acted as a TPO sink [17]. The current study was conducted in children, and the maturity of the spleen in children was slightly different from adults, so the result made sense. In addition to that, this was the first study which was conducted among Sudanese (mixed between Arabs and Africans) population, thus the diverse ethnic groups might play a major role, that is why the findings of this study conflicted with the previous studies.

In the present study, there was a significant negative correlation between the mean plasma of TPO and the platelet count at the baseline (before treatment) and also the post steroid treatment.

A lot of previous studies have investigated TPO and other megakaryocytopoietic cytokines in ITP to understand the pathogenesis of the disease and the role of the cytokines in megakaryocytopoiesis [18], however the results were controversial, slightly elevated, normal, and even low values of TPO have been reported in patients with ITP [16].

In the present study, baseline IL-11 concentration was significantly higher in the ITP children than in the controls suggesting a compensatory purpose. This was consistent with many studies that found similar results such as Wada et al [19] Chang et al [20] Nomura et al [21], and Haznedaroglu et al [22].

Following the steroid treatment, IL-11 concentration in ITP patients was significantly decreased. Throughout the study, there were no correlations between IL-11 levels and platelets count.

Consistent with the results of this study, Cremer et al [23] found IL-11 concentrations to be decreased in patients with ITP after the intravenous immunoglobulin treatment. They also found no correlation between the platelet counts and the IL-11 levels. Normal levels of IL-11 were found by the other investigators who studied the patients who had undergone bone marrow transplantation or myelosuppressive chemotherapy [24]. The results of this study were in agreement with Chang et al [20], who found that IL-11 levels in children with acute ITP were significantly increased at diagnosis. Following the treatment with intravenous gamma globulin or

steroids, the platelet counts returned to the normal, but the circulating IL-11 levels remained elevated for 6 months after diagnosis. In contrast to the present study, Chang et al. [20] demonstrated a significant correlation between IL-11 levels and platelet counts in patients who had undergone myeloablative therapy and subsequent bone marrow transplantation. The elevated levels of IL-11 could also be related to non-hematological responses, such as febrile reactions or acute-phase reactions [25].

In the present study, baseline sP-selectin concentration was significantly higher in the ITP children than in the controls. Following the steroid treatment, sP-selectin concentration in ITP patients was significantly decreased. Throughout the study, a significant negative correlation was observed between sP-selectin levels and platelets count. Following the treatment, also a significant negative correlation was observed between post steroid sP-selectin levels and platelets count, and no correlations were found between sP-selectin and IL-11 or TPO.

These results were in agreement with the previous studies [22]. These results suggested that the residual platelets were activated in ITP, which offered a relatively benign clinical course compared to the other thrombocytopenias. Olcay et al. [26] evaluated TPO, soluble P-selectin, soluble P-selectin per platelet in children with ITP before the treatment in 16 acute and 22 chronic cases, and after the treatment in 10 acute and chronic cases who received mega-dose methylprednisolone. The post treatment TPO declined, but the soluble P-selectin and platelet count increased.

Considering the results of this study, it could be concluded that determining the status and kinetics of TPO, IL-11, and sP-selectin, which were involved in platelet production and function during the clinical course of ITP, might be helpful for the treatment and understanding the pathophysiology of the disease.

Author's Disclosures of Potential Conflicts of Interest;

No potential conflicts of interest relevant to this article were reported.

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