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Vitamin D Status in Pediatric Patients with Newly Diagnosed Acute Lymphoblastic Leukemia in University Hospital of Split

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Objective – Vitamin D mediates and enhances the immune system through a vitamin D nuclear receptor present in almost all types of immune cells. It has antiangiogenic, proapoptotic and antiproliferative effects. We investigated the vitamin D status of pediatric patients with newly diagnosed acute lymphoblastic leukemia. **Patients and Methods** – Newly diagnosed patients aged 0-18 years with acute lymphoblastic leukemia, hospitalized between September 1st, 2013 and June 30th, 2017 on the Hematology and Oncology Ward, Department of Pediatrics, University Hospital of Split were included in this study. Serum vitamin D levels were measured upon admission to the hospital. Vitamin D >75 nmol/L was considered sufficient, vitamin D between 50 and 75 nmol/L was considered insufficient, while vitamin D <50 nmol/L was considered as a deficiency. **Results** – Twenty patients were included in the study. Only two patients had a sufficient level of vitamin D. Vitamin D deficiency was found in 14 and insufficiency in 4 patients. The mean concentration of vitamin D in children with acute lymphoblastic leukemia in this study was 42.6±21.2 nmol/L. **Conclusion** – The prevalence of vitamin D insufficiency/deficiency in pediatric patients with a malignant disease is very high. Therefore adequate replacement therapy should be started as soon as possible to avoid a further decrease in vitamin D levels, as this may have an impact on the outcome in these patients.

Introduction

Vitamin D (VD) level is the best indicator of overall vitamin status because its measurement reflects total VD from both dietary intake and sunlight exposure. Besides having an important role in the regulation of serum calcium and phosphorus concentrations, VD has a very important non-skeletal role: it mediates and enhances immune system action (1). It exerts its biological action by binding to a vitamin D nuclear receptor (VDR) spanning the innate and adaptive immune response (2). Vitamin D nuclear receptors are present in almost all types of immune cells, including antigen-presenting cells (APCs): macrophages and dendritic cells (DCs) and also in activated CD4+ and CD8+ T cells, B cells and neutrophils (3, 4). On the other hand, VDR expression is controlled by immune signals in some immune cells. Although naive T cells show low VDR expression, this receptor is abundantly present during T cell activation (3, 5). Vitamin D directly changes the cytokine profiles of T cells by inhibiting the

production of inflammatory Th1-cytokines, such as IL-2 and IFN-g, as well as the Th17 cytokines IL-17 and IL-21, thereby it slowly shifts T cell polarization from Th1 and Th17 towards Th2 response: antibody mediated immunity (6-9). Although modulation of T cells will inevitably affect the B cells, these cells are directly affected by 1,25(OH)2D3 as well. The active form of vitamin D can inhibit B cell proliferation, memory B cell generation, plasma-cell differentiation and immunoglobulin secretion (IgG and IgM), and also induce B cell apoptosis (10). There is a decrease in VDR-expression during differentiation of monocytes into macrophages or other DCs, which makes these cells less sensitive to 1,25(OH)2D3 when they mature (11, 12). The high abundance of receptors for active vitamin D throughout the immune system and their regulation by immune signals encourages the thought that vitamin D is a strong immunological modulator.

There is also evidence that there is a difference in the expression of 1,25(OH)2D3 receptors between normal resting and malignant or activated B and T lymphocytes (3). Vitamin D3 could be an important factor in leukemic cell differentiation because, in contrast to normal lymphocytes, established lines of malignant human B, T and non-B, non-T lymphocytes have this receptor. Also, it has been demonstrated that T and B lymphocytes obtained from normal humans and activated by mitogenic lectins and Epstein Barr virus also express VDR (3). Among healthy children and adolescents in the United States, the prevalence of vitamin D deficiency (VDD) is 9%–18%, while among critically ill children rates are 35%–70% (13-17). In 1988, Shu et al. showed that long-term pre-conceptual consumption of cod liver oil (rich in A and D vitamins) is associated with a lower risk of developing acute leukemia in children. However, it has not yet been established which exact substance in cod liver oil has this protective effect (18). Animal studies have shown that severe vitamin D deficiency or VDR gene deletion increases the risk of developing cancer (19). The anticancer effects of vitamin D include various mechanisms at the cellular level such as: its antiproliferative effect, apoptosis regulation, angiogenesis suppression, cell differentiation stimulation, anti-inflammatory activity, DNA repair and immunomodulatory action (20). However, for now there is not enough evidence to link vitamin D concentration and the incidence of any malignant disease in children.

Referring to the existing evidence, it is reasonable to try to prove the hypothesis that children with malignancy have low levels of vitamin D. In this study we investigated the VD status of pediatric patients diagnosed with acute lymphoblastic leukemia. We correlated the data with the patients' ages and gender.

Patients and Methods

Newly diagnosed patients aged 0-18 years with acute lymphoblastic leukemia, hospitalized between September 1st, 2013 and June 30th, 2017 on the Pediatric Hematology/Oncology Ward, Department of Pediatrics, University Hospital of Split, were included in this study. As a control group we used randomly selected healthy patients admitted to the Department of Pediatric Surgery for elective one-day minor operations in the period from June 12th, 2015 to June 23rd, 2015. Children were excluded from the study if they were on VD supplementation or medical records were incomplete. All the patients had blood drawn upon admission to the hospital. Research was conducted by retrospective cross-sectional analysis of medical records. Data collected from medical records included age, sex, and VD concentrations. A venous blood sample was collected using standard sampling tubes or tubes containing separating gel. After that the serum was separated for analysis.

Serum VD levels were measured using a commercially available Elecsys® Vitamin D total assay (Roche Diagnostics International Ltd., Rotkreuz, Switzerland). The assay used is intended for quantitative determination of VD using a competitive electrochemiluminescence binding technique. The measuring range of the test was 7.5-175 nmol/L. There is no standard definition or consensus about optimal VD status in children. The VD sufficient level is recommended to be >75 nmol/L (13, 20). Vitamin D insufficiency is present with levels of 25-OH VD between 50 and 75 nmol/L, while levels ≤50 nmol/L are considered inadequate and reflect a state of deficiency.

Ethics Statement

The study was approved by the University Hospital of Split Ethics Committee (Number 2181-147-01/06/MB-16-2) and the children's parents submitted informed written consent for their participation in the study and collection of data from their medical records.

Statistical Analyses

The data were analyzed using descriptive statistics by Microsoft Office Excel 2016. Differences between continuous variables were tested for statistical significance using the Mann Whitney test. The values of categorical variables were compared between groups using the Fischer exact test. The value of $p<0.05$ was considered statistically significant.

Results

In the period in question, 60 children diagnosed with a malignant disease were admitted to the Department of Pediatrics of the University Hospital in Split. The diagnosis of ALL was confirmed in 22 patients. In 2 patients the VD concentration was not available and, therefore, 20 patients were enrolled in the study group. Of them, 11 (55%) were males and 9 (45%) were females. The mean age at the time of drawing blood was 93.3±57.1 months (7.8±4.8 years). The youngest child was 33 months old (2.8 years) and the oldest 208 months (17.3 years). The mean age of the female subjects was 69.4±50.4 months and of the males 112.8±56.9 months. The control group consisted of 11 patients, 2 females (18.2%) and 9 (81.8%) males. The mean age at the time of drawing blood was 144.4±66.5 months (12±5.5 years).

Only 2 patients in the study group were found to be VD sufficient. Vitamin D deficiency was found in 14 and vitamin D insufficiency in 4 patients. The mean concentration of vitamin D in children with ALL in this study was 42.6±21.2 nmol/L. The mean VD concentration was 47.5±19.5 nmol/L in males and 36.6±22.8 nmol/L in females. In the control group, 1 patient was found to be VD sufficient, deficiency was found in 6, and insufficiency in 4 patients. The mean concentration of vitamin D in the control group was 53.2±30.2 nmol/L and there was no statistical difference from the study group (Mann Whitney test, p=0.34). The mean VD concentration was 55±32.5 nmol/L in males and 45±21.8 nmol/L in females. Table 1 shows the VD status in the study group according to gender. There is no statistically significant difference between the groups (Fischer exact test; P>0.05).

We further divided the study group subjects into 2 age groups. Vitamin D status

Table 1. Vitamin D status by gender groups (N=20)

Vitamin D status	Gender	
	Female (N=9)	Male (N=11)
VDD+VDI	8 (88.9%)	10 (90.9%)
VDS*	1 (11.1%)	1 (9.1%)

VDD=Vitamin D deficiency; VDI=Vitamin D insufficiency; VDS=Vitamin D sufficiency.

Table 2. Vitamin D status by age groups

Vitamin D status	Age group (years)	
	1-9 (N=13)	>10 (N=7)
	N (%)	N (%)
VDD	10 (76.9)	4 (57.1)
VDI	2 (5.4)	2 (28.6)
VDS	1 (7.7)	1 (14.3)

VDD=Vitamin D deficiency; VDI=Vitamin D insufficiency; VDS=Vitamin D sufficiency.

depending on the age group of the patients is shown in Table 2. The mean concentration of vitamin D in the group of subjects aged between 1 and 9 years was 40.6±22 nmol/L, in the age group above 10 years it was 46.4±20.8 nmol/L.

Discussion

The results of this study showed that pediatric patients diagnosed with acute lymphoblastic leukemia, as well as the group of control children, are more likely to have low VD levels, despite the fact that all of them originate from southern Croatia, a region along the Adriatic Coast with a Mediterranean climate and many sunny days.

There are studies which indicate that most children with cancer show some degree of VDD at diagnosis or develop deficiency during therapy (21-24). In survivors of pediatric ALL, VDD is also prevalent (25). It is possible that pediatric oncology patients are at further risk for developing deficiency in the course of their treatment due to poor nutrition or loss of appetite with decreased oral intake of dietary VD, lack of sun exposure due to seasonal and climate variations, and possible other effects of treatment (26).

As corticosteroid treatment is integral to some forms of pediatric cancer, it is important to recognize whether patients have VD insufficiency or VDD (27). This is important since both of these factors contribute to increased risk for osteopenia/osteoporosis. There are also other risk factors contributing to VDD in childhood malignancies, such as malaise and fatigue leading to more indoor activities, low levels of active VD due to the renal or hepatic involvement of the disease, genetic factors (darker skin color), developmental issues (infancy and adolescence with accelerated growth, obesity), mucositis leading to limited absorption of VD through the gut, drug – VD interaction, and persistent pre-existing factors (20, 28, 29). The impact of modern life has to be taken in account, with more and more children spending their free time in front of TVs and computers. Although there are only rare studies evaluating VD levels in patients with pediatric malignancies, our results are similar to others previously reported. Sinha et al. (23) found significantly lower VD levels in children with solid tumors, leukemia and lymphomas, compared to the control group of children.

Since some studies have indicated that VD may have a beneficial impact on survival following cancer (30, 13), it is important to assess VD levels in patients and to start VD supplementation, when needed, as soon as possible. This could be achieved by including VD in the recommended screening schedule for pediatric hematology and oncology patients.

To our knowledge, there are no contraindications for VD supplementation during ALL treatment. However, the in vitro influence of VD on dexamethason cytotoxicity in human pre B ALL has been shown, and further studies are required (31). The link between VD and infections or sepsis also supports the need for VD supplementation when needed. Among children, in randomized controlled trials, it was shown that VD reduced rates of recurrence of respiratory tract infections at 3 months (14) and decreased the incidence of influenza A infection (15). Also, a study of intensive care unit patients showed increased mortality in VDD

patients (16). Another study suggested that VDD patients are more likely to have severe sepsis and organ dysfunction (17).

Limitations of the Study

There are some shortcomings and limitations of this research. It is a retrospective study of VD status in children suffering from ALL, and comparable data on the VD status of the healthy subjects in the control group are limited. The sample of subjects is small and our research should continue enrolling more patients. Also, all the blood samples in our study subjects were drawn at the time of admission, so there are no data about VD status after ALL treatment. However, we may presume that VD concentrations will continue to decline in ALL patients due to poor nutrition, and as a consequence of chemotherapy or corticosteroid treatment.

Conclusion

Children with cancer are definitely at risk of having VDD or VDI, and adequate replacement should be started to avoid a further decrease in VD levels. However, further studies are warranted to determine the full potential role of VD in disease outcome.

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