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THE PREVALENCE OF VITAMIN D DEFICIENCY IN A PEDIATRIC HOSPITAL IN ROMANIA

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Abstract: Vitamin D deficiency in childhood represents an important link not only in the pathophysiology of rickets, but also of non-skeletal diseases with immune-mediated pathogenesis. The present study is a cross-sectional analytical one that was carried out on 2464 patients aged from 0 to 17 years old who were hospitalized in the Children's Emergency Clinical Hospital ″St. Ioan″, Galati. The study duration was between 01.11.2021 - 31.10.2022 and involved the measurement of serum vitamin D levels using the YHLO I - FLASH 1800A device, by chemiluminescence technique, or with the GEMINI device, through ELISA technique. The prevalence of vitamin D insufficiency, deficiency, and toxicity among children was 11.32%, 1.78%, and respectively 0.64%.. The cases of insufficiency and vitamin D deficiency were more common in girls (55.55%; 65.90%) than in boys (45.90%; 34.10%) whereas the cases with toxic levels were more common in boys and within the 1-3 years age group. The children from urban areas were more predisposed to hypovitaminosis. Although vitamin D deficiency was found in only 11% (279) of the patients, the management of pathological vitamin D values is very important and should be assessed for every child, especially where sex, age, and place of origin are significant risk factors.

Keywords: vitamin D, insufficiency, deficiency, toxic level, children.

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Introduction

Vitamin D is a fat-soluble vitamin that plays a key role in calcium homeostasis and the process of bone mineralization regulation. Vitamin D acts similarly to steroid hormones, modulating bone metabolism at a distance by directing and interacting with other hormones. Vitamin D also acts locally as a cytokine, contributing to an immune response, cellular differentiation, and proliferation, suggesting the extraskelatal effects of this hormone (Norman, 2008; Prietl *et al*., 2013). Vitamin D is composed of a group of ten well-studied sterolic compounds, and the major physiologic forms are represented by vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D_2 is produced by irradiation with ultraviolet B (UVB) radiation of ergosterol in plants and fungi (mushrooms). Vitamin D_3 is synthesized in the skin and is produced from 7-dehydrocholesterol (7-DHC) through a two-step process in which the B ring is opened by UV rays (280-315 nm). This process forms previtamin D_3, which later isomerizes into vitamin D_3 (Bikle, 2014). Vitamin D can be produced in the skin as a result to sunlight exposure or absorbed through diet or vitamin D supplements (Vanchinathan *et al*., 2012; Kochupillai, 2008; Gilchrest, 2008). The primary source of vitamin D is the skin, where vitamin D synthesis is dependent on a range of geographic (latitude, season, degree of sunshine and pollution) and personal factors (time spent outdoors, age, body composition, degree of skin pigmentation, genetic factors) (https://courses.lumenlearning.com/suny-nutrition/chapter/12-11-environmental-factors-that-impact-vitamin-d3-synthesis/ accessed 2022). For adequate vitamin D synthesis during summer months, dark-skinned infants require 30 min of sun exposure per week (diaper only), without the use of sunscreens, or 2 hours per week dressed with their head uncovered. Dietary sources of vitamin D include fatty fish (mackerel, salmon, tuna), fish liver oil (cod), mushrooms, egg yolk, beef liver, foods artificially fortified with vitamin D (breakfast cereals, orange juice, milk formulas). Breast milk contains less than 40 IU/L of vitamin D, and cow's milk contains less than 20 IU/L, both of which are considered as insufficient amounts.

The requirement for vitamin D is expressed in international units (IU) or micrograms (1mcg = 40 IU). Daily intake should range from 400-800 IU in sunny regions and to 1000-1200 IU in colder regions. According to the recommendations of the Endocrine Society of the United States - 2011, infants up to 1 year of age need at least 400 IU/day of vitamin D, children over 1 year - 600 IU/day and pregnant and breastfeeding mothers need a minimum of 600 IU/day and a maximum of 1500 IU/day of vitamin D. Obese children and those undergoing chronic treatment with anticonvulsants, corticosteroids, antifungals, or antiretroviral therapy require triple doses of vitamin D.

It is estimated that one billion people worldwide either have insufficiency or deficiency of vitamin D. Vitamin D deficiency is widespread in the European population (Holick, 2007). An inadequate vitamin D diet in combination with inadequate sun exposure causes vitamin D deficiency. Severe vitamin D deficiency in children causes rickets, a softening and weakening of the bones, which is a rare disease in the developed world (Cashman *et al*., 2016). Deficiency rickets is a condition of the growth cartilage characterized by a decreased ossification (deficiency of maturation of the bone protein matrix) and chondrocyte apoptosis, the final consequence being represented by the deformation of the growth cartilages, late closure of the fontanelles, delayed tooth eruption, dental enamel hypoplasia, hypertrophy of the costochondral joints (rachitic rosaries), widening of the costal margin (Harrison's groove), deformation of the long bone diaphyses, and cupping of the radius metaphysis ("rachitic bracelets"). Epidemiologically, rickets has a high frequency in infants, approximately 10%. It occurs between 3-18 months and is more frequent in premature infants and boys. Risk factors are represented by breastfeeding (breast milk contains insufficient amounts of vitamin D), hyperpigmentation, and reduced sun exposure. It occurs more frequently in developing countries, while in developed ones, the correct prophylaxis has decreased its incidence to under 1%. In the United Kingdom, at the beginning of the 21st century, a general incidence of 7.5 per 100,000 children under the age of five was reported (Callaghan *et al*., 2006). Rickets represents a major risk factor causing infant morbidity and mortality.

The serum concentration of 25(OH)D – the main metabolite of vitamin D, indicates the vitamin D status. The total concentration of 25(OH)D reflects the supply of vitamin D from both sources (cutaneous, oral) and is expressed in ng/mL or nmol/L (1 ng/mL = 2.5 nmol/L). In children, the thresholds for sufficient (optimal, 20-100 ng/mL), insufficiency (12-19 ng/mL), and vitamin D deficiency (<12 ng/mL) are based on associations between 25(OH)D levels and clinical evidence of rickets (In line with the 2016 recommendations from the Global Consensus and the Paediatric Endocrine Society (2011), serum concentrations of 25(OH)D). The indicated toxic level of vitamin D is >100 ng/mL.

Currently, it is recommended to maintain serum 25(OH)D levels of at least 20 ng/mL, given that the clinical diagnosis of rickets is made at values between 12-19 ng/mL. Measurement of serum 25(OH)D concentrations is performed using recent analytical methods, which are generally applied on fully automated platforms used in biochemistry laboratories. For routine/clinical testing of serum 25(OH)D concentration, the methods that measure both 25(OH)D_2 and 25(OH)D_3 are recommended, with the result being a total of both. In healthy individuals, routine testing of 25(OH)D_2 is not recommended. Indications for testing include pregnant women (under 18 years, over 35 years, multiparous (more than 3 pregnancies), with diabetes mellitus, or obesity), lactating women who had severe 25(OH)D deficiency during pregnancy. In the cases of vitamin D deficiency or insufficiency, substitution therapy is necessary for children who have low vitamin levels in their blood (serum values) below 20 ng/mL or for rickets. Both vitamin D_2 and D_3 can be used.

Recent studies have suggested that vitamin D insufficiency and deficiency in children are widespread worldwide. However, the status of vitamin D among Romanian children is relatively rarely investigated, with the last national study being conducted in 2018. Therefore, the aim of this present study was to evaluate the prevalence and risk factors of vitamin D insufficiency, deficiency, and toxicity among children hospitalized in the Emergency Clinical Hospital for Children "St. Ioan", Galați for conditions that suggest hypovitaminosis or hypervitaminosis D.

Materials and methods

Sample collection

The study was a retrospective one, which included 2464 paediatric patients aged between 0-17 years, present at the Emergency Clinical Hospital for Children "St. Ioan", Galați between 01.11.2021-31.10.2022. Venous blood was collected from patients according to the laboratory's collection manual, in anticoagulant-free vacutainers, for the determination of the 25(OH)D concentration.

Determination of vitamin D concentrations

Vitamin D levels were obtained from the hospital laboratory records, and clinical observation sheets were used to determine age, gender, and place of origin. The 25(OH) D analysis was performed in the hospital's clinical laboratory using the YHLO I - FLASH 1800A apparatus by chemiluminescence technique or the GEMINI apparatus through ELISA technique. The results from 01.11.2021 - 31.01.2022 were obtained using the GEMINI apparatus (ELISA), while those from 01.02.2022 - 31.10.2022 were obtained using the YHLO I - FLASH 1800A apparatus (chemiluminescence). Only one serum sample was analysed for each patient.

Data analysis

Data collection was performed from the hospital's electronic archive, and the results were analysed by Excel (Microsoft Office 2016). Analysis and interpretation were performed according to the WHO 1240/2019 data, in the form of graphs and tables, with the classification

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RESEARCH ARTICLE

of values as follows: optimal level: 20-100 ng/mL, insufficient: 12-19 ng/mL, deficiency < 12 ng/mL, toxicity > 100 ng/mL. Descriptive statistics and univariate analyses were used.

Results and discussions

From the analysis of data regarding the vitamin D levels for the 2464 paediatric patients, it was found that the majority (86.24% representing 2125 cases) had optimal serum vitamin D levels, 279 patients (11.32%) had insufficient levels, 44 patients (1.78%) presented a deficiency, and 16 patients (0.64%) had toxic levels (Figure 1).

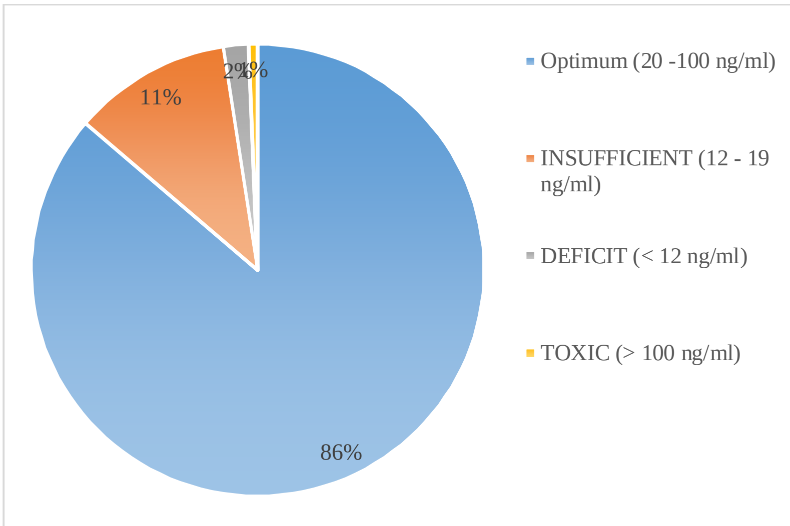

Figure 1. Plasma concentration of vitamin D

Our results showed that female with insufficient vitamin D levels predominated, with a prevalence of 55.55% (155 patients), while male had a lower percentage of 44.45% (124 cases). Depending on the area of residence, 191 cases (68.45%) with insufficient vitamin D levels were recorded in urban areas and 88 cases (31.55%) in rural areas.

Patients with insufficient vitamin D levels were distributed into 6 age groups: <1 year; 1-3 years; 4–6 years; 7–10 years; 11–14 years; and 15–17 years. The distribution of these patients by age groups shows a clear predominance of the age group between 11–14 years with a percentage of 32.97% (92 children), followed by the 7–10 years and 15–17 years age groups with 22.22% and 18.64%, respectively. The fewest cases were observed in children under 1 year old (5 cases; 1.79%). The other two age groups of 1–3 years and 4–6 years had 27 cases (9.68%) and 41 cases (14.70%), respectively (Figure 2).

Innovative Romanian Food Biotechnology Vol. 23, 2023
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RESEARCH ARTICLE

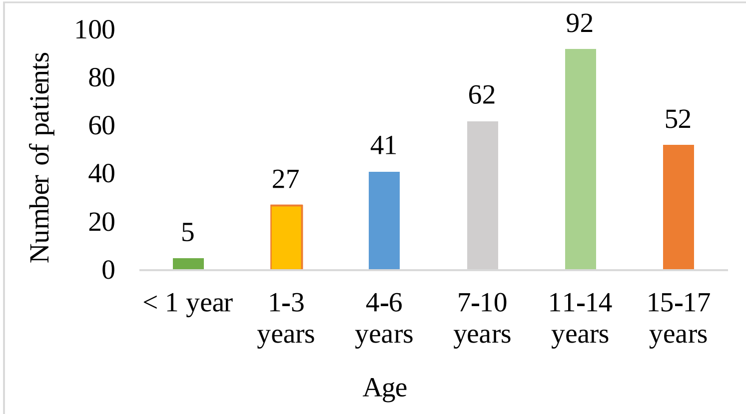

Figure 2. Distribution of patients with insufficient vitamin D levels, by age group

Children with serum vitamin D deficiency represented only 1.78% (44 cases) of the total of 2,464 patients. The majority of children with deficiency were girls (29 cases; 65.90%), and the predominant area of origin was urban (31 cases; 70.45%). Regarding the children with insufficient levels of vitamin D, most patients were diagnosed during the period of December to February (20 cases; 45.45%) (Figure 5). The distribution of the 44 patients across age groups shows a similar incidence for the age categories of 11-14 years and 15-17 years, with 12 cases (27.27%) each.

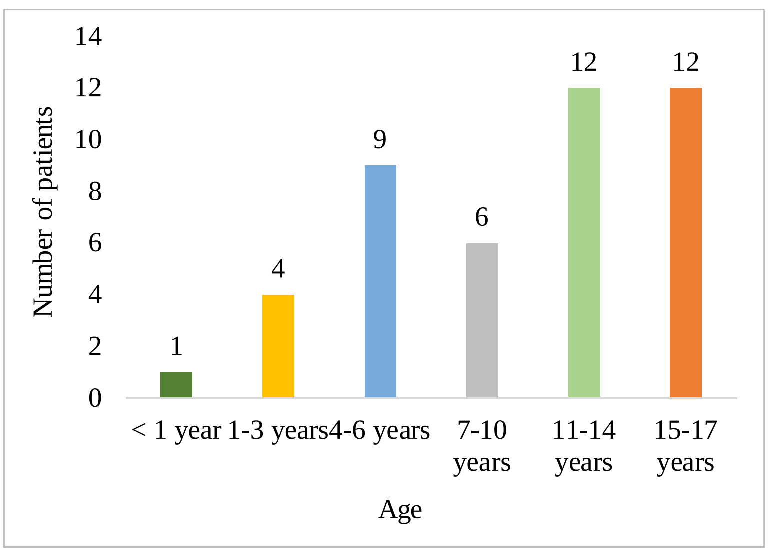

Figure 3. Distribution of patients with vitamin D deficiency, by age group

These groups are followed by the age groups of 4-6 years with 9 cases (20.46%), 7-10 years with 6 cases (13.64%), and 1-3 years with 4 cases (9.09%). Only one patient (2.27%) was found among the children under 1 year old (Figure 3). The lowest number of patients in the study were those with a toxic level of vitamin D in the serum - 16 cases (0.64%). Regarding the toxic levels of vitamin D, contrary to the previous situations, our results have shown that most cases were found in boys (12 cases; 75%) and only 4 cases in girls (25%), while the distribution by the place of origin showed an equal distribution between urban and rural areas (8 cases; 50%). The age group with the most cases was between 1-3 years old (12 patients; 75%), followed by the 4-6 years age group with 3 cases (18.75%) and the 7-10 years age group with 1 case (6.25%) (Figure 4).

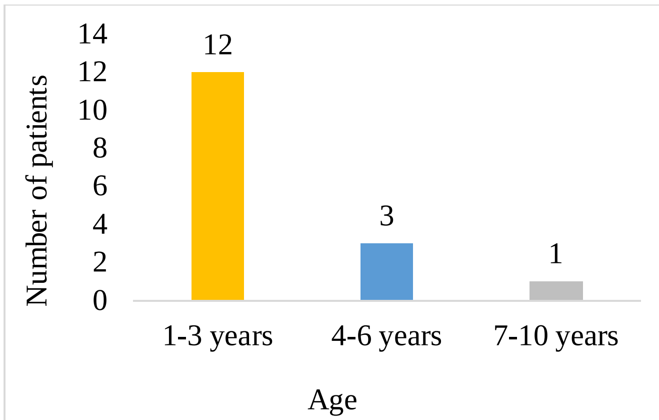

Figure 4. Distribution of patients with toxic levels of vitamin D, by age group

Additionally, the period with the highest number of cases was represented by the winter months, December-February (14 cases; 88%) (Figure 5).

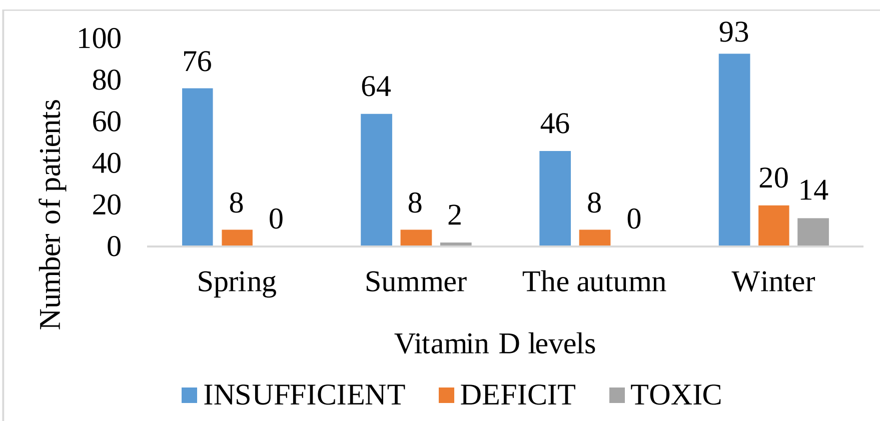

Figure 5. The correlation between the plasma level of vitamin D and the seasons

Most of the patients with insufficient levels of vitamin D out of the total of 279 cases were diagnosed during the winter months, December to February (93 cases; 33.33%) (Figure 5).

 Vol. 23, 2023
Received December /Accepted March 2023

Discussion

This study was a cross-sectional one, based on the evaluation of the prevalence of vitamin D insufficiency, deficiency, and toxicity in children aged 0-17 years, taking into account the period of vitamin D level measurement, gender of patients, and place of origin. The results of this study indicate a much lower prevalence of vitamin D insufficiency (11.32%) compared to the values reported among the children in the UK (35.1%; age 4-18 years). Unlike the reports of Absoud *et al.* (2011), who found no significant difference in gender among children, the undertaken study revealed that a relatively higher prevalence among girls compared to boys. Furthermore, similar to their results, an increasing risk of vitamin D insufficiency in older age groups was also observed in this study. This may be due to the fact that there are no recommendations for vitamin D supplementation in older children. In addition, older children may have less sun exposure due to fewer opportunities to play outdoors. It should be noted that the periods of vitamin D level measurement were different. Our investigations took place in winter, in the months of December-February, compared to the summer months reported in the previous study.

The deficiency and insufficiency of vitamin D are common in some regions of Africa, with some studies reporting insufficiency in 19% of 10-year-old children and deficiency in 7% (Poopedi *et al.*, 2011). In Saudi Arabia, a study found that 59% of healthy children aged 4 to 15 years were deficient and insufficient in vitamin D (28%) (Mansour *et al.*, 2012).

Comparing the results of our study to those reported in a study from Romania from 2018 regarding the deficiency and toxic levels of vitamin D (Ene *et al.*, 2018) (Table 1 and 2), higher values for certain age groups were observed in the former study: [8.9% for vitamin D deficiency (14-18 years) and 11.5% for toxicity (0-1 year)] compared to those found in our study [deficiency: 0.48% for age groups 11-14 and 15-17 years, and toxicity: 0 cases for 0–1-year-olds].

Table 1. Prevalence of deficiency and toxic level of vitamin D in a study conducted in Romania 2018

Age group with vitamin D deficiency (years)	Prevalece (%)
0 – 1	1.92
1 – 3	0.66
4 – 6	1.96
7 – 10	0.24
11 – 14	4.30
15 – 17	8.90

Age group with toxic level of vitamin D (years)	Prevalence (%)
0 – 1	11.50
1 – 3	4.90

Table 2. Prevalence of vitamin D deficiency and toxic level in Emergency Clinical Hospital for Children, "St. Ioan", Galați

Age group with vitamin D deficiency (years)	Prevalece (%)
0 – 1	0.04
1 – 3	0.16
4 – 6	0.36
7 – 10	0.24
11 – 14	0.48
15 – 17	0.48
Age group with toxic level of vitamin D (years)	**Prevalence (%)**
1 – 3	0.48
4 – 6	0.12
7 – 10	0.04

Each patient who presented serum vitamin D levels outside the normal range underwent treatment for correction. Those with vitamin D deficiency and insufficiency received supplementation according to the customized treatment regimens, as appropriate for their comorbidities. Those with toxic levels of vitamin D had their vitamin D supplementation immediately discontinued and were administered isotonic NaCl solution.

Conclusion

The prevalence of vitamin D insufficiency in the pediatric patients included in our study was 11.32%. The prevalence of vitamin D deficiency among our study participants was 1.78%, and the toxicity was 0.64%. Both patients with insufficiency and deficiency of vitamin D presented higher percentages in boys and urban areas. The prevalence of vitamin D insufficiency varied by age, with a higher prevalence in the age group between 11-14 years. The assessment of vitamin D levels was performed during the critical winter period, from December to February, when sun exposure was limited. Vitamin D toxicity was more common in boys and during the winter months (December, January, February). In this case, the age group most affected was between 1-3 years. When some diseases are associated with vitamin D deficiency, urgent attention should be given to clothing and dietary behaviours, and new strategies for vitamin supplementation or the fortification of foods should be developed. A continuous monitoring and surveillance of the vitamin D levels in the pediatric population is important for broader public health programs and policies.

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