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Vitamin D Status in Pediatric Patients with Newly Diagnosed Malignant Disease: Preliminary Results

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Objectives – Vitamin D (VD) has an impact on the immune system via vitamin D nuclear receptor (VDR) present in various types of immune cells. Its anti-angiogenic, pro-apoptotic, and anti-proliferative effect have been found as well. We investigated VD status of paediatric patients with malignant disease such as leukaemia, lymphoma, Langerhans cell histiocytosis, and solid malignant tumours. **Materials and Methods** – In children with malignant disease from the case cohort, total 41, serum VD level was measured upon first admission to the hospital. They were subdivided into those with leukaemia / lymphoma and those with solid malignant tumours (body and central nervous system). Langerhans cell histiocytosis was included in leukaemia /lymphoma group. The optimal level for VD was recommended to be >75 nmol/L. The insufficiency was presented with levels of 25-OH VD between 50 and 75 nmol/L, while levels ≤50 nmol/L were recognized as deficiency. We further categorized deficiency as strong (30 – 49.9 nmol/L), significant (20 – 29.9 nmol/L) and extreme (<20 nmol/L). **Results** – Only three patients had optimal level of VD. All others (92.8%) suffered from VD insufficiency 11 (26.8%) and various levels of deficiency: 10 (24.4%) from strong, 11 (26.8%) significant, and 6 (14.6%) from extreme. **Conclusions** – The prevalence of VD insufficiency/deficiency in paediatric patients with malignant disease is very high especially in patients with solid malignant tumour. Such condition, regarding that VD insufficiency/deficiency can debilitate immune system may have a negative impact on these patients.

Introduction

Vitamin D (VD) has an important role in regulation of serum calcium and phosphorus concentrations. In addition, it has a very important extra skeletal role mediating and enhancing the immune system action (1). Its biological action is achieved by binding to a vitamin D nuclear receptor (VDR) present in nearly all types of immune cells, spanning the innate and adaptive immune response (2). VD is a strong immunological modulator. It also has anti-proliferative effects, potentiate apoptosis, and inhibit angiogenesis (3). Its serum level is the best indicator of overall vitamin status because its measurement reflects total VD in the body. 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% (4-8). Those, at greatest risk of VDD, include patients with chronic

illnesses (e.g. chronic kidney disease (CKD), cystic fibrosis (CF), asthma, and sickle cell disease), dark-pigmented skin, poor nutrition, and infants who are exclusively breast-fed (9, 10). An increasing body of evidence demonstrates an association between several VDR gene polymorphisms and cancer risk and progression (11). Research in animals has shown that severe VDD or deletion of the VDR increases cancer risk (12).

In this study, we investigated the VD status of paediatric patients diagnosed with a malignancy. We correlated the data with patients' gender, and cancer type.

Materials and Methods

Subjects

Children from the case cohort were recruited from patients newly diagnosed with malignancy and hospitalized and treated from 1 January 2012 until 20 April 2015 at the Paediatric Haematology/Oncology Division, Department of Paediatrics, University Hospital of Split. Children were excluded from the study if they were on VD supplementation. Patients were subdivided into those with leukaemia/lymphoma and those with solid malignant tumour (body and central nervous system). The patients with Langerhans cell histiocytosis (LCH) were included in leukaemia/lymphoma group. As a control group, we used randomly selected healthy patients admitted to Department of Paediatric Surgery for elective one-day minor operation in the period from June 12th, 2015 until June 23rd, 2015. Research was conducted as case-control study. Data collected on the demographic information of our patients included age and gender. Data were also collected on disease characteristics of the study population.

Determination f Serum 25-Hydroxy Vitamin D Levels

Participants from patient and control group provided a blood sample for 25-hydroxyvitamin D determination, which is the standard indicator of VD status (19). All of the patients had their blood drawn upon first admission to our division. Venous blood sample was collected using standard sampling tubes or tubes containing separating gel. After that, serum was separated for the analysis. 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 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 (13, 14). VD 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. We further categorized VD deficiency as strong (30–49.9 nmol/L), significant (20–29.9 nmol/L) or extreme (<20 nmol/L) (20).

Ethics Statement

The study was approved by the University Hospital of Split Ethics Committee and the children parents submitted informed written consent for their participation in the study and the collection of the data from their medical charts.

Statistical Analyses

Data are presented as individual data points and medians. Comparison of groups is based on Kruskal-Wallis and Dunn's multiple com-

parison test. All applied test were two-tailed, and the results were considered as a statistically significant if P value was less or equal 0.05.

Results

In the analysed period, 41 children were admitted at the Department of Paediatrics with newly diagnosed malignant disease. Out of them, 20 were females and 21 were males. The mean age at the time of blood draw was 115.6 months (SD=65.3 months). VD was significantly low in sick girls then in boys (Mann-Whitney U-test, P=0.010). Median value for girls was 26.8 nmol/L, and 49.2 nmol/L for boys. Patient's diagnosis and the level of VD are presented in Table 1 and Fig. 1 and 2, while the distribution of VD level (by category) in patients with specific tumour type is presented in Table 2.

Table 1. Patient's Diagnosis

Tumour type	Patients
	N (%)
Acute lymphoblastic leukaemia	16 (39)
Acute myeloid leukaemia	3 (7.3)
Lymphoma	4 (9.8)
Langerhans cell histiocytosis	6 (14.6)
Solid tumours*	12 (29.3)

*Astrocytoma, Neuroblastoma, Ewing sarcoma, Medulloblastoma, Dysgerminoma Ovarii, Yolk Sac, Tumour mixtus testis and Juvenile Granulosa Cell Tumour.

The majority of patients suffered from acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), lymphoma and LCH. Only three patients had optimal lev-

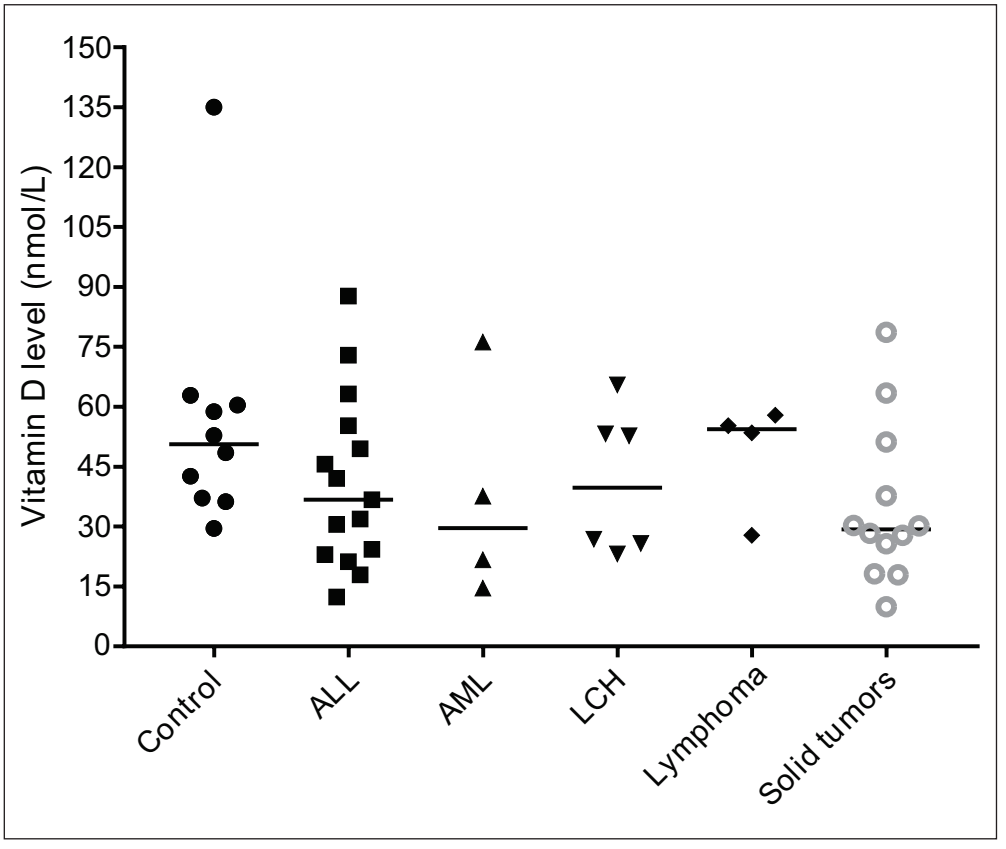

Fig. 1. *Patient's Diagnosis and the Level of VD.*

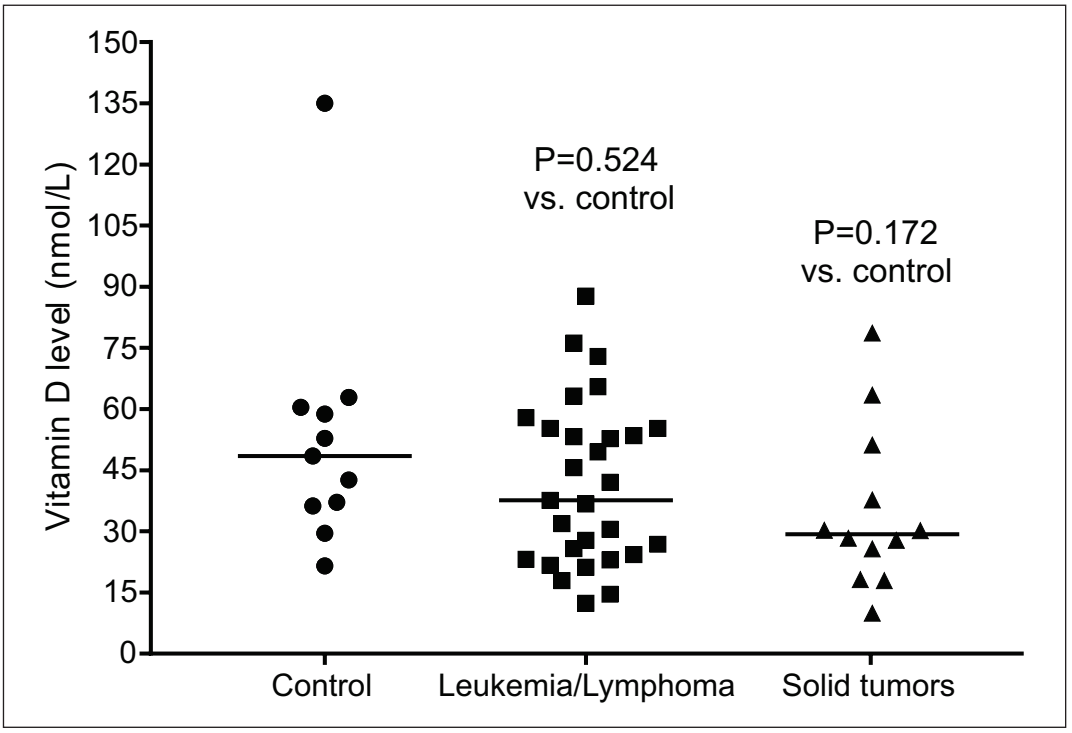

Fig. 2. *Patient's Diagnosis and the Level of VD for Pooled Groups.*

Table 2. Distribution of Vitamin D Level (by category) in Patients with Specific Tumour Type

Vitamin D level by categories ALL		Number of patients by tumour type and category of vitamin D level						
		AML	LYPH	LCH	MB	Other ST	Total (%)	
Optimal		2	-	-	-	-	1	3 (7.3)
Insufficiency		3	-	3	3	-	2*	11 (26.8)
Deficiency	Strong	6	1		-	-	3†	10 (24.4)
	Significant	3	1	1	3	1	2‡	11 (26.8)
	Extreme	2	1	-	-	1	2§	6 (14.7)

Optimal >75 nmol/L; Insufficiency between 50 and 75 nmol/L; Deficiency: Strong 30-49.9 nmol/L; Significant: 20-29.9 nmol/L; Extreme: <20 nmol/L.ALL=Acute Lymphoblastic Leukaemia; AML=Acute Myeloid Leukaemia; LYPH=Lymphoma; LCH=Langerhans cell histiocytosis; MB=Medulloblastoma; ST=Solid Tumour:*Neuroblastoma; †Ganglioma Astrocytoma; ‡Dysgerminoma Ovarii and Ewing Sarcoma; §Yolk Sac and Juvenile Granulosa Cell Tumour.

el of VD (two patients suffering from ALL and one patient suffering from tumour mixtus testis). All others (92.8%) suffered from various level of VD insufficiency / deficiency. Out of those 10 (24.4%) had a strong, 11 (26.8%) had a significant, and 6 (14.6%) had an extreme VD deficiency. The mean concentration of VD in control group was 53.2±30.2 nmol/L (mean±SD), there is no statistical difference versus leukaemia/lymphoma (41.6±20.1 nmol/L), and solid tumours group (34.9±20.0) (Fig. 2). However, in the solid tumours group the mean value of VD was in average 18.26 nmol/L lower in comparison to control group.

Discussion

The results of this study showed that vast majority (92.8%) of our newly diagnosed paediatric haemato-oncology patients and control group have decreased VD levels. This finding was not expected since all the participants in this study live in southern part of Croatia, along the Adriatic Coast with many sunny days per year.

There are studies, which indicate that most of the children with cancer show some degree of VDD at diagnosis or develop deficiency during therapy (15-18). VDD is also prevalent in survivors of paediatric ALL (19). We do not know the reason why our patients have so high prevalence of VD insufficiency/deficiency at time of diagnosis. It is very likely that paediatric oncology patients are at risk for developing deficiency. During their treatment it is expected that they will have poor nutrition, and that their appetite will be affected leading to decreased oral intake of dietary VD (21). In addition, they will probably lack sun exposure due to treatment and seasonal or climate variations (21). As corticosteroids are integral part of treatment in some forms of paediatric cancer, it is important to recognize whether patients have VD insufficiency or VDD (22). This is important since these factors contribute to increased risk for osteopenia/osteoporosis and can have the impact to disease outcome. All the blood samples in our patients were drawn at the time of admission when the malignancy diagnosis was set, and it can be assumed that this is not entirely due to the poor nutrition and definitely not a consequence of the chemotherapy or corticosteroid treatment. What is happening with immune system during VDD? Can it contribute to onset of malignant disease? There are so many questions to ask and the science in further investigation may give us the answer. There are also other risk factors contributing to VDD in childhood malignancies, like malaise and fatigue leading to more indoor activities, low levels of active VD due to renal or hepatic involvement of the disease, genetic factors, obesity, mucositis leading to limited absorption of VD, drug – VD interactions (3, 23, 24).

Although there are only rare studies evaluating the VD levels in patients with paediatric malignancies, our results are similar to others previously reported. Significantly, lower VD levels were found in children with solid tumours and leukaemia and lymphomas compared to the control group of healthy subjects (17). Suboptimal VD levels were found as common in paediatric patients scheduled to receive haematopoietic stem cell transplantation and were associated with lower overall 1-year survival (25). Therefore, since some studies have indicated that VD may have beneficial impact on survival following cancer it is important to assess VD levels in patients and to start VD supplementation when needed (26, 27). This could be achieved by including VD in recommended bundle of tests for paediatric haemato-oncology patients. The connection between VD and infections or sepsis supports this need especially because it was found that VDD patients treated in intensive care units have higher mortality (28). That was also confirmed by meta-analysis which suggested that VDD increases susceptibility for severe infections and mortality of the critically ill (29).

Limitation of the Study

This study has some limitations, which have to be pointed out. The first one is the limited patient population studied. Larger series with long-term follow-up are needed to confirm our conclusions. Finally, our study did not compare results of the malignant disease treatment with or without VD supplementation. The major weakness of this study is that our control group is very small so we do not know if those with malignancy were more at risk for VD deficiency than healthy chil-

dren of the same age and gender. However, our preliminary results will be the subject of ongoing studies and our limitation may be someone else's inspiration for the new study.

Conclusion

In conclusion, children with cancer are definitely at risk of having VDD and adequate replacement should be started as soon as malignancy has been diagnosed to avoid further VD level decrease. However, further studies are warranted to determine the full potential role of VD to disease outcome. Hence, measuring serum levels of VD could be considered in the routine assessment of children with malignant disease. We propose to undertake a long-term study in the near future in order to determine the effect of VD supplementation on malignancy relapse prevention and to establish whether a cause and effect relationship exists between VD and malignant disease in children.

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Conflict of interest: The authors declare that they have no conflict of interest.

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