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ORIGINAL ARTICLE

Study of the Role of Antiplatelet Antibodies in Children with Immune Thrombocytopenic Purpura

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ABSTRACT

Background: Platelet counts below 100×10^9/L characterize immune thrombocytopenia (ITP)in children, an uncommon autoimmune bleeding condition affecting 2 to 6 children per 100,000 annually. Fifty percent of kids get an infection first before they start showing signs of bleeding and developing thrombocytopenia. This study aimed to detect the possible correlation between anti-platelet glycoprotein-specific IgM and IgG antibodies and different types of ITP at initial diagnosis and at follow up.

Methods: We carried out this prospective cohort study at pediatric Hematology Oncology Unity, Faculty of Medicine, Zagazig University, on 30 children who had ITP. Anti-PLT antibodies (Ab) was assessed for all early diagnostic and chronic cases of ITP using Human anti-platelet antibodies (anti-PA Ab) ELISA Kit

Results: Regarding the type of ITP, acute was (40.0%), chronic was (46.7%) and persistent was (13.3%). Antiplatelet antibodies ranged between 41.19 and 244.73 with a mean (120.50± 59.57). There was a statistically significant increase among PLT at initial than follow-up (p=0.011). Regarding treatment, IVIG was (16.7%), Nplate was (33.3%), Revolade was (26.7%), Steroid was (10.0%), and Steroid and IVIG was (13.3%). A statistically significant difference was found between initial and follow-up treatment (p=0.004). **Conclusions:** The increased levels of antiplatelet antibodies in children with acute ITP suggest that these factors could play a role in ITP pathogenesis. Anti-platelet antibody testing could potentially be used as a rule-in test for childhood ITP, although anti-platelet antibody testing cannot be used to exclude a diagnosis of ITP.

Keywords: Antiplatelet Antibodies; Children; Immune Thrombocytopenic Purpura

INTRODUCTION

Platelet counts below 100×10^9/L characterize immune thrombocytopenia (ITP) in children, an uncommon autoimmune bleeding condition affecting 2 to 6 children per 100,000 annually. About half of newborns are thought to develop self-directed anti-platelet immunity before the start of thrombocytopenia and bleeding symptoms, and this may be due to epitope spreading or molecular mimicry. Platelet self-antigens provoke humoral and cellular immune responses, which contribute to the pathogenesis of ITP. Consequently, anti-platelet antibody testing could improve the diagnosis and prognosis of ITP [1].

It was found that a transferrable component in the plasma of adult ITP patients caused

thrombocytopenia in healthy individuals, indicating that anti-platelet antibodies play a pathophysiological role in ITP. Human gamma globulin included a component that could bind to platelets in its 7S fraction. The fibrinogen receptor glycoprotein (GP) IIb/IIIa has been identified as the first platelet antigen targeted by ITP anti-platelet antibodies, according to studies employing platelets from individuals with Glanzmann disease. Multiple platelet glycoproteins, such as GP Ib/IX and GP V complexes, are implicated. The prevalence of IgM anti-platelet antibodies in pediatric ITP has been hypothesized to be at least comparable to that of anti-platelet IgG antibodies. Anti-glycoprotein antibodies not only disrupt platelet clearance, but they can also functionally impede the aggregation of platelets by preventing the fibrinogen receptor contacts, and they can even decrease efficient thrombopoiesis by blocking GPIb-mediated hepatocyte thrombopoietin synthesis [2].

Because platelet-protein non-specific adsorption causes false-positive results in patients without immune-mediated thrombocytopenia, it was determined that early antibody tests focusing on detecting antibodies bound to entire platelets were not helpful. Subsequently, advances were made in the field of antigen-specific antibody testing, with methods such as monoclonal antibody-immobilization of platelet antigens (MAIPA) and immunobead technology being two examples, Because of their higher specificity, these are now widely used instead of the older whole-platelet assays. IgG anti-GP IIb/IIIa antibodies were found in twenty-seven percent of cases in the first glycoprotein-specific study of 15 children with acute ITP. However, no anti-GP Ib/IX antibodies were found. In a more recent study, researchers looked at the presence of IgG glycoprotein-specific antibodies in seventy-four newly diagnosed children with ITP [3].

Thirty-six percent of kids tested positive for IgG anti-GP IIb/IIIa antibodies, while thirty percent tested positive for IgG anti-GP Ib/IX antibodies. There was no correlation between short-term and long-term disease occurrences in this analysis. The difficulty in the clinical context liaising these data stems from the lack of description of inclusion criteria, controls, clinical features, and follow-up of included patients. Anti-GP Ib/IX antibodies among children who were newly diagnosed with ITP may be related to chronic disease. In contrast, according to a study conducted in China, anti-GP IIb/IIIa antibodies may be associated with temporary disease. Despite these studies suggesting a possible application, the diagnostic and prognostic efficacy of anti-platelet antibody testing is unknown [4].

This study aimed to detect the role of anti-platelet glycoprotein-specific IgM and IgG antibodies in the severity and prognosis of ITP.

METHODS

We carried out this prospective cohort study between September 2021 and September 2022 in the pediatric hematology oncology unit and clinical pathology department at Zagazig University hospitals. This study followed the guidelines [the World Medical Association's Code of Ethics (Declaration of Helsinki) for human studies]. All parents of participants provided informed and written consent. The Institutional Review Board has approved this research (#5753)

Inclusion Criteria: Children from both sexes aged between 1 year to 18 years, who have been newly diagnosed cases of ITP (up to 3 months after diagnosis), or persistent cases of ITP (from 3 months to 12 months after diagnosis), or chronic cases of ITP (more than 12 months after diagnosis).

Exclusion Criteria: Children who had secondary ITP (due to medications or because of a concurrent disease like systemic lupus erythematosus (SLE), autoimmune, infectious, or oncologic etiology).

All the included children were subjected to history taking, including personal, complaint, present, past, and family history. Clinical examination was done, including a general examination, with the classical petechial rash that did not blanch when pressure was applied with mucosal bleeding or hemorrhage. Mucocutaneous bleeding presented as ecchymosis on the skin, purpura, or petechiae.

Laboratory tests included Routine laboratory investigations, including complete blood count (CBC), bone marrow (BM) aspiration if indicated, and Anti-PLT Ab for all early diagnostic and chronic cases of ITP using Human anti-platelet antibodies (anti-PA Ab) ELISA Kit**.**

Anti-platelet antibodies (anti-PA Ab) in human serum were measured using this kit. Human anti-platelet antibodies (anti-PA Ab) are measured using a double-antigen sandwich enzyme-linked immunosorbent test (ELISA) with this kit. After pre-coating an enzyme well with human anti-platelet antibodies (anti-PA Ab)antigen, adding anti-platelet antibodies (anti-PA Ab), incubating, adding biotin-labeled PA-Ab antigen mixed with Streptavidin-HRP to form an immune complex, incubating again to remove uncombined enzyme, and so on. When Chromogen Solutions A and B are

added, the liquid turns blue; the color changes to yellow when acid is added. Human anti-platelet antibodies (anti-PA Ab) concentration was strongly linked with sample color intensity. All children were followed for 6 months to assess the prognosis of ITP.

STATISTICAL ANALYSIS:

Coding, entering, and analyzing the data obtained from the history, basic clinical examination, laboratory investigations, and outcome assessments were all done in Microsoft Excel. SPSS (Statistical Package for the Social Sciences) version 24 was used to analyze the data after it was imported. The significance of differences was examined using the following tests. The Chi-square test was used to analyze the differences between qualitative frequency variables and percentage differences between the groups. Parametric two-group comparisons of means (quantitative variables) using the t-test.

RESULTS

As regards age, it ranged between 2 and 14 years with mean (7.43± 3.56). Regarding sex, females were (56%) and males were (43.3%), regarding to duration, it ranged between .25 and 48 years with mean (14.82± 16.49). Regarding to type of ITP, acute were (40.0%), chronic were (46.7%) and persistent were (13.3%) (Table 1).

Antiplatelet antibodies ranged between 41.19 and 244.73 with a mean (120.50± 59.57) (Table 2).

There was a statistically significant increase among PLT at initial than follow-up (p=0.011). In contrast, non-statistically substantial differences were found between HB, WBCs, and antiplatelet antibodies at initial and follow-up (Table 3).

A statistically significant difference was found between initial and follow-up treatment (p=0.004) (Table 4).

Non statistically significant differences were found between sex, type of ITP and Antiplatelet antibodies (Table 5).

Table (1): demographic data among the studied cases (N=30).

		Range	Mean ± SD
Age (years)		2 - 14	7.43± 3.56
		No. (n=30)	**%**
Sex	**Female**	17	56.7
	Male	13	43.3
		Range	**Mean ± SD**
DURATION (months)		.25- 48	14.82± 16.49
		No.	**%**
TYPE OF ITP	**acute**	12	40.0
	chronic	14	46.7
	persistent	4	13.3

Table (2): Antiplatelet antibodies among the studied cases.

	Range	Mean ± SD
Antiplatelet antibodies (ng/ml)	41.19- 244.73	120.50± 59.57

Table (3): Relation between CBC, Antiplatelet antibodies at initial and follow up.

		Initial	follow up	t.test	P. value
HB	**Mean ± SD**	11.22± 1.17	11.46± .839	0.834	0.365
	Rang	8.90- 13.20	10.30- 13.20		
WBCS (10^3)	**Mean ± SD**	8.10± 3.21	9.02± 3.77	1.036	0.313
	Rang	5.50- 22.50	5.00- 22.50		
PLT	**Mean ± SD**	26700± 19001.17	16433.33± 9726.19	6.940	0.011
	Rang	4000- 71000	4000- 46000		
Antiplatelet antibodies	**Mean ± SD**	120.50± 59.57	138.85± 56.76	1.492	0.227
	Rang	41.19- 244.73	50.49- 244.73		

HB: hemoglobin, WBCS: white blood cells, PLT: platelets

Table (4): Relation between treatment at initial and follow up.

			Initial	follow up	X^2	P. value
Treatment	IVIG	No.	5	0	8.4090	.004
		%	16.7%	0%		
	Nplate	No.	10	16		
		%	33.3%	53.3%		
	Revolade	No.	8	14		
		%	26.7%	46.7%		
	Steroid	No.	3	0		
		%	10.0%	0%		
	Steroid&IVIG	No.	4	0		
		%	13.3%	0%		

Table (5): Relation between sex, type of ITP and Antiplatelet antibodies.

		Antiplatelet antibodies Mean ± SD	t.test	P. value
Sex	Female	125.82± 65.8	0.306	0.584
	Male	113.53± 51.96		
TYPE OF ITP	Acute	110.33 ± 48.22	0.495	0.615
	Chronic	122.27 ± 63.68		
	Persistent	144.77± 83.62		
	Present	122.51± 60.88		

DISCUSSION

Testing for glycoprotein-specific anti-platelet antibodies may help assess the prognosis and responsiveness to IVIg in children with newly diagnosed ITP. The most common type of anti-platelet antibody found in the blood is IgM. Short-lived IgM antibodies do not show signs of class change to IgG [4].

In the current study, regarding age, it ranged between 1 and 18 years with mean (7.43± 3.56). Regarding sex, females were (56.7%) and males were (43.3%). Two previous Pakistani investigations, by Farid et al. [5] and Mushtaq et al. [6], found that females were more likely to be diagnosed with acute ITP. However, a recent Japanese research of 7774 ITP cases between 2004 and 2007 found that boys were significantly overrepresented in the 0–4 age range [7].

Our study showed that, at Initial ITP, petechiae & bruises were present in (90.0%). Regarding bleeding, from the nose was (16.7%), nose &teeth were (13.3%), heavy menses, nose teeth &gum, teeth &gum were (10%) each, blood in stool was (6.7%), blood in urine &stool, gum was (3.3%) each while no bleeding was present in (26.7%). Regarding, intra cranial bleeding was present in (3.3%), and no Cranial bleeding was present in (96.7%).

Even though over half of the patients in one big study group reported either no bleeding or a minor amount of bleeding, Zafar et al. [8] stated that most of the patients who presented had a clinically substantial bleed that required medical treatment.

In this study, antiplatelet antibodies ranged between 41.19 and 244.73 with mean (120.50± 59.57). Investigating patients with pediatric ITP compared to healthy controls and non-immune thrombocytopenic controls revealed moderate sensitivity and good specificity, suggesting that antibodies were present in some but not all of these individuals. [9,10]. Schmidt et al. [10] focused on determining the frequency and clinical importance of IgM and IgG antibodies against platelet

glycoproteins. Using a recently developed glycoprotein-specific IgM MAIPA, they found a significant prevalence of IgM anti-platelet antibodies. Anti-platelet antibodies in ITP patients frequently attack several platelet GPs [11].

Our study revealed that, regarding treatment, IVIG was (16.7%), Nplate was (33.3%), Revolade was (26.7%), Steroid was (10.0%), and Steroid & IVIG was (13.3%).

Evidence-based practice recommendation for immune thrombocytopenia from the American Society of Hematology, 2011: Platelet count is not a consideration for managing children with no bleeding or minor bleeding (defined as cutaneous symptoms alone, such as bruises and petechiae). A single dose of intravenous immunoglobulin (0.8-1 g/kg for 1 or 2 days, or 0.4 g for 3-5 days) or a brief course of corticosteroids are recommended as initial treatments for pediatric patients. Rh-positive, non-splenectomized children can be treated well with a single dosage of anti-DChildren and adolescents with ITP who continue to bleed heavily while receiving IVIG, anti-D, or standard dosages of corticosteroids may benefit from high-dose dexamethasone. Surgical options like splenectomy or those with a high risk of complications should be avoided for at least a year [12].

The present study found a non-statistically significant correlation between antiplatelet antibodies (ng/ml) and other numerical data.

Platelet-associated antibodies (PA IgG and PA IgM), activated platelets, and serum leptin were studied by Badrawy et al. [13] in children with acute ITP. They discovered that the percentage of activated platelets was negatively correlated with the overall number of platelets, which could indicate that their patients experienced acute ITP in conjunction with platelet activation. Because platelet activation may serve as a compensatory strategy to decrease bleeding that occurs due to thrombocytopenia, many ITP patients may not experience substantial bleeding despite severe thrombocytopenia.

Patients with ITP have platelet auto-antibodies that stimulate their healthy platelets [14]. The percentage of CD62p-positive platelets was considerably higher in ITP patients than in other patients when assessed by Shao et al. [15].

The current study found a non-statistically significant difference between type OF ITP and Antiplatelet antibodies.

Patients with acute and chronic ITP had greater levels of PAIgG than controls. In comparison, patients with acute ITP had considerably higher levels of PAIgM than controls, according to research by Yildirmak et al. [16]. Patients with ITP had higher levels of PAIgG, PAIgM, and PAIgA than controls, and these levels fell after therapy, as described by Shao et al. [15].

Simultaneously developing several platelet antibodies in childhood, ITP provides serological evidence for the previously noted link between anti-GPIIb/IIIa and anti-GPIb IgG-producing B cells in circulation [17].

According to our study, antiplatelet antibodies were not significantly different between the initial and follow-up assessments. Once age and history of infection are accounted for, Schmidt et al. [10] found that having IgM anti-platelet antibodies was related to a better prognosis for a disease's spontaneous recovery.

Evaluation of glycoprotein-specific IgG antibodies in 74 children with either short- or long-term illness courses by Biglino et al. [18] revealed no correlation with outcome. Surprisingly, they found that greater than 30% of patients had circulating IgG antibodies, which could be due to changes in the patient group they studied (selection bias) or to variations in the technical performance of MAIPA.

Results from various low-risk-of-bias studies, including case-control and prospective cohort designs, led Schmidt et al. [19] to conclude that anti-platelet antibody testing has good diagnostic accuracy. The intermediate sensitivity seen in different investigations may be due to differences in the pathomechanisms involved, such as anti-platelet clearance mediated by antibodies or T cells [21]. Potential reasons include insufficient antibody levels in the bloodstream during platelet-antibody immune complex active clearance, poor antibody avidity, test insensitivity, and variation in the underlying path mechanisms.

Several investigations found that anti-platelet antibody levels were lower in individuals evaluated later in the disease course or after therapy, suggesting that testing delays could affect diagnostic accuracy [22].

The role of platelet autoantibody testing in diagnosing ITP was recently examined in a systematic review and meta-analysis published by Vrbensky et al. [22]. A positive autoantibody test result can help rule in ITP, but a negative impact does not rule out ITP, the researchers found on testing for autoantibodies in patients with ITP. Similar conclusions were achieved for autoantibody detection in a recent systematic review of platelet

autoantibody tests in pediatric ITP by Schmidt et al. [19].

Limitations

There are certain limitations in our study. Firstly, the sample size might be relatively small, with 30 cases. The results may not apply to a broader population because of this. Secondly, since the study was conducted in a single outpatient clinic of a specific hospital, there is a potential for selection bias. The patient population might not fully represent the diversity and characteristics of all individuals with ITP. This could affect the external validity of the study.

CONCLUSIONS

Children with acute ITP had higher than normal levels of antiplatelet antibodies, which may indicate a role for these variables in the pathogenesis of ITP. Antiplatelet antibody testing could be used as a rule-in test for pediatric ITP; however, this method cannot rule out ITP.

Conflict of Interest: Nothing to declare.

Financial Disclosures: Nothing to declare

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Citation

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