The Contribution of Tumor Necrosis Factor-α (TNF-α) to Pathogenesis of Childhood Primary Immune Thrombocytopenia: Single Center Study

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ABSTRACT

Background: Primary immune thrombocytopenia (ITP) is an autoimmune disease mediated by antiplatelet autoantibodies that cause platelet destruction and suppression of platelet production. Tumor necrosis factor alpha (TNF-α) is a multifunctional cytokine and is involved in the promotion of inflammatory responses. It also plays critical roles in the pathogenesis of autoimmune diseases. **Objective:** To assess the level of TNF-α in children with primary ITP and its contribution to pathogenesis of the disease. **Patients and Methods:** A case control study was conducted on 21 ITP patients (13 males and 8 females) and twenty age- and sex-matched healthy children as a control group at the Outpatient Clinic of Hematology Unit of Pediatric Department and Clinical Pathology Department at Zagazig University Hospitals, Sharkya, Egypt in the period from November 2019 to April 2020. All patients and control were subjected to full medical history, thorough clinical examination and laboratory investigations in the form of complete blood picture, PT, PTT, C3, ANA, anti-DNase and serum TNF-α by ELISA kit. **Results:** There was statistically significant higher serum TNF- α in ITP cases than controls with highest level in the newly diagnosed ITP cases followed by persistent then chronic cases. TNF alpha showed statistically significant negative correlation with platelets count and disease duration and positive correlation with WBCs and response to treatment. **Conclusion:** Significant differences in TNF alpha levels between ITP patients and healthy controls indicates that TNF alpha disturbances might be involved in the pathogenesis of ITP in pediatric patients.

Keywords: Immune thrombocytopenia, TNF-α, bleeding disorders and platelet destruction.

INTRODUCTION

Primary immune thrombocytopenia (ITP) is an acquired hematological disorder that is developed secondary to the production of auto-antibodies against platelets leading to isolated thrombocytopenia (peripheral platelet count <100,000/ml) in the absence of other causes of thrombocytopenia such as drugs, infections, malignancy, or other autoimmune diseases [(1)]. Over the last two decades, several investigators have contributed to an improved understanding of the complex pathophysiology of this condition in an attempt to develop an individualized treatment approach for affected patients [(2)].

Studies have shown that patients with ITP exhibit antiplatelet self-reactive T cells and an imbalance in cytokine levels, which suggests loss of peripheral tolerance [(3)]. Dysfunction of T cells in ITP may be contributed to loss of tolerance, and impairment of the delicate balance of specific cytokine and serum cytokines may play a role in the pathogenesis of ITP [(4)]. Understanding the role of T-cell subsets will permit a better control of autoimmunity through manipulation of their cytokine network [(5)]. Dysfunction at cellular immunities is evaluated by the levels of cytokines, e.g. TNF-α and IL-6. Measurement of levels of cytokines may help in prediction of the course of ITP[(6)].

Tumor necrosis factor-alpha [TNF-α] is a pleiotropic cytokine produced primarily by macrophages and T cells, and has a range of inflammatory and immunomodulatory activity. It is considered an inflammatory cytokine that is involved in the inflammatory response and protective immune response to infectious agents. It participates also in the pathogenesis of many autoimmune diseases e.g. ITP[(7)]. Therefore, the present study aimed to assess the level of TNF-α in children with primary ITP and its contribution to pathogenesis of the disease.

SUBJECTS AND METHODS

A case control study carried out at Hematology and Oncology Unit, Pediatric Department, Zagazig University Hospital during a period from (November 2019 to April 2020).

The sample size was 42 using OPEW EPI at power 80% and C.I 95%. As seaming that the mean ± SD of (TNF-α) in acute ITP is 0.16±0.12 versus 0.08±0.05 in control group. The subjects were divided into two groups: Group (A): composed of 21 children diagnosed with ITP (newly diagnosed, persistent and chronic) and Group (B): composed of 21 age- and sex-matched healthy children as a control group.

Ethical approval:

The present study was conducted in accordance with the ethical standards of the Helsinki Declaration of 1964, as revised in 2000, and was approved by ethical committee of Zagazig University . Informed consent was obtained from the study participants.

Inclusion and exclusion criteria: Children from 1-18 years of both sexes who were diagnosed with primary ITP (newly diagnosed, persistent and chronic) were included in the study. While, children below 1 year or above 18 years and patients with secondary immune thrombocytopenia were excluded.

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Operational interpretation: All patients in this study were subjected to complete history taking including disease duration, bleeding tendency and complications, and family history. Complete clinical examination was done with special emphasis on site and shape of bleeding and presence of organomegaly and lymphadenopathy.

Response to treatment was classified in the studied cases according to: (a) Complete response (platelet >100×109/l) at least 6 weeks post treatment. (b) Response (platelet between 30×109/l and 100) and double base line platelet count. (c) No response (platelet <30×109/l or less than double base line platelet count.

Laboratory investigations were performed including complete blood count (CBC) (Initially at diagnosis, at discharge and after 3 month) using Sysmex Xp-300. Bone marrow examination if indicated and laboratory investigation to exclude secondary causes as retics% , PT, PTT, C3, ANA , anti-DNase and H.pylori) in ITP patients . Measuring of serum tumor necrosis factor – α by ELISA kit.

Tumor necrosis factor-α measurement:

Serum TNF-α was assessed using ELISA Kit (sunRed Corporation, China): allow the serum to clot for 10-20 minutes at room temperature, centrifuge (at 3000 RPM) for 20 minutes, and collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again. The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay ELISA to assay the level of human tumor necrosis factor α (TNF-α) in samples. The chroma of color and the concentration of the human substance tumor necrosis factor α (TNF-α) of sample were positively correlated.

Statistical analysis:

Collected data were analyzed using computer using SPSS version 22 (SPSS Inc. Chicago, IL, U.S.A). Data were presented in tables and graphs and summarized as median and mean ± standard deviation for quantitative variables and as number and percentage for qualitative variables. Shapiro-Wilk's W-test was applied for checking the normality assumption of continuous variables. Fisher's exact test was used to compare qualitative data. Kruskal Wallis test was used for comparing the median. P-value <0.05 was considered significant. P-value <0.001 was considered as highly significant.

RSULTS

Concerning bleeding manifestation, purpuric rash was the commonest bleeding manifestation (Table 1).

Table (1): Bleeding manifestations among the ITP cases

Bleeding manifestations	The ITP cases	
	NO (21)	%
Purpuric rash	15	71.4%
Epistaxis	8	38.1%
Gingival bleeding	7	33.3%
Ecchymosis	14	66.7%
Bruises	5	23.8%
Sub-conjunctival hge	1	4.8%
Hematuria	1	4.8%

After 3 months follow up, there was statistically significant higher platelets count among newly diagnosed than persistent and chronic cases (Table 2).

Table (2): Comparison between ITP cases regarding initial and follow up platelets count

Variable	Newly diagnosed ITP (NO.=7)	Persistent ITP (NO.=7)	Chronic ITP (NO.=7)	K.W test	P	LSD
Initial platelets count ($X10^9$/L) mean ± SD	7.6±1.5	12.4±1.1	16.7±3.4	1.9	0.2	0.3(1) 0.2(2) 0.6 (3)
Platelets count at discharge ($X10^9$/L) mean ± SD	40.0±5.5	44.4±4.9	35.3±6.4	0.2	0.7	0.7(1) 0.7(2) 0.5 (3)
Follow up platelets count at 3 months ($X10^9$/L) mean ± SD	286.7±15.9	128±13.6	86.6±8.9	8.7	002*	0.006*(1) 0.001**(2) 0.4 (3)
Paired W.S	15.3	12.4	9.6			
P-value	0.001**	0.002*	0.007*			

(1) Newly diagnosed versus persistent, (2) Newly diagnosed versus chronic and (3) Persistent versus chronic, K.W=Kruskal-Wallis test, W.S=Wilcoxon signed rank test, *Statistically significant difference (P ≤ 0.05), **Statistically highly significant difference (P ≤ 0.001).

There was statistically significant increase of TNF alpha among ITP cases compared to control group. Also, there were highly significant cases in newly diagnosed cases compared to persistent and chronic cases (Figure 1).

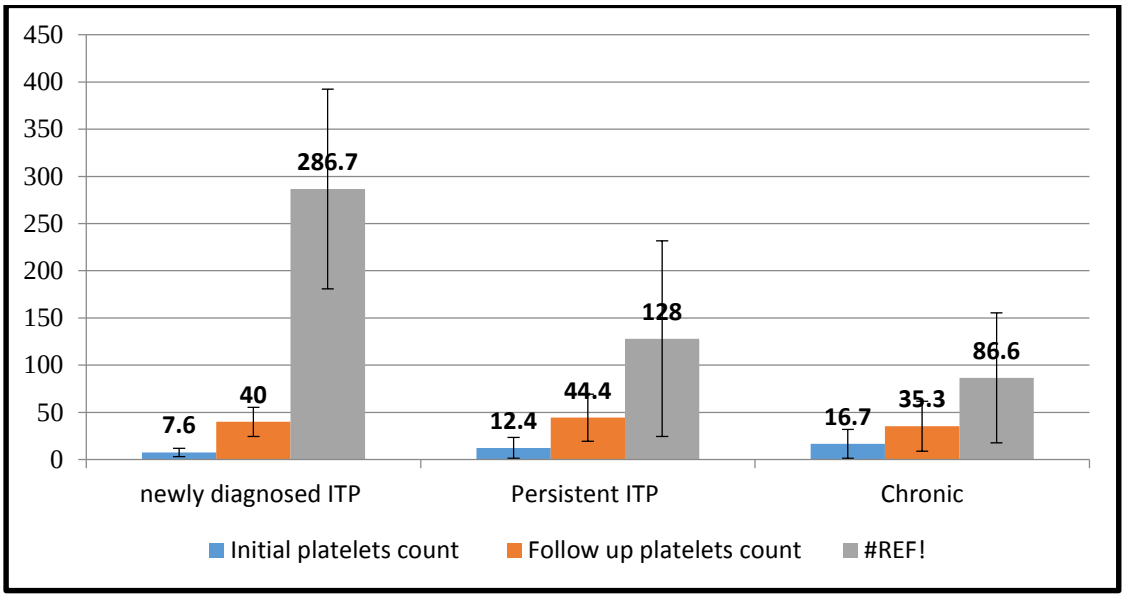

Figure (1): Bar chart for initial, at discharge and follow up platelets count of ITP cases among the studied groups

There was statistically significant increase of TNF alpha among ITP cases compared to control group. Also, there were highly significant cases in newly diagnosed cases compared to persistent and chronic cases (Table 3).

Table (3): Comparison between the studied groups regarding tumor necrosis factor alpha

The studied groups	Number of patients (21)	TNF alpha (µg/ml) mean ± SD Range	Kruskal Wallis Test	p-value	LSD
Control	21	91.1±23.8	24.1	0.001**	0.001**(1) 0.04*(2) 0.02* (3) 0.001**(4) 0.001**(5) 0.7 (6)
Newly diagnosed ITP	7	478.8±30.4			
Persistent ITP	7	179.8±9.1			
Chronic ITP	7	170.6±9.1			

(1) Control versus newly diagnosed, (2) Control versus persistent, (3) Control versus chronic, (4) Newly diagnosed versus persistent, (5) Newly diagnosed versus chronic and (6) Persistent versus chronic, *Statistically significant difference ($P \leq 0.05$), **Statistically highly significant difference ($P \leq 0.001$).

There was no statistically significant difference between the ITP cases regarding response to treatment (Table 4).

Table (4): Comparing disease response to treatment between the ITP cases

Response	Newly diagnosed ITP		Persistent ITP		Chronic ITP		χ^2	p
	NO. (7)	%	NO. (7)	%	NO. (7)	%		
Complete response (7)	5	71.4	0	0.0	2	28.6	8.9	0.06
Response (9)	2	28.6	4	57.1	3	42.9		
No response (5)	0.0	0.00	3	42.9	2	28.6		

TNF-α is a good predictor marker for prediction of the course and prognosis of ITP with (86.0%) ability to diagnose ITP truly, (72.0%) ability to exclude truly negative ones and with total accuracy of (79.0%) (Table 5).

Table (5): Accuracy of TNF alpha in detection of ITP course and prognosis:

Variable	Cut off	AUC	P	95% CI
TNF alpha	>102.9	0.83	<0.001**	0.69-0.98

Variable	Sensitivity	Specificity	PVP	PVN	Accuracy
TNF alpha	86.0%	72.0%	75.4%	83.7%	79.0%

TNF alpha showed statistically significantly negative correlation with platelets count, Hb level and disease duration and significantly positive correlation with WBCs and response to treatment (Table 6).

Table (6): Correlation between TNF alpha and clinical and laboratory data among the ITP cases

Variables	TNF alpha		
	r	p	Sig
Age	0.3	0.1	NS
Disease duration	-0.7	0.001**	HS
WBCs	0.4	0.02*	S
RBCs	0.01	0.9	NS
Hb	-0.4	0.04*	S
Platelets	-0.7	0.001**	HS
Response to TTT	0.4	0.03*	S

*Statistically significant difference ($P \leq 0.05$)
**Statistically highly significant difference ($P \leq 0.001$)

DISCUSSION

Immune thrombocytopenia (ITP) is an immune-mediated platelet disorder in which autoantibody-coated platelets are removed from the blood by monocytic phagocytes, resulting in a remarkable decrease of platelet count, accompanied by impaired platelet production [(8)]. The majority of childhood cases are acute and resolve successfully without therapy within 6 months [(9)]. The term "peripheral tolerance" refers to mature reactive cells that escaped negative selection in the thymus and are suppressed from peripheral blood by a particular class of immunoregulatory cells, the regulatory T cells (Tregs) [(10)]. Studies have shown that patients with ITP exhibit antiplatelet self-reactive T cells and an imbalance in cytokine levels, which suggests loss of peripheral tolerance [(11)].

Tumor necrosis factor (TNF) is a critical cytokine, which contributes to both physiological and pathological processes. It is considered an inflammatory cytokine that is involved in the inflammatory response and protective immune response to infectious agents. It participates also in the pathogenesis of many autoimmune diseases, e.g. ITP [(12)].

In our study, chronic ITP cases had higher age than other groups (control group, newly diagnosed and persistent cases) and this agree with the finding of the cross-sectional case–control study, conducted at Ain Shams University, by **Fatma *et al.*** [(13)] where patients with chronic ITP were statistically older, with mean age of 7.7±4.19, than those with acute ITP, with mean age of 3.95±2.21 and this is most probably because of the chronic nature of the disease as children with the chronic form of the disease are diagnosed for a much longer period of time, while children with acute ITP are usually diagnosed at a younger age .

Regarding bleeding manifestations among the ITP cases; purpuric rash was the commonest bleeding manifestation (71.4%) followed by ecchymosis (66.7%) then epistaxis (38.1%). **Fatma *et al.*** [(13)] reported that all studied patients presented with cutaneous bleeding, and only one of the patients with chronic ITP developed intracranial hemorrhage owing to head trauma.

Talaat *et al.* [(14)] reported that clinical features were present in all patients (100%) in the form of petechial rash and 91·4% (32 of 35) with epistaxis. It was reported previously that petechiae and ecchymosis were present in 99% of patients with childhood ITP and epistaxis was the most frequent hemorrhagic manifestation.

Concerning platelets, there was highly statistically significant decrease in ITP cases (newly diagnosed, persistent and chronic cases) compared to control group with no statistically significant difference between newly diagnosed, persistent and chronic cases. In the case control study of **Čulić *et al.*** [(15)] on 45 children

with ITP, 26 (58%) had a platelet count below 10×109/L.

In agreement with **Talaat *et al.*** [14] our patients showed a significant decrease in platelet count in acute and chronic stages, with a maximum reduction in acute patients .

Tumor necrosis factor-alpha is a critical cytokine in the inflammatory response to infection. Accordingly, any genetic variability in the production of TNF-a after an infectious stimulus could have a significant impact on the degree of inflammatory response and therefore potentially influence the clinical outcome [16].

In our study there was statistically significant difference in TNF alpha between the control group and all ITP cases and this is consistent with **Čulić *et al.*** [15] who found that there was statistically significant difference in TNF alpha between healthy controls and children with ITP. Also, our results showed that the TNF-α was statistically significantly higher in newly diagnosed cases compared to persistent and chronic cases.

Fatma *et al.* [13] found that TNF-α was increased in all patients with acute ITP and in 70% of those with chronic ITP showed a highly statistical significant association compared with controls, and this may highlight its role in the pathogenesis of ITP.

Also, many studies reported higher levels of TNF-α in the plasma/serum from ITP patients and they concluded that TNF-α is the most informative variable for discrimination between healthy children and those with ITP. This could be related to activation of macrophages, which have been reported to be stimulated in ITP patients by platelet autoantigen and lead to activation of T cells [14, 17].

Correlation between TNF alpha with clinical and laboratory characteristics of our ITP cases showed that TNF alpha was statistically significantly negatively correlated with platelets count and disease duration as TNF alpha was markedly increased in newly diagnosed cases and decreased with chronicity and this data agrees with **Fatma *et al.*** [13] who found a significant negative correlation between TNF-α and platelet count .

CONCLUSION

Immune thrombocytopenia (ITP) is one of the most common acquired bleeding disorders of childhood. The finding of significant differences in TNF alpha levels between ITP patients and healthy controls (significantly higher in ITP cases) indicates that TNF alpha disturbances might be involved in the pathogenesis of ITP in pediatric patients.

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