Vitamin D Status in Children with Immune Thrombocytopenic Purpura (ITP) in Assiut University Children's Hospital

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Abstract

Introduction: Vitamin D (VD) and its prohormones have been the focus of a growing number of studies in the past few years, demonstrating their function not only in calcium metabolism and one formation but also their interaction with the immune system, which is not surprising since VD receptors are expressed in different tissues, such as brain, heart, skin, bowel, gonads, prostate, breast and immune cells, bones, kidneys, and parathyroid.

Aim: To assess VD status in acute and chronic immune thrombocytopenic purpura (ITP) pediatric patients compared to normal-control children and to find any association between VD deficiency and ITP.

Patients and Methods: This study included 48 children diagnosed with ITP admitted to the Hematology Unit at Assiut University Children's Hospital and 30 normal matchable controls. Our patients were subjected to full history taking and thorough clinical examination. ITP patients were classified as acute and chronic. Both patients and controls were subjected to complete blood count (CBC) and serum total 25-OH vitamin D.

Results: VD levels were lower in patients with acute and chronic ITP than in normal healthy controls but not statistically significant (P = 0.6). VD deficiency was significantly more in patients with chronic ITP in comparison with normal healthy controls (P = 0.044).

Conclusion: We concluded that vitamin D deficiency may have a role in the pathogenesis of immune thrombocytopenia, especially chronic ITP. Further studies are needed to investigate whether vitamin D supplementation is helpful in the treatment of ITP by modulating the immune system.

Keywords: Assiut Children University Hospital, idiopathic thrombocytopenic purpura, vitamin D.

Introduction

Immune thrombocytopenic purpura (ITP) describes an autoimmune disorder in which the number of circulating platelets is reduced [1]. This is due to their increased destruction and sometimes also due to reduced production [2].

ITP in children most commonly occurs following a viral infection or occasionally following immunization. It is usually a self-limiting condition that follows a benign course and resolves spontaneously within 6–

Eight weeks [3]. The clinical manifestations range from no symptoms to life-threatening intracranial hemorrhage. Treatment decision is based on clinical symptoms and not on the platelet count alone since there is rarely a significant risk of bleeding in children with severe thrombocytopenia [4].

The role of vitamin D (VD) and its prohormones in calcium metabolism and bone formation, as well as their interaction with the immune system, have been the focus of an increasing number of studies in recent years. This is not surprising, as VD receptors are expressed in a variety of tissues, including the brain, heart, skin, bowel, gonads, prostate, breast, and immune cells, in addition to bones, kidneys, and parathyroid [5].

A few autoimmune disorders, including rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, systemic lupus erythematosus, and insulin-dependent diabetes mellitus, have been related to VD deficiency in some recent studies. Based on these associations, it has been proposed that VD is an extrinsic factor that can influence the prevalence of autoimmune diseases [6].

The mechanisms underlying the link between VD and autoimmunity are not completely identified but probably are related to its anti-inflammatory and immune-modulatory functions.

VD appears to interact with the immune system through its action on the regulation and differentiation of cells like macrophages, lymphocytes, and natural killer cells, in addition to interfering in the in vivo and in vitro production of cytokines. The decrease in interferon-γ synthesis induced by VD may be one of the methods of VD immune-modulatory action. New insights regarding the effect of vitamin D3 on the interferon-γ gene repression further emphasize the role of the vitamin D3 derivatives as an immunomodulator [7].

Several studies have shown that VD downregulates tumor necrosis factor (TNF)-α-associated genes. Higher serum VD levels correlated with lower TNF-α levels.

There is an inverse association between TNFα levels and VD in a healthy population, and a protective association has been found for VD against inflammatory diseases such as heart disease and rheumatoid arthritis. However, additional studies are needed to accurately characterize the relationship between VD and TNFα. VD treatment may hold promise as an adjunct to anti-TNFα drugs in autoimmune diseases such as ITP [8].

The ideal VD level required to ensure normal immune system function has not yet been determined. This level is likely different from the level needed to prevent VD insufficiency or maintain calcium hemostasis. It has been suggested that VD and its analogs prevent the development of autoimmune diseases and can be used therapeutically to treat them [9].

VD can be administered as a new immune-modulatory therapy in children and adults with ITP. Bockow and Kaplan described two adult patients with refractory ITP initially treated with high-dose corticosteroids that were successfully treated with mega-dose VD-replacement therapy and hydroxychloroquine. An association between VD deficiency and ITP was found, but they could not explain the pathophysiological mechanism of immune-modulatory action [1].

In this study, our objectives were to assess VD status in acute and chronic

immune thrombocytopenic purpura (ITP) pediatric patients compared to normal-control children and to find any association between VD deficiency and ITP.

Patients and Methods

This case-control study included 48 cases of children diagnosed as immune thrombocytopenic purpura (ITP) at the Hematology Unit at Assiut University Children's Hospital and 30 normal age and sex-matchable controls.

The study received ethical committee approval (IRB: 04-2024-200872) from the Faculty of Medicine Assiut University, Egypt. Written consent was obtained from all the children's caregivers.

Our patients were subjected to full history taking and thorough clinical examination. ITP patients were classified as acute and chronic according to the duration and mild, moderate, and severe according to the severity of bleeding. Both patients and controls were subjected to the following:

(1) Complete blood count (CBC).

(2) Serum total 25-OH vitamin D.

A 3 ml volume of venous blood was collected after complete aseptic conditions into a plain tube, left to clot for 30 min at 37° C, and centrifuged at 3000 rpm; the separated serum was divided into aliquots for routine investigations. EDI measured the total 25-OH vitamin D (vitamin D2 and vitamin D3), and the total 25-OH vitamin D EIA kit was supplied by EDI ref no. 815.

The principle of this test was designed, developed, and produced for quantitative measurement of a total of 25-OH vitamin D2 and D3 in serum utilizing the competitive immunoassay technique. This assay used a monoclonal antibody bound to both 25-OH vitamin D2 and 25-OH vitamin D3 equally.

According to the serum 25-(OH) D3 level, the three groups were further divided into deficiency (< 20ng/ml), insufficiency (20-29 ng/ml), and sufficiency (30-100ng/ml) [10].

Statistical Analysis

Data entry and analysis were done using Statistical Package for the Social Sciences program, version 20 (SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean + standard deviation (SD) of the mean. Differences between the groups were examined for statistical significance using the Mann-Whitney test. A p-value of less than 0.05 was considered statistically significant.

Results:

The present study included 24 children with acute ITP and 24 children with chronic ITP in addition to 30 normal controls. **Table 1** shows the demographic data of the 3 studied groups, with patients with chronic ITP significantly older than those of acute and control groups. There were no statistically significant differences between the three groups as regards sex and residence. **Table 2** showed no statistically significant difference between acute and chronic ITP groups regarding type of bleeding or degree of severity. **Table 3** shows the CBC data of the studied groups and reveals no statistically significant difference between acute and chronic ITP patients regarding platelet count. We found that vitamin D deficiency was more common in patients with ITP (acute and chronic) than in normal healthy controls but without statistical significance ($P = 0.6$), as shown in **Table 4**. However, VD deficiency was significantly higher in

patients with chronic ITP in comparison with normal healthy controls ($P = 0.044$).

Table 1 Data collected from 48 patients and 30 control in relation to age, sex, and residence

	Acute ITP [*n* (%)]	Chronic ITP [*n* (%)]	Control [*n* (%)]	P_1	P_2	P_3	P_4
Age (years)							
Range	2-12	2.4-18	2-13	0.003**	0.039*	0.238	0.026*
Mean ± SD	5.31±3.39	9.2±4.58	6.64±3.55				
Sex							
Male	12 (50)	12 (50)	18 (60.0)	0.690	0.773	0.646	0.646
Female	12 (50)	12 (50)	12 (40.0)				
Residence							
Rural	22 (91.7)	18 (75)	23 (76.7)	0.263	0.245	0.270	0.859
Urban	2 (8.3)	6 (25)	7 (23.3)				

ITP, idiopathic thrombocytopenic purpura; P_1, comparison between ITP patients (acute and chronic) and control groups; P_2, comparison between acute ITP and chronic ITP; P_3, comparison between acute ITP and control; P_4, comparison between chronic ITP and control.

*Statistically significant difference ($P < 0.05$). **Highly statistically significant difference ($P < 0.01$).

Table 2 Clinical manifestations and therapeutic intervention

	Acute ITP (n=24) [*n* (%)]	Chronic ITP (n=24) [*n* (%)]	P_1	P_2	P_3	P_4
Ecchymosis	14 (58.3)	22 (91.7)	0.243	0.243	-	-
Mucosal bleeding	13 (54.2)	19 (79.2)	0.377	0.377	-	-
Purpura	18 (75)	21 (87.5)	0.749	0.749	-	-
Bleeding after circumcision	0	1 (4.2)	-	-	-	-
Intran cranial hemorrhage	0	2 (8.3)	-	-	-	-
ITP grading according to severity						
Mild	21 (87.5)	21 (87.5)	<0.001**	0.364	<0.001**	<0.001**
Moderate	3 (12.5)	1 (4.2)				
Severe	0	2 (8.3)				
Treatment						
No treatment	7 (29.2)	4 (16.7)	<0.001**	0.242	<0.001**	<0.001**
IVIG	3 (12.5)	1 (4.2)				
Corticosteroid	14 (58.3)	6 (25)				
Others (platelet-rich plasma)	0	2 (8.3)				

ITP, idiopathic thrombocytopenic purpura; P_1, comparison between ITP patients (acute and chronic) and control groups; P_2, comparison between acute ITP and chronic ITP; P_3, comparison between acute ITP and control; P_4, comparison between chronic ITP and control.

*Statistically significant difference ($P < 0.05$). **Highly statistically significant difference ($P < 0.01$).

Table 3 Laboratory data of the investigated cases

	Acute ITP (*n*=24)		Chronic ITP (*n*=24)		Control (*n*=30)		P_1	P_2	P_3	P_4
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD				
WBCS	3.7-15.4	7.67±2.87	4.1-11.4	7.02±2.32	4.4-14.4	9.02±2.86	0.025*	0.074	0.009	0.409
Hb (mg/dl)	8.4-12.6	10.93±1.12	9-13.3	11.24±1.03	9.5-15	11.81±1.13	0.114	0.323	0.061	0.334
PLT 10^3/µl	3-100	20.31±36.71	10-50	10.02±15.22	179-0.489	314±72.85	0.006**	0.211	< 0.001**	< 0.001**
MPV	5.3-26.3	8.81±4.4	4.5-36	9.95±6.47	4.1-10.1	7.27±1.37	0.093	0.222	0.032*	0.396
Bone marrow biopsy [*n* (%)]	3(12.5)		24 (100)		0		<0.001**	<0.001**	0.163	<0.001**

Hb, hemoglobin; ITP, idiopathic thrombocytopenic purpura; P1, comparison between ITP patients (acute and chronic) and control groups; P2, comparison between acute ITP and chronic ITP; P3, comparison between acute ITP and control; P4, comparison between chronic ITP and control; PLT, platelet; WBC, white blood cells. *Statistically significant difference (P < 0.05). **Highly statistically significant difference (P < 0.001).

Table 4 Serum vitamin D level regarding the degree of deficiency of vitamin D in the three studied groups

	Acute ITP (*n*=24) [*n* (%)]	Chronic ITP (*n*=24) [*n* (%)]	Control (*n*=30) [*n* (%)]	P_1	P_2	P_3	P_4
Serum vitamin D level							
Deficiency	18 (75)	19 (79.17)	19 (63.33)	0.698	0.835	0.652	0.044*
Insufficient	4 (16.67)	4 (16.67)	7 (23.33)				
Sufficiency	2 (8.33)	1 (4.17)	4 (13.33)				

ITP, idiopathic thrombocytopenic purpura; P1, comparison between ITP patients (acute and chronic) and control groups; P2, comparison between acute ITP and chronic ITP; P3, comparison between acute ITP and control; P4, comparison between chronic ITP and control.

Discussion

Our study included 3 groups (acute ITP, chronic ITP, and normal controls). There were no statistically significant differences between the three groups regarding sex and residence. Still, the children of the chronic group were older than those of the acute and control groups. In our study, platelet values did not follow the VD level. In 2017, Yon Chul Park and colleagues did a study illustrating the association between VD level, platelet count, and mean platelet volume (MPV) in adults. They found that VD insufficiency and sufficiency groups had a significantly lower platelet count and MPV than the deficiency group; they also found that platelet count was inversely related to VD level in their study. No previous studies have demonstrated increased platelet count in patients with VD deficiency [11].

In the present study, VD levels were lower in patients with acute and chronic ITP than in normal healthy controls but not statistically significant (P = 0.6). Our results agree with the results of Soliman and Fattizzo and colleagues, who found significantly lower mean 25(OH) D levels among ITP adults compared with the healthy control group [12,13].

Regarding the degree of deficiency of VD in the three groups, it was found that VD levels were significantly deficient in our patients with chronic ITP in comparison with normal healthy controls (P = 0.04). Still, there was no statistically significant difference between the levels in acute ITP cases and controls. This is inconsistent with the results of Mu and colleagues, who studied 25-hydroxyvitamin D3 [25(OH)D3] and 1,25-dihydroxy vitamin D3 [1,25(OH)2D3] levels in 45 ITP patients and 30 normal controls. The result showed that the levels in newly diagnosed ITP patients were lower than those of normal controls [14].

Another study performed by Čulić and colleagues on pediatric patients included an assessment of vitamin D status in children with ITP. The study was done on a total of 21 cases of chronic ITP and newly diagnosed ITP patients. Only three patients were newly diagnosed, and none with

chronic ITP had normal VD levels. VD deficiency was found in 11 patients with newly diagnosed ITP and seven patients with chronic ITP. Newly diagnosed ITP patients had statistically significantly higher values ($P < 0.044$) of VD than the patients with chronic ITP [15].

Vitamin D deficiency may contribute to immunological abnormalities that lead to the development of chronic form of ITP, and vitamin D supplementation may lower the risk of chronic disease by modifying the immune system [5].

Conclusion:

VD deficiency is common in children with ITP and may have a role in its pathogenesis, especially chronic forms of ITP. Therefore, vitamin D supplements may be used as adjuvant therapy and immune-modulating agents in chronic ITP children.

Recommendations:

We recommend VD assay for ITP cases, which are either acute or chronic. VD supplementation for vitamin D deficient cases. Further prospective studies with a larger number of patients are necessary.

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Conflicts of interest

All authors have no conflict of interest to report.

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