Seminars in Hematology 53 (2016) S67–S69

Contents lists available at ScienceDirect

Seminars in Hematology

journal homepage: www.elsevier.com/locate/enganabound

Serum vitamin D levels in children with newly diagnosed and chronic immune thrombocytopenia

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ARTICLE INFO

Available online 8 April 2016

Keywords:
Vitamin D
Children
Chronic
Immune thrombocytopenia

ABSTRACT

The primary objective of the study was to assess the vitamin D (VD) status of patients suffering from ITP. Children from the case cohort (total 21) were recruited from chronic ITP patients (followed as outpatients) and newly diagnosed ITP (prospective study) patients. VD deficiency (values <75 nmol/L) was detected in 11 patients with newly diagnosed ITP, and seven patients with chronic ITP. Only three patients with newly diagnosed, and none with chronic ITP had normal VD values. Newly diagnosed ITP patients had statistically significantly higher values ($P < .044$) of VD than the patients with chronic type of ITP. Platelets values did not follow VD level. VD deficiency is very common in children with either newly diagnosed or chronic ITP form. Therefore there is a utility supplementing VD in these patients. To investigate the role of VD as an immune modulating drug for patients with ITP, a prospective randomized placebo-controlled trial needs to be performed.

1. Introduction

In recent years more and more is being investigated about noncalcemic role of vitamin D (VD) that modulates immune and inflammatory responses. Immune cells express VD nuclear receptor (VDR) and are capable of metabolizing VD. This hormone is a secosteroid (steroid with an opened B-ring) that is not limited to its well-known role in calcium homeostasis. Like other steroid hormones, its action is mediated by the VDR, which belongs to the superfamily of steroid and thyroid hormone receptors [1].

VD also functions as a regulator of the hematopoietic system, modulates lymphocyte activation and proliferation, induces the differentiation of promyelocytes into monocytes, and inhibits secretion of several cytokines in T cells [2].

In particular, different reports have demonstrated the ability of VD to repress the synthesis of interferon-γ (IFN-γ) and interleukin-2 (IL-2) in peripheral blood lymphocytes (PBL) and T-cell lines [3]. Epidemiologic evidence links poor VD status to susceptibility to infection and autoimmune diseases [4]. Results show that VD can prevent and suppress experimental autoimmune encephalomyelitis, rheumatoid arthritis, systemic lupus erythematosus, and inflammatory bowel disease [5]. Its influence in immunomodulation is discussed [6].

This hormone stimulates interleukin 4 (IL-4) production and suppress inflammatory T-cell activity [6]. VD deficiency skews the immunologic response towards loss of tolerance [7].

Accumulating evidence suggests that T helper cells and the cytokines they produce play a key role in pathogenesis of immune thrombocytopenia (ITP). Significant positive correlations between age and serum levels of IFN-γ, age and $CD4^+$ lymphocytes, age and natural killer cell count were observed in ITP patients [8]. Cytokine abnormalities were found in newly diagnosed patients with ITP, especially in children. Serum level of IL-4 was significantly higher in both children and adults with newly diagnosed ITP. Significant differences in serum cytokine levels between pediatric patients and healthy controls indicate that cytokine disturbances-especially changes in IL-2, IL-3 and tumor necrosis factor (TNF)-α might be involved in the pathogenesis of newly diagnosed ITP. TNF-α is the most informative variable for discrimination between healthy children and those with ITP [9].

The goal of this study was to establish whether children afflicted by newly diagnosed and chronic ITP suffer from serum VD deficiency.

2. Materials and methods

Children from the case cohort (total 21, aged 2–18 years) were recruited from chronic ITP patients (followed as outpatients) and newly diagnosed subsequent ITP (prospective study) patients from

This supplement was sponsored by the Intercontinental ITP Study Group.
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http://dx.doi.org/10.1053/j.seminhematol.2016.04.020
0037-1963/$/

Table 1
The level of VD (above or below optimal values) in relation to gender and ITP status.

Patients	VD concentration	
	≥ 75 nmol/L (%)	< 75 nmol/L (%)
ITP		
Male	2 (9.5)	6 (28.5)
Female	1 (4.8)	12 (57.2)
Newly diagnosed ITP	3 (14.3)	11 (52.4)
Chronic IPT	0	7 (33.3)

December 13, 2013 until May 20, 2015. Newly diagnosed patients were treated in the pediatric hematology/oncology division at the Department of Pediatrics of the University Hospital Centre Split. The study was approved by University Hospital Centre Split Ethics Committee and the children parents gave informed written consent for their participation in the study and the collection of the data from their medical charts.

Children were excluded from the study if they were on VD supplementation. Data collected on the demographic information of our patients included age and sex. Season of laboratory blood draw was categorized as winter (December–February), spring (March–May), summer (June–September), and autumn (October–November).

Patients with newly diagnosed ITP had their blood drawn at Department of Medical Laboratory Diagnostics of University Hospital Centre Split upon admission to division. Venous blood samples were collected using standard sampling tubes or tubes containing separating gel. After that serum was separated for analyses. Serum VD level was measured using a commercially available Elecsys Vitamin D total assay (Roche Diagnostics International Ltd., Rotkreuz, Switzerland). The assay used is intended for the quantitative determination of VD using a competitive electrochemiluminiscence binding technique. Measuring range of the test is 7.5–175 nmol/L. There is no standard definition or consensus about the optimal VD status in children. The preferred and optimal level for VD is recommended to be > 75 nmol/L [10,11]. VD insufficiency is present with levels of VD between 50 and 75 nmol/L, while levels ≤ 50 nmol/L are considered inadequate and reflect a state of deficiency [12]. We further categorized deficiency as being strong (30–49.9 nmol/L), significant (20–29.9 nmol/L), or extreme (< 20 nmol/L).

Data are presented as box-and-whisker plots, and were analyzed using two-tailed *t* test. The difference between groups is statistically significant ($P = .0444$).

3. Results

Data were collected from a total of 21 patients. The mean patients age at the time of blood draw was 84 months (SD 66.3 months). With regards to the season of laboratory blood draw (which did not correlated with the level of VD) 13 samples were drawn during the winter, three during spring, five during summer, and none during autumn.

Table 2
Distribution of VD levels.

VD (nmol/L)		No. of patients (%)
≥ 75	Optimal value	3 (14.3)
50 – 74.9	Insufficiency	9 (42.9)
30 – 49.9	Strong deficiency	4 (19.0)
20 – 29.9	Significant deficiency	2 (9.5)
< 20	Extreme deficiency	3 (14.3)

Table 3
VD level in acute and chronic ITP.

ITP	No. of patients (%)	VD level (nmol/L) (mean ± SD)
Newly diagnosed	14 (66.7)	59.1 ± 26.12
Chronic	7 (33.3)	34.9 ± 19.5

The level of VD (above or below optimal values) in relation to gender and ITP status is shown in Table 1. Of the 21 patients tested, the majority (N = 13) were girls. Also, the majority of patients (N = 14), predominantly girls, suffered from a newly diagnosed form of ITP. VD insufficiency/deficiency (values < 75 nmol/L) was detected in 11 patients with newly diagnosed ITP, and seven patients with chronic ITP. Only three patients (two boys and one girl) with newly diagnosed, and none with chronic ITP had normal VD values (≥ 75 nmol/L).

Table 2 presents VD values distribution according to the categories: optimal value, insufficiency, and strong, significant, and extreme deficiency. Only three patients had optimal VD values. All other patients suffered from some type of vitamin insufficiency/deficiency.

The VD levels in newly diagnosed and chronic ITP are shown in Table 3 and Fig. 1. Newly diagnosed ITP patients had statistically significantly higher values ($P < .044$) of VD (mean ± SD 59.1 ± 26.12) than patients with chronic-type ITP (34.9 ± 19.50).

Platelets values did not follow VD level.

4. Discussion

VD as an immunomodulatory agent, target various immune cells such as monocytes, macrophages, dendritic cells (DCs), as well as T lymphocytes and B lymphocytes, hence modulating both innate and adaptive immune responses. VD also reduces a risk of developing autoimmune diseases. There is also the evidence that autoimmune diseases are VD-sensitive. VDR is found in significant concentrations in T-lymphocyte and macrophage populations and its role in T cell–mediated immunity is important. Furthermore, serum levels of VD have been found to be significantly lower in patients suffering from several autoimmune or immune-mediated diseases than in the healthy population.

There is a poor review of literature about VD and ITP, pathogenesis and clinical treatment. Mu et al investigated the level of 25-hydroxylate vitamin D_3 [25(OH)D_3] and 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] in peripheral blood from 45 ITP patients and 30 normal controls. The result showed that the levels in peripheral blood of newly diagnosed ITP patients were lower than those of

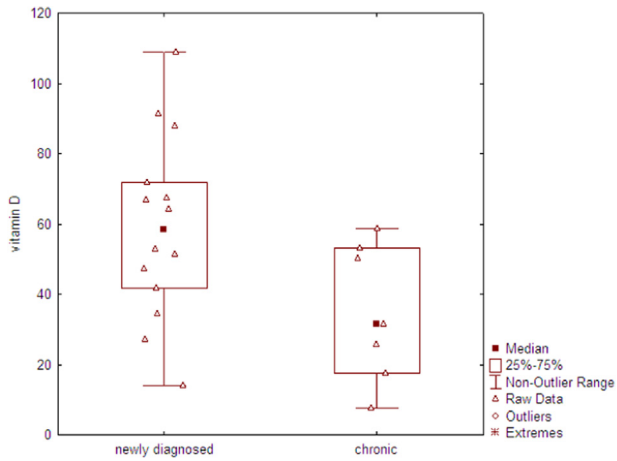

Fig 1. The VD levels in newly diagnosed and chronic ITP.

normal controls [13]. In our cohort of children with newly diagnosed form of ITP (N = 14) VD deficiency (values < 75 nmol/L) was detected in 11 patients. However, there is an obvious limitation of our study as we did not have the control group of patients. It is interesting area of research that VD can be administered as a new immunomodulatory therapy in children and adults with ITP. Bockow and Kaplan described two adult patients with refractory ITP initially treated with high-dose steroids that were successfully treated with high-dose VD replacement therapy and hydroxychloroquine. They found an association between VD deficiency and ITP but could not explain the pathophysiological mechanism of immunomodulatory action [14].

The reduction in IFN-γ synthesis induced by VD may be one of the modes of VD immunomodulatory action. New insights regarding the effect of vitamin D_3 on the IFN-γ gene repression further emphasize the role of the vitamin D_3 derivatives as an immunomodulator [15]. VD is applied as a new pharmacological tool, especially for chronic inflammatory autoimmune disorders as multiple sclerosis, where the presence of increased levels of IFN-γ plays an important role in disease pathogenesis [16]. Also the application of VD can be beneficial to patients with newly diagnosed and chronic ITP [14].

Some studies found that VD downregulates TNF-α–associated genes. Higher serum VD levels correlated with lower TNF-α levels. There is an inverse association between TNF-α and VD levels in a healthy population and there is the protective association found for VD against inflammatory diseases such as heart disease and rheumatoid arthritis. However, more studies are needed to precisely characterize the relationship between VD and TNF-α. Vitamin D therapy may show promise as adjunct to anti–TNF-α in autoimmune disease such as ITP [9,17].

Hypovitaminosis D may give the impact to immune abnormalities in the development of chronic form of ITP and supplementation of VD might reduce chronic disease risk by modulating the immune system [18].

5. Conclusion

VD deficiency is very common in children with either newly diagnosed or chronic ITP form. Therefore there is a utility supplementing VD in ITP patients. To investigate the role of VD as an immune-modulating drug for patients with ITP, a prospective randomized placebo-controlled trials needs to be performed.

Conflicts of interest

All authors disclose that they do not have any financial or any other relationship with the company that produces a compound used in the study, or any other party.

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