

Study of Serum Vitamin D Levels in Children with Immune Thrombocytopenic Purpura

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Immune thrombocytopenic purpura (ITP) is described by an immune responding versus the host's own platelets, in recent years is progressively studied the non-calcemic roles of vitamin-D (VD) that controls immune and inflammation responding.

Aim and objectives: The current work aimed to study VD-level in children with ITP and influence of VD supplementing upon the responding of the thrombopenia to conventional therapy of ITP.

Subjects and methods: This study is a cross-sectional observational work which included 30 ITP-children who were attendants to Hematology and Oncology Unit, Pediatric Department, Tanta University Hospitals with ages from 2 to 16-yrs with mean ageing of 6.43 ± 3.75 -yrs, for all patients and controls serum-levels of 25-hydroxyvitamin D (25[OH]D) were measured.

Results: A significant change was found among the studied groups in regard to VD-levels with lower values among patients compared with controls, a statistically significant negative association was found among platelet counts and each of vitamin D level and serum Ca, the mean platelet count after conventional therapy was significantly increased in group one ITP patients.

Conclusion: VD lack is very frequent in children with recently identified or chronic ITP form. Consequently, there are advantages of supplement VD in ITP-cases.

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1. INTRODUCTION

Immune thrombopenia (ITP) of children is described by isolate thrombopenia (platelet counts <100,000/mm³ with ordinary white blood cells counts and hemoglobin). The reason of ITP still unclears in most patents, but it could be generated by virus infecting or other immunological or environment trigger [1].

It is an immune-mediating disease produced by auto-antibodies versus glycol-proteins (GP) IIb/IIIa and GPIb/IX originate in the platelet membranes [2].

In accordance to latest strategies from the International Working Group, ITP is clinically sorted into 3-subtypes, anew diagnosed (within 3-mths from diagnosing), persisting (for children with ITP lasting from 3 to 12-mths from diagnosing; this comprises cases that don't reached spontaneous remission or don't preserve comprehensive responding of treatment), and chronic ITP (durable for at least 12-mths) [2].

Severe ITP is characterized by the existence of blood losing symptoms at presentation that command treating or incidence of novel blood losing needing new treatments like platelet enhancement agents or growing the dosages of earlier utilized medications [2].

VD is a fat-soluble vitamin that is obviously existing in very few foods. VD could be formed from skin upon sunlight exposures; VD in foods is biologically inert and must undergoes 2 hydroxylation in the body for activating. The first occurrence in the liver and adapts vitamin D [25(OH)D], as well known as calcidol. The 2nd occurs initially in the kidney and procedures the physiologically active 1,25 dihydroxy-vitamin D [1,25(OH)2D], as well known as calcitriol [3].

In recent years is progressively studied the non-calcemic roles of vitamin-D (VD) that controls immune and inflammation responding. Immune cells expressing VD nuclear receptors (VDR) and are capable for metabolization of VD. This hormone is a secosteroid (steroid with an opened B-ring) that is unlimited to its familiar roles in calcium homeostasis. Similar to other steroid hormones, its actions are intermediated by the VDR, that belonging to the super-family of steroid and thyroid hormones receptors [4].

VD as well has a role in regulating the hematopoietic-system modulating lymphocyte activating and proliferating, inducing the differentiating of promyelocytes into monocytes, and prevents secreting many cytokines in T-cells [5].

In specific, various studies have been reported the ability of VD to suppress the synthesis of interferon- γ (IFN- γ) and interleukin-2 (IL-2) in peripheral blood lymphocyte (PBL) and T-cell line [5].

Epidemiological idea would be to link low levels of VD with a tendency towards infections and autoimmune diseases [6].

These hormones stimulating interleukin 4 (IL-4) producing and Suppressing inflammation of T-cell activity [6].

The current work aimed to study VD-level in children with ITP and influence of VD supplementing upon the responding of the thrombopenia to conventional therapy of ITP.

2. PATIENTS AND METHODS

The current study was a cross-sectional observational work which included 30 children with immune thrombocytopenic purpura who were attendants to Hematology and Oncology Unit, Pediatric Department, Tanta University Hospitals, Tanta, Egypt from 13 March 2019 to 7 July 2019. This study included also 30 children healthy of matching controls regarding ages and gender.

2.1 Inclusion Criteria

ITP children aged 2 - 18 yrs.

2.2 Exclusion Criteria

Children with other causes of thrombopenia and Children with ITP on Vitamin D supplementation.

2.3 Methods

All cases in the current work were exposed to the next:

2.3.1 A- A full history-taking with special emphasis on

History of upper respiratory tract infections before the beginning of the disease, date of

disease onset, Duration of disease, History of recurrence, Detailed nutritional and developmental history and History of vaccination before the onset of the disease.

2.3.2 B - Clinical examination with special emphasis on

General look and appearance of the child, Important signs including Heart rates, breathing rates, pressure of the blood and temp., Anthropometrical measures including body mass, tallness, head perimeter, mid upper arm circumference, Complete systemic examination including chest , heart , central nervous system and abdomen with especial comment on liver and spleen state, Skin , soft tissues , LNs for Purpura, Echymosis and Lymphadenopathy, Clinical examination for different presentation of immune thrombopenia: Purpura, Echymosis and Mucous membrane bleeding and Clinical examination for different clinical manifestations of vitamin D deficiency and rickets Broad epiphysis, Ricketic rosaries, Box shaped head, Bow legs, Marfan sign, Genua varum deformity, Pigeon breast, Frontal bossing and Delayed teething.

2.3.3 C- Laboratory investigations including

Complete blood count including platelet, Bone marrow aspiration, Serum level of 25 hydroxyvitamin D, calcium, phosphorus, magnesium, Alkaline phosphatase and Parathyroid hormone.

2.4 Administrative and Ethical Design

A written informed consent was obtained from the all involved case's parents. The current work had been permitted by the ethics committee on research concerning human subjects of Medicine Faculty, Tanta University.

2.5 Data management and Statistical Analysis

Collected data were recorded then presented and analyzed statistically by computer using SPSS-22(SPSS Inc. Chicago, IL, U.S.A) as follow: Editing and coding, Data entry in computer. Data were summarized and presented in tables and graphs and summarized as median and mean \pm standard deviation (SD) for

quantitative variables and as numbers and percentages for qualitative variable. P value < 0.05 were considered significant.

3. RESULTS

Regarding sex and ages, nonsignificant differences was found among the groups involved in this work Table (1).

A significantly lower platelet count was found in studied patients compared with controls while nonstatistical changes regarding levels of hemoglobin, leukocyte counts, and RBCs counts, mean corpuscular size, mean corpuscular hemoglobin concentration were found but mean reticulocyte counts was significantly high in studied cases in comparison to controls. Table (2).

There was statistically significant change among the studied groups regarding vitamin D level with lower values among studied patients compared with controls. Table (3).

most of the studied patients have normocellular bone marrow (two thirds of cases) while one third of studied patients shows Hyper cellular bone marrow, with increased megakaryocytes in 26.7% of the studied patients and weak productive capacity in 76.7 % of studied patients. Table (4).

A statistically significant positive correlation was found between platelet count and vitamin D level and serum Ca. There is no correlation between platelet count and serum magnesium. Table (5).

The mean platelet count after conventional therapy was significantly higher than before conventional therapy in group one ITP patients and the mean platelet count after conventional therapy with VD was significantly high in comparison to before conventional therapy with VD while before induction of treatment in the two groups, There was statistically non-significant difference between them concerning platelet count and after induction of treatment in the studied cases , the mean platelet counts was significantly high in group two ITP cases under conventional therapy with vitamin D than in group one ITP patients under conventional therapy Table (6).

Table 1. Comparison among the study groups in regard to demographic info

Demographic data	Patients (n=30)		Controls (n=30)		Test of sig.	P
	No.	%	No.	%		
Sex						
Males	15	50	11	36.7	$\chi^2=1.086$	0.297
Females	15	50	19	63.3		
Age (yrs)						
Min. –Max Rang.	16 –2		15 –2		t=1.197	0.236
Mean ± SD.	6.43 ± 3.75		7.55 ± 3.46			
Median (IQR)	5 (9 –4)		8 (9–5)			
Disease duration (weeks)						
Min. –Max Rang.	2 –11		-		-	-
Mean ± SD.	5.4 ± 2.47		-			
Median (IQR)	5 (4 –8)		-			

χ^2 : Chi square testing t: Student t-testing IQR: interquartile range
p: p-value for comparison of the study groups

Table 2. Comparison among the study groups regarding complete blood count

Routines investigation	Patient (n=30)	Control (n=30)	t	P
Platelet count ($\times 10^3$)				
Max.–Min.	78 – 6	420 – 200	27.449*	<0.001*
Mean ± SD.	23.75±44.93	52.86±331.67		
Median (IQR)	45 (33 – 65)	335 (290 – 370)		
Hb % (g/dl)				
Max.–Min.	13 – 8.5	11 – 9	0.915	0.365
Mean ± SD.	10.38 ± 1.04	10.58 ± 0.67		
Median (IQR)	10.5 (10– 11)	10.75 (10 – 11)		
Leukocyte count				
Max.–Min.	12000 – 5600	12700 – 6700	0.89	0.377
Mean ± SD.	1488.83±8834.5	1367.04±9163		
Median (IQR)	8900(8200 – 9700)	9150(8600 – 9800)		
RBCS count				
Max.–Min.	5.1 – 4.5	5.1 – 4.8	2.199*	0.034*
Mean ± SD.	4.93 ± 0.15	4.99 ± 0.05		
Median (IQR)	5 (4.9 – 5)	5 (4.9 – 5)		
MCV (fl)				
Max.–Min.	99 – 77	90 – 80	1.587	0.12
Mean ± SD.	86.53 ± 7.54	84.10 ± 3.69		
Median (IQR)	86 (79 – 90)	85 (80 – 87)		
MCH (pg)				
Max.–Min.	33 – 28	32 – 28.5	1.084	0.283
Mean ± SD.	29.62 ± 1.16	29.93 ± 1.1		
Median (IQR)	29.75 (29 – 30)	30 (29 – 31)		
MCHC (g/dl)				
Max.–Min.	35.5 – 33.4	35.4 – 33.9	0.118	0.907
Mean ± SD.	34.28 ± 0.62	34.3 ± 0.47		
Median (IQR)	34 (33.9 – 34.9)	34 (34 – 34.7)		
Reticulocyte count				
Max.–Min.	4 – 0.5	1.9 – 0.8	2.875*	0.007*
Mean ± SD.	1.73 ± 0.88	1.24 ± 0.28		
Median (IQR)	1.5 (1 – 2.5)	1.1 (1 – 1.5)		

t: Student t-testing, Hb: Hemoglobin, MCV: mean corpuscular size
MCH: mean corpuscular hemoglobin RBCs: Red blood cells
MCHC: mean corpuscular hemoglobin concentrations
p: p-value for comparison of the study groups IQR: interquartile range
*: significant at p-value ≤ 0.05

Table 3. Comparison among the study groups regarding vitamin D level

Specific data	Patients (n=30)	Controls (n=30)	T	P
VD levels ng/MI				
Min. –Max.	30 – 4.7	48 – 4.1	2.461*	0.02*
Mean ± SD.	10.37 ± 5.4	28.69 ± 11.31		
Median (IQR)	8.5 (7 – 12)	9.8 (6.2 – 33)		

t: Student t-testing, IQR: interquartile range
 p: p-value for comparison of the study groups
 *: significant at p-value ≤ 0.05

Table 4. Bone marrow cellularity in studied patients with ITP

Bone marrow	No.	%
Cellularity		
Normo cellular	20	66.7
Hyper cellular	10	33.3
Megakaryocytes (increased)	8	26.7
Productive capacity (weak)	23	76.7

Table 5. Correlation among platelet counts and specific data in patient group

Specific data	Platelet count	
	R	P
Vitamin D level	0.34	0.046*
Serum total Ca (mg/dl)	0.371	0.044*
Serum Mg (mg/dl)	- 0.179	0.345

r: Pearson coefficient
 *: significant at p-value ≤ 0.05

Table 6. Comparison among the study groups in regard to platelet count before and after treatment

Platelet count (x10 ³)	Before treatment	After treatment	T	P
ITP group one under conventional therapy (n= 15)				
Min. –Max.	78 – 6	111 – 45	6.526*	<0.001*
Mean ± SD.	44.93 ± 23.74	79.6 ± 20.27		
Median (IQR)	45	77		
ITP group two under conventional therapy with vitamin D** (n= 15)				
Min. –Max.	70 – 14	156 – 70	9.907*	<0.001*
Mean ± SD.	49.93 ± 17.29	107.87 ± 25.3		
Median (IQR)	60	103		
p ₁	0.515	0.002*		

t: Paired t-test
 p: p value for comparison among before and afterward
 p₁: p-value for Student t-testing for comparison among the study groups
 *: significant at p-value ≤ 0.05

** : Vitamin D is given at a dose of 3000 IU per day for one month or at a dose of 50.000 IU every two weeks for two doses then platelet count was measured after one week from the last dose.

4. DISCUSSION

Immune thrombocytopenic purpura (ITP) is immune-mediated disorders in which platelets are opsonized by auto-reactive anti-bodies and prematurely destroyed by the reticulo-endothelial system [7].

The goal of the current work study was to study VD level in 30-Cases with ITP and 30 apparent healthy controls matching in gender and ages and to investigate the effect of vitamin D supplementation upon the response of the thrombopenia to conventional therapy of ITP.

The study revealed nonsignificant change among the groups of the study in regard to ages, gender and anthropometric measures

In the current study, the Mean platelet counts in studied ITP-cases was 47433.3 ± 20567.3 before treatment.

Saeidiet al, [8] revealed that mean platelet counts before treatment in childhood ITP patients were $33000/\mu\text{L}$. Platelet counts less than $20000/\mu\text{L}$ were more frequent in children (35.6%) compared to adults at diagnosis (24.8%), while in Al-Mulla et al [9] report, that assessed the patterns of ITP (acute/chronic) and to define present features and clinical characteristic of the disease in kids of maximum age 14-yrs old in a anew developed Arabic societies and they found that 68 percent of the kids with ITP exhibited a platelet counts less than $20 \times 10^9/\text{L}$ at the period of presenting.

The current work results in accordance to Mu et al [10] who investigated VD levels in 45-cases with ITP compared to 30 apparently healthy controls and found significantly lower VD levels in studied cases compared with control and Čulić et al [5] who found significantly lower vitamin D level in their cases of anew diagnosed ITP.

Findings of another work revealed that in 20 pediatric cases with chronic ITP, the mean VD levels was $46.4 \text{ nmol}/\pm 25.8$. 40% of these were lacking and 35% inadequate, while in 6-cases with acute ITP, the mean VD levels was $47.5 \text{ nmol}/\text{L} \pm 29.6$. Total, 83.3% of those examined were lacking or inadequate and this have not been correlated with severity of thrombopenia.

VD is used as a novel pharmacological tool, particularly for chronic inflammation of auto-immune disorder as multi-sclerosis, where the

existence of amplified level of IFN γ has a significant function in diseases pathogenesis, as well the applications of VD could be help anew diagnosed cases and chronic ITP [11].

Bone-marrow is nonfrequent examining in ITP-cases. In accordance to the American Society of Hematology (ASH) strategies, it is unnecessary in kids with standard characteristics of ITP and in those who not succeed in responding to intravenous immunoglobulin (IVIg). Important signs for bone-marrow aspirating includes uncommon clinical or laboratory characteristics at presenting that propose malignancy or bone-marrow fail, treatment-refractory ITP, and novel results that emerges through following-up that are unpredictable with ITP [12].

The current study revealed statistically significant negative correlation between platelet counts and each of Vitamin D level and Serum Ca, while there was no correlation between platelets count and Serum magnesium.

In agreement with our results, Park et al [13] studied if VD lack is accompanying with a grown platelet-counts (PC) and mean platelet volumes (MPV) and they concluded that platelet counts was inversely correlated with VD level. There was no previous studies confirmed raised platelet counts in cases with VD lack. These detected relations might be because of the near association among oxidative-stress and platelet counts.

Hypovitaminosis D might give the influence to immune anomalies in developing of the chronic grade of ITP and supplementing VD may decrease chronic diseases risks by modifying the immune-system [14].

The current work presented that a statistically significant change was found in platelets count in patient with ITP before and after conventional treatment and platelets count in patient with ITP under conventional treatment plus vitamin D supplementation.

Čulić et al [5] studied serum level of vitamin D in patient with ITP including anew detected and chronic ITP and they concluded VD lack in recently diagnosed and chronic ITP with significantly lower VD levels in chronic ITP compared with recently detected ITP with nonsignificant association among VD and platelet counts.

5. CONCLUSION

VD lack is very frequently found in children with either anew diagnosed or chronic form of ITP. Vitamin D supplementation improves platelets count in ITP.

ETHICAL APPROVAL AND CONSENT

A written informed consent was obtained from the all involved case's parents. The current work had been permitted by the ethics committee on research concerning human subjects of Medicine Faculty, Tanta University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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