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IGFBP2 Cell Death Marker in Children with Immune Thrombocytopenia

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Abstract:

Introduction: Pediatric Immune thrombocytopenia (ITP) is a condition in children where the immune system attacks and destroys platelets, leading to a temporary or ongoing drop in platelet count. According to the time that thrombocytopenia lasts, ITP can be classified as "acute" which resolves before six months, versus "chronic" ITP which lasts more than 6 months- 12 months. Multicellular animals undergo apoptosis, a complicated process of regulated cell death. It involves a series of intricate processes, which rely on energy and consist of a cascade of molecular events. Multiple studies have investigated the involvement of apoptosis in individuals with ITP. Platelet apoptosis may occur as a result of autoantibodies targeting proteins on the surface of platelets.

Aim of the study: We intended to correlate insulin growth factor binding protein type-2 (IGFBP-2) levels with clinical outcomes in young patients with ITP, so we could predict their outcome since diagnosis.

Subjects and Methods: This study is a cross-sectional study that was conducted in Fayoum governorate, the research involved a total of 40 children with newly diagnosed ITP. All Cases were subjected at presentation to a thorough full history, physical examination, and laboratory investigations. IGFBP2 levels were measured using enzyme-linked immunosorbent assay (ELISA) technique. Our ITP cases were followed-up for six months.

Results: There were significantly increased levels of IGFBP2 in the newly diagnosed resolved pediatric ITP cases compared to chronic ITP cases (p <0.001).

Conclusions: The outcome of pediatric ITP cases could be predicted using the IGFBP2 apoptotic marker.

Keywords: ITP; Apoptosis; Outcomes.

1. Introduction

A condition known as pediatric immune thrombocytopenia (ITP) causes a reduction in the platelet count in children, either temporarily or for a longer period, due to the immune system's response [1].

ITP is categorized as either primary or secondary. The absence of a recognized cause or trigger is a hallmark of primary ITP, which is sometimes called idiopathic ITP. In contrast, a preexisting cause or trigger is a hallmark of secondary ITP, such as medication usage, immunizations, infections, and so on [2].

Multicellular animals undergo apoptosis, a complicated process of regulated cell death. It involves a series of intricate processes and relies on a cascade of chemical events that need energy [3].

Typically, there are two primary channels via which apoptosis occurs: the extrinsic pathway, which includes the death receptor pathway, and the intrinsic system, which involves the mitochondrial pathway [4].

Previous research using a mouse model demonstrated an increased occurrence of platelet death when exposed to antibodies targeting integrin aIIbb3. However, this impact was successfully inhibited by administering IVIG therapy [5].

Insulin-like growth factors (IGFs) are peptides that have a role in the development, growth, and cellular processes of mammals, including proliferation and differentiation [6].

IGFs often form complexes with IGF-binding proteins (IGFBPs), which consist of six well-defined high-affinity IGFBP members known as IGFBP1 through 6 [7].

IGFBP2 has a crucial function in several physiological and metabolic processes, including development, cell proliferation, differentiation, migration, and the prevention of apoptosis. However, its exact role is still uncertain and not well-defined [8].

Our research aimed to investigate the significance of IGFBP2 in predicting the fate of ITP patients. We want to contribute to adding more apoptotic indicators in conjunction with existing predictors.

2. Subjects & Methods

2.1. Study design

This cross-sectional research was conducted in the outpatient facility of hematology at the pediatric unit of Fayoum University Hospital, located in Fayoum, Egypt, between the years 2023 and 2024.

2.2. Subjects

The present research included forty children who were clinically diagnosed with primary ITP, a kind of thrombocytopenic purpura distinguished by a platelet count below 100 $x10^9$/L, without any other identifiable reasons.

Inclusion Criteria

Newly diagnosed cases of ITP in children aged from six months to thirteen years.

Exclusion Criteria

- Patients > 13 years old.
- In acute instances, the presence of organomegaly is inconsistent with a diagnosis of primary immune thrombocytopenia (ITP), or the features suggestive of viral infections as EBV.
- Existence of profound or perhaps fatal hemorrhaging.

Pre-treatment, blood samples were collected. The individuals in this group were systematically monitored for six months after being diagnosed. Consequently, each patient with immune thrombocytopenia (ITP) was classified into one of two groups: "resolved, non-chronic" or "chronic," depending on the length of time they had low platelet count. Thrombocytopenia resolving before 6 months is an acute (resolved ITP), while unresolved for more than six months is a chronic one [9].

2.3. Methods

All ITP cases, at the diagnosis, were subjected to:

- Thorough medical history including drug intake, preceding febrile illness, and bleeding manifestations.
- Physical examination including organomegaly, lymphadenopathy and all signs of apparent bleeding manifestations, its site and extension.
- Laboratory investigations:

  - Complete blood count (CBC).

  - Direct blood film examination.

- Activated partial thromboplastin time (aPTT) and INR.

- ESR.

- Quantitative assessment of IGFBP2 Using ELISA.

- Laboratory findings and clinical manifestations were the primary foci of the data analysis for the two study groups.

2.4. Statistical Methods

We entered the data into Microsoft Access twice after we arranged it so it would be simple to manipulate. The data analysis was carried out using SPSS software, version 22, on a Windows 7 operating system. The program was developed by SPSS Inc. of Chicago, IL, USA. Quantitative and percentage-based descriptions of qualitative data are part of descriptive analysis. It also includes using arithmetic means to assess central tendency and standard deviations to quantify the dispersion of quantitative descriptive data. The t-test for independent samples was employed for contrasting quantitative measurements across two distinct groups in the case of quantitative data. The Chi-square test is used for comparing between two or more qualitative groups when dealing with Qualitative Data. For testing the association between the variables, the Bivariate Pearson correlation test was used. The sensitivity and specificity of a novel test may be assessed using the Receiver Operating Characteristic (ROC) curve. The *P*-value< 0.05 was considered as a statistically significant.

3. Results

Our study included 40 newly diagnosed children with primary ITP with demographic details as shown in (**Table 1**) and (**Figure 1**). There was a significant decrease in platelet count in the cases (12.7 ±12.5 × 10^9 /L), and the hemoglobin concentration also was significantly decreased (10.2 ±1.4 gm/dl). Whereas, the mean platelet volume (MPV) was significantly increased in the cases (9.4 ±1.3 fl) (**Table 2, Figures 2 & 3**). All patients (100%) had cutaneous symptoms, namely petechiae and ecchymosis. There were no instances of organomegaly (enlarged liver or spleen) or severe lymphadenopathy seen in any of our patients. (**Table 3, Figure 4**).

Table 1: Demographic Details of the Investigated ITP Patients.

Variables		Cases (n =40)	Chronic (n =21)	Non-chronic (n =19)	*P*-value
Age (years)		6.2 ±3.2	7.3 ±3.7	5 ±1.9	0.014*#
Gender	female	21 (52.5%)	11 (52.4%)	10 (52.6%)	0.987##
	male	19 (47.5%)	10 (47.6%)	9 (47.4%)	

*#Independent-t test, ## Chi squared test, *Significant at p<0.05.*

Table 2: Laboratory Findings (Hgb, TLC, PLT count, MPV & ESR) of the Studied ITP Cases.

Variables	Cases (n=40)
Hgb conc. (gm/dl)	10.2 ±1.4
TLC ($\times 10^9$/L)	8.2 ±2
PLT count ($\times 10^9$/L)	12.7 ±12.5
MPV (fl)	9.4 ±1.3
ESR	23.1 ±12.8

SD: standard deviation, Hgb conc.: hemoglobin concentration, TLC: total leucocytic count, PLT: platelet, MPV: mean platelet volume, ESR: erythrocyte sedimentation rate.

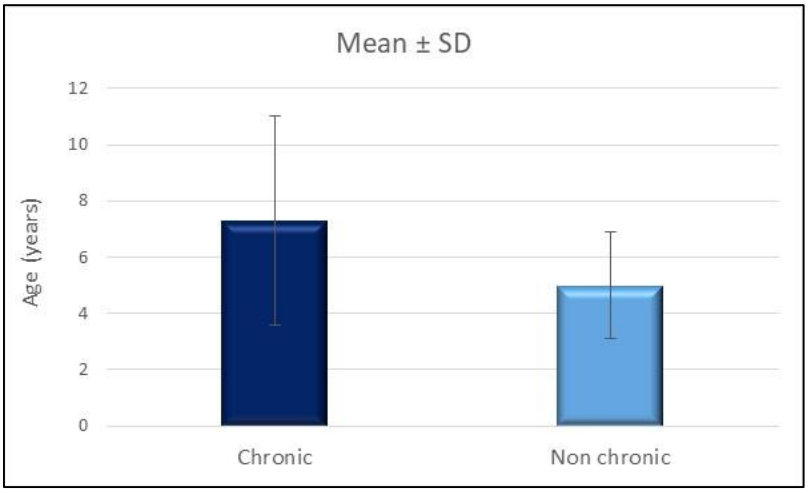

Figure 1: Age Chart of the Cases.

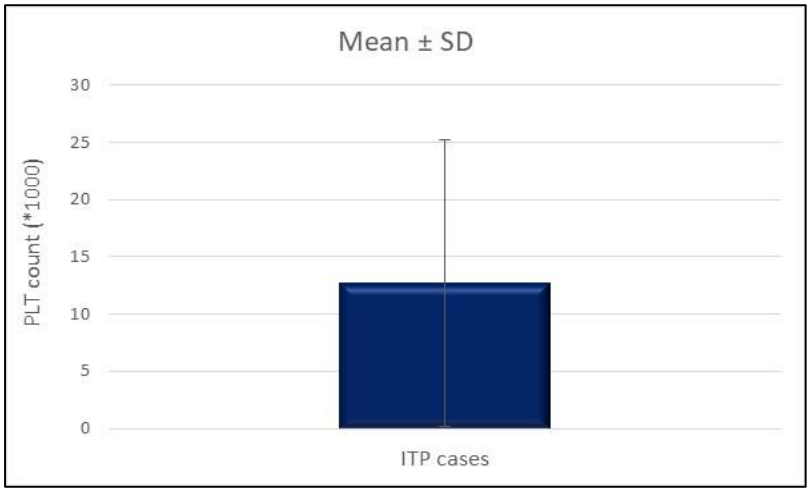

Figure 2: Platelet count (PLT count) in ITP cases.

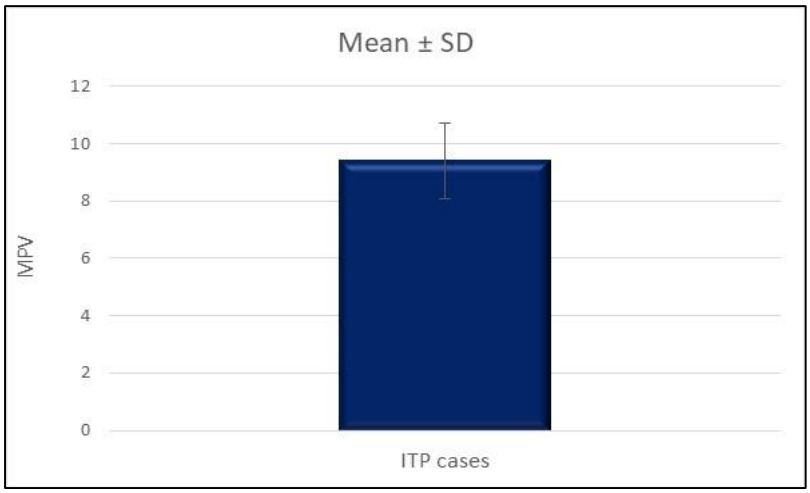

Figure 3: Mean platelet volume (MPV) in the cases.

Table 3: Clinical Data of the Studied ITP Cases:

Variables	ITP Cases
Preceding febrile illness	11 (27.5%)
Cutaneous bleeding (Petechiae & ecchymosis)	40 (100%)
Epistaxis	13 (32.5%)
Hematuria	2 (5%)
Oral bleeding	2 (5%)
Bleeding per rectum	1 (2.5%)
Organomegaly or lymphadenopathy	0 (0%)

Our results also showed that a high percentage of cases received corticosteroids (95%) and a low percentage (7.5%) of whom received immunosuppressive, 17.5 % received IVIG and 20% received thrombopoietin (TPO) receptor agonists among our cases **(Table 4, Figure 5).** Our data also found that 52.5% of the 40 newly diagnosed ITP patients were most likely to develop chronic ITP during the 6-month follow-up period **(Figure 6).** Additionally, our results showed that there was a significant statistical difference in IGFBP2 levels between the resolved non-chronic cases and the chronic cases (p <0.001).

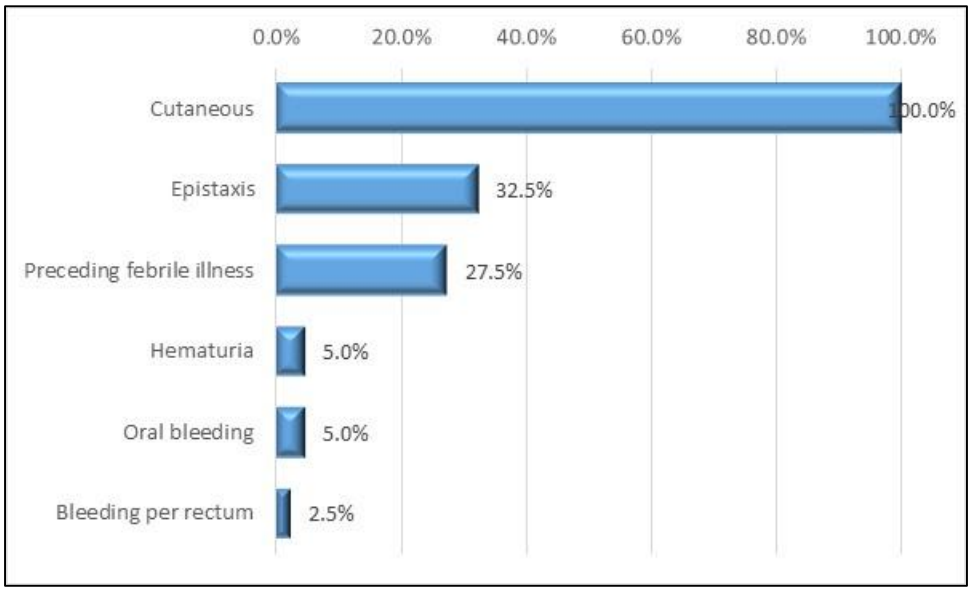

Figure 4: Clinical Data of the Studied ITP Cases.

Table 4: Lines of Treatment Administrated by the Studied ITP Patients:

Variables	N	%
IVIG	7 (17.5%)	17.5%
Corticosteroids (Oral or IV)	38 (95%)	95.0%
Immunosuppressive	3 (7.5%)	7.5%
Thrombopoietin receptor agonist	8 (20%)	

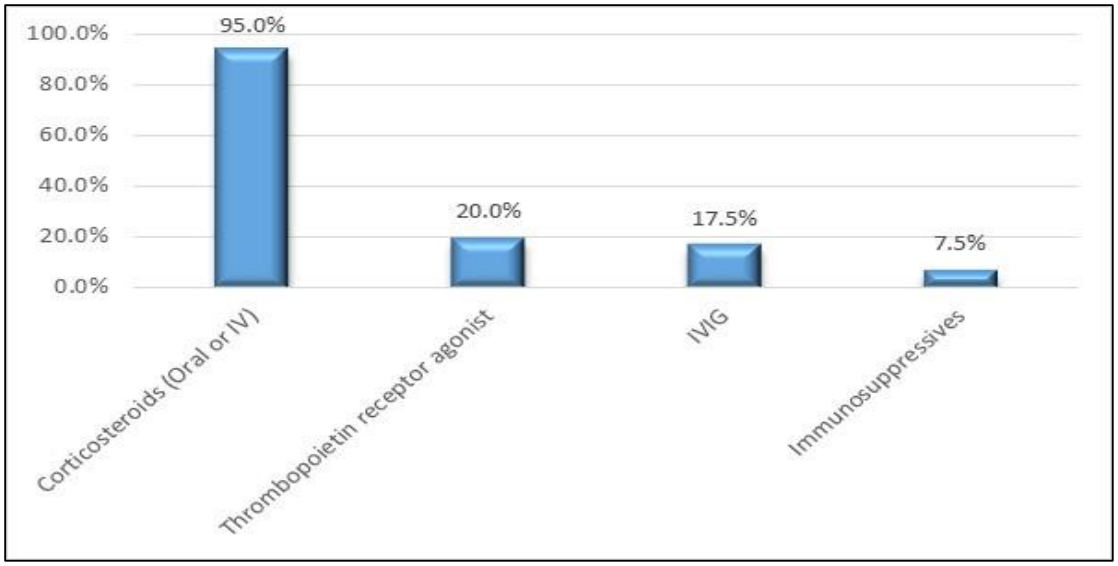

Figure 5: Lines of Treatment Administrated by the Studied ITP Patients.

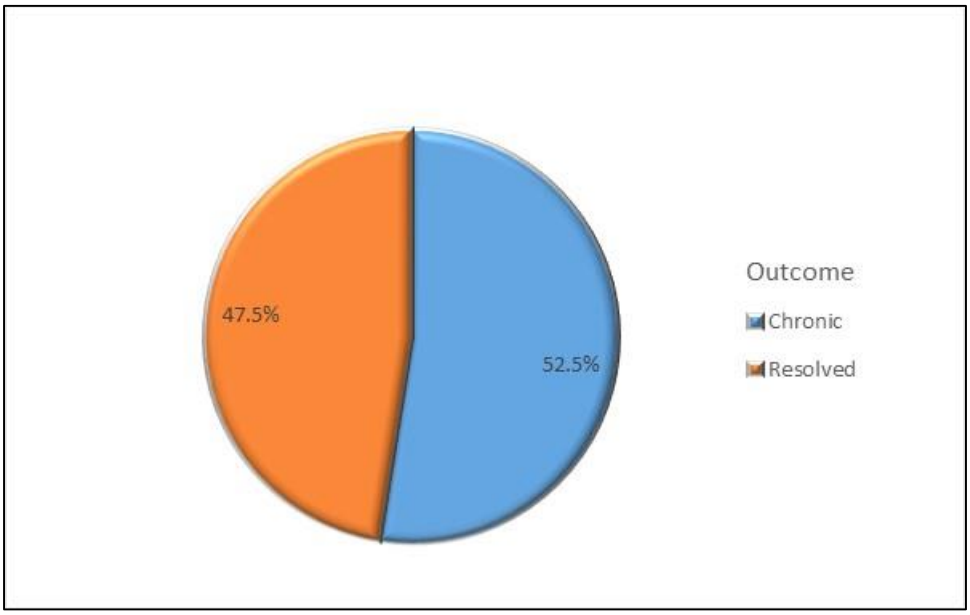

Figure 6: The Outcome of the Studied ITP Cases.

4. Discussion

Idiopathic thrombocytopenic purpura (ITP) is a hematological disorder characterized by a deficiency in the platelet numbers, resulting from the generation of auto-antibodies that attack platelets. This leads to isolated thrombocytopenia, without any other identifiable causes including

drugs, infections, tumors, or other forms of autoimmune illness [10].

Several investigations have shown evidence of programmed platelet cell death in children with acute ITP, including the activation of caspase 3, 8, and 9. This apoptosis was improved by the administration of an intravenous immunoglobulin infusion [11].

A study conducted in 2016 found that platelets from individuals with ITP showed increased membrane mitochondrial depolarization, active caspase 3, and contact with phosphatidylserine (PS). These findings were identified as markers of apoptosis. The study also ruled out basal platelet activation, evaluated by procaspase triggering compound 1 (PAC-1) binding and P-selectin externalization, as a possible cause of the increased PS expression [12].

Subsequent investigations validated these findings and demonstrated elevated levels of the pro-apoptotic protein BAK and BAX, whereas the anti-apoptotic component BCL-2 or BCL-XL was reduced [13].

The investigation was done at the Pediatric department of Fayoum University Hospital. It included 40 children from ITP cases.

In our study, females are affected more than males with their mean age (6.2 ± 3.2 years). This agrees with Al-Suheel, et al. (2014), who demonstrated the female predominance among ITP children [14].

On the other hand, Sutor et al. (2003), and Kuhne et al. (2001), reported that ITP in children has an equal impact on men and girls, however during infancy, boys are more often afflicted than females. Similarly, Lee et al. (2017), mentioned in a Korean study the incidence of ITP was higher in boys than girls among children under the age of 10 [15-17].

Cutaneous manifestations (petechiae and ecchymosis) were present in our patients' cohort at the first presentation which goes along with a previous study [15].

During monitoring our patients, we discovered that they could be categorized into two distinct categories. One cohort consisted of patients with a short period of thrombocytopenia lasting less than 6 months (47.5%), whereas the second cohort consisted of individuals with a longer duration of thrombocytopenia lasting more

than 6 months (52.5%). Badrawy, et al. (2013) have observed similar findings [18].

According to Edslev, et al. (2007), this group of patients with a short period of thrombocytopenia had a younger age, lower total leukocyte count, and lower platelet count compared to those with a longer duration [19].

The majority of our ITP cases with a history of preceding febrile illness recovered within six months and had resolved non-chronic course. This goes ahead with the findings of other previous studies [20, 21].

Regarding epistaxis manifestation at first diagnosis that was found more among those who had chronic outcomes in the present ITP cohort, unlike other studies reported by Al-Mulla et al., and Glanz et al. found that the presence of mucosal bleeding symptoms was negatively correlated with the likelihood of developing chronic ITP [22, 23].

We observed significantly higher levels of IGFBP2 at presentation in the non-chronic patients than the chronic patients after follow-up for 6 months, this agrees with Sarina Levy-Mendelovich, et al. (2021), who reported that When comparing the blood of chronic ITP patients with those of resolved non-chronic-juvenile-ITP patients, the Human Apoptosis Array revealed elevated levels of five apoptotic proteins: BIM, CD40, IGFBP2, P21, and SMAC [24].

5. Conclusion

In conclusion, the outcome of pediatric ITP cases could be predicted using the IGFBP2 apoptotic marker. We recommend to use IGFBP2 as a predictive marker of ITP course as in our study there was a significant statistical difference in its levels between non-chronic and chronic cases, at a sensitivity of 90.5% and a specificity of 68.4%. Also, to extended large-scale multicenter studies with more apoptosis markers to generalize our results. And finally, to extend the period of follow-up to report the effect of different lines of treatment on levels of apoptotic markers and outcome, which may help in the treatment plan.

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