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Research Article

Relationship Between Serum Vitamin D Levels and Clinical Findings: What We Know and Unknown in Pediatric Cases

Serum D Vitamini Düzeyleri ile Klinik Bulgular Arasındaki İlişki: Pediatrik Olgularda Bildiklerimiz ve Bilmediklerimiz

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Abstract
Vitamin D plays a crucial role in calcium homeostasis, influencing bone health and overall well-being. Despite universal supplementation recommendations, vitamin D deficiency remains highly prevalent, particularly among pediatric populations. This study investigates the symptoms and clinical findings associated with vitamin D levels in children, focusing on recurrent respiratory infections (RRIs), night sweats, rib flare, and genu varum. Electronic medical records of 396 children (aged 3.5 days to 17.6 years) from the pediatric clinic of a private hospital were analyzed. Serum levels of vitamin D, parathyroid hormone, calcium, phosphorus, and alkaline phosphatase were evaluated, and symptomatic cases underwent further assessment. Among the patients, 76.26% exhibited low vitamin D levels. Symptoms such as night sweats and RRIs were correlated with vitamin D insufficiency and deficiency (80.28% and 80.87%, respectively). Specific clinical findings, including rib flare (75.12%) and genu varum (78.26%), were also associated with low vitamin D levels. The study revealed weak positive correlations between vitamin D levels and both calcium ($r=0.287$, $p<0.001$) and phosphorus levels ($r=0.211$, $p<0.001$), a weak negative correlation with parathyroid hormone levels ($r=-0.219$, $p<0.001$), and no significant correlation with alkaline phosphatase levels ($r=0.083$, $p=0.100$). This study highlights a significant association between low vitamin D levels and specific symptoms and clinical findings in pediatric patients. Understanding these associations is essential for timely intervention and emphasizes the importance of assessing vitamin D status in children presenting with RRIs, night sweats, rib flare, and genu varum.
Keywords: Night Sweats, Recurrent Respiratory Infections, Rib Flare, O-bain, Vitamin D.

Özet
D vitamini, kalsiyum homeostazında önemli bir rol oynar ve hem kemik sağlığını hem de genel refahı etkiler. Evrensel takviye önerilerine rağmen, D vitamini eksikliği özellikle pediatrik popülasyonlarda yaygınlığını korumaktadır. Bu çalışma, çocuklarda D vitamini düzeyleriyle ilişkili semptom ve klinik bulguları araştırmakta, özellikle tekrarlayan solunum yolu enfeksiyonları, gece terlemeleri, arcus costalis belirginliği ve genu varuma odaklanmaktadır. Özel bir hastanenin çocuk kliniğinden 396 çocuğun (yaş aralığı: 3,5 gün–17,6 yıl) elektronik kayıtları incelendi. D vitamini, paratiroid hormonu, kalsiyum, fosfor ve alkalen fosfataz düzeyleri değerlendirildi; semptomatik çocuklar ayrıca analiz edildi. Hastaların %76,26'sında düşük D vitamini düzeyleri saptandı. Gece terlemeleri ve tekrarlayan solunum yolu enfeksiyonları gibi semptomlar, sırasıyla %80,28 ve %80,87 oranında D vitamini yetersizliği veya eksikliği ile ilişkiliydi. Arcus costalis belirginliği (%75,12) ve genu varum (%78,26) gibi klinik bulgular da düşük D vitamini düzeyleriyle ilişkilendirildi. D vitamini düzeylerinin kalsiyum ($r=0,287$, $p<0,001$) ve fosfor ($r=0,211$, $p<0,001$) düzeyleriyle zayıf pozitif, paratiroid hormonu ile zayıf negatif korelasyon gösterdiği; alkalen fosfataz düzeyleriyle ise anlamlı bir ilişki bulunmadığı saptandı ($r=0,083$, $p=0,100$). Bu çalışma, pediatrik hastalarda düşük D vitamini düzeyleri ile belirli semptom ve klinik bulgular arasındaki önemli ilişkiyi ortaya koymakta ve tekrarlayan solunum yolu enfeksiyonları, gece terlemeleri, arcus costalis belirginliği ve genu varum ile başvuran çocuklarda D vitamini durumunun değerlendirilmesinin önemine dikkat çekmektedir.
Anahtar Kelimeler: Gece Terlemeleri, Tekrarlayan Solunum Yolu Enfeksiyonları, Arcus Costalis Belirginliği, O-bain, D Vitamini.

Introduction

Vitamin D3 (cholecalciferol) is a fat-soluble vitamin synthesized in the skin, known for its resistance to heat, acid, alkali, and oxidation. Its intestinal absorption requires bile, and it becomes active through hydroxylation in the liver and kidney. On the other hand, Vitamin D2 is obtained from plants and yeast. Both forms are biologically equivalent (1, 2). The primary function of vitamin D is facilitating the absorption of calcium (Ca) and phosphorus (P) from the gastrointestinal tract and regulating their resorption with a direct impact on bones (2).

Deficiency of vitamin D in growing children may lead to rickets, osteomalacia, tetany, and convulsions. On the other hand, an excess of vitamin D may result in vomiting, loss of appetite, pancreatitis, hypertension, arrhythmia, central nervous system effects, polyuria, kidney stones, and kidney failure (2). Additionally, it has been reported that sufficient vitamin D is necessary for proper muscle function (3). To prevent deficiency, it is recommended that all infants receive 400 units/day of cholecalciferol from birth to one year of age, and this supplementation is provided free of charge in Türkiye (4).

Vitamin D deficiency remains a widespread public health concern. A meta-analysis indicates that the prevalence of Vitamin D deficiency in our country varies between 24% and 99%, with our region reporting a rate of 63.5% (below 30 ng/ml) (5, 6).

Rickets, osteomalacia, osteoporosis, chronic kidney disease, liver failure, malabsorption syndromes, hyperparathyroidism, medications, individuals of African and Hispanic descent, pregnant and breastfeeding women, elderly adults with a history of falls, elderly adults with non-traumatic fractures, obese children and adults (BMI > 30 kg/m2), disorders causing granuloma formation, and certain lymphomas are generally considered indications for investigating vitamin D levels (7). However, the criteria for requesting vitamin D level tests in children and adolescents are not adequately clear (8).

In our study, we aimed to investigate the symptoms and clinical findings of pediatric patients who presented with different complaints and had their vitamin D levels requested, in addition to the indications for vitamin D testing mentioned in the literature. Among the symptoms, we focused on recurrent respiratory infections (RRIs) and night sweats, while among the clinical findings, our attention was on rib flare and O-bain deformities.

Material and Method

This retrospective study was conducted over a 1.5-year period (between November 2016 and May 2018). Electronic records of children who were brought to the pediatric clinic of a private hospital (Mediclinic Hospital, Denizli, Türkiye), for whom the 25-hydroxy vitamin D (25(OH)D) test was requested for any reason, were extracted. Patients with chronic liver disease, renal insufficiency, or those using antiepileptic medications were excluded from the study. Ethical approval was obtained from the local committee.

Serum Vitamin D levels were categorized into four groups as sufficient (≥ 30 ng/ml), insufficient (21–29 ng/ml), deficient (<20 ng/ml), and high (>100 ng/ml) (7). Serum parathyroid hormone (PTH), Ca, P, and alkaline phosphatase (ALP) levels were grouped as high, normal, and low according to age groups (6, 9). Cases in which all these tests were not conducted together were excluded.

Serum vitamin D levels were measured using the chemiluminescence method on the Abbott Architect i2000SR immunoassay analyzer (2015, USA). Serum calcium (Ca) and phosphorus (P) levels were determined photometrically, while alkaline phosphatase (ALP) levels were measured enzymatically using the Abbott Architect C8000 biochemistry analyzer (2015, USA).

Patients presenting with night sweats, or a complaint of frequent respiratory tract infections (more than six respiratory diseases a year), or clinical findings of rib flare (RF) or genu varum (O-bain) during the physical examination were evaluated for their vitamin D levels (10, 11).

All statistical analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA) and Microsoft Excel 365. Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables as number (n) and percentage (%). The distribution of continuous variables was assessed using the Shapiro–Wilk test. Laboratory parameters (PTH, Ca, P, ALP) were compared between groups by Kruskal Wallis H test and descriptive statistics were shown as median (min-max). Pearson's correlation coefficient (for normally distributed variables) and Spearman's rank correlation coefficient (for non-normally distributed variables) were used to assess relationships between Vitamin D levels and other biochemical

markers (PTH, Ca, P, ALP). Significance level: A p-value < 0.05 was considered statistically significant.

Results

During the study period, Vitamin D, PTH, Ca, and ALP tests were requested for 396 out of 3932 children examined in the outpatient clinic (10%).

Of our patients, 40.4% were female (n=160), and 59.60% were male (n=236). The age distribution of the children ranged from a minimum of 3.5 days to a maximum of 17.6 years, with a mean age of 5.7 ± 4.19 years.

Laboratory levels for Vitamin D, PTH, Ca, and P levels are provided in Table 1.

No significant difference was observed between girls and boys in terms of vitamin D level groups, symptoms, and findings.

Vitamin D levels showed weak positive correlations with Ca (r=0.287, p<0.001) and P(r=0.211, p<0.001), a weak negative correlation with PTH (r=−0.219, p<0.001), and no significant correlation with ALP (r=0.083, p=0.100) (Table 2).

Vitamin D deficiency was observed at the highest rate in children with RRIs, at 80.87%. This was followed by night sweats at 80.28%, genu varum (O-bain) at 78.26%, and rib flare (RF) at 75.2%. In the total patient group, 76.26% had low Vitamin D levels (31.31% insufficient + 44.95% deficient) (Table 3).

Table 1. Laboratory levels

N= 396	Minimum	Maximum	Mean	Standard deviation
25(OH) D (ng/ml)	4.25	145.12	24.26	14.29
PTH (ng/L)	3.00	219.30	31.51	19.76
Ca (mg/dL)	6.9	11.9	10.01	0.65
P (mg/dL)	2.68	7.67	4.69	0.66
ALP (iU/L)	20.90	1065.32	223.56	94.79
Reference levels (23)				
25-hydroxy vitamin D: ≥30 ng/mL (≥75 nmol/L) indicate sufficiency, 20–29 ng/mL (50–74 nmol/L) indicate insufficiency, and <20 ng/mL (<50 nmol/L) indicate deficiency.				
(PTH) in serum is generally 10–65 pg/mL (1.1–6.9 pmol/L)				
Ca: 9.0–11.5 mg/dL (2.25–2.88 mmol/L) in cord blood, 9.0–10.6 mg/dL (2.30–2.65 mmol/L) in newborns aged 3–24 hours, 7.0–12.0 mg/dL (1.75–3.00 mmol/L) at 24–48 hours, 9.0–10.9 mg/dL (2.25–2.73 mmol/L) at 4–7 days, 8.8–10.8 mg/dL (2.20–2.70 mmol/L) in children, and 8.4–10.2 mg/dL (2.10–2.55 mmol/L) thereafter				
P: 4.8–8.2 mg/dL (1.55–2.65 mmol/L) in newborns aged 0–5 days, 3.8–6.5 mg/dL (1.25–2.10 mmol/L) in children aged 1–3 years, 3.7–5.6 mg/dL (1.20–1.80 mmol/L) at 4–11 years, 2.9–5.4 mg/dL (0.95–1.75 mmol/L) at 12–15 years, and 2.7–4.7 mg/dL (0.90–1.50 mmol/L) at 16–19 year				
ALP: 145–420 for children aged 1–9 years, 140–560 for ages 10–11, 200–495 in males and 105–420 in females for ages 12–13, 130–525 in males and 70–230 in females for ages 14–15, and 65–260 in males and 50–130 in females for ages 16–19 (iU/L)				

Abbreviations: 25(OH)D – 25-hydroxy vitamin D; PTH – parathyroid hormone; Ca – calcium; P – phosphorus; ALP – alkaline phosphatase.

Table 2. Correlation of laboratory levels

N=396		Vitamin D	PTH	Ca	P	ALP
25(OH) D	r	1	-0.219**	0.287**	0.211**	0.083
	p		<0.001	<0.001	<0.001	0.100
PTH	r	-0.219**	1	-0.171**	-0.055	0.039
	p	<0.001		0.001	0.272	0.444
Ca	r	0.287**	-0.171**	1	0.236**	0.070
	p	<0.001	0.001		<0.001	0.163
P	r	0.211**	-0.055	0.236**	1	0.209**
	p	<0.001	0.272	<0.001		<0.001
ALP	r	0.083	0.039	0.070	0.209**	1
	p	0.100	0.444	0.163	<0.001	

** Correlation is significant at the 0,01 level (2-tailed). Abbreviations: 25(OH)D – 25-hydroxy vitamin D; PTH – parathyroid hormone; Ca – calcium; P – phosphorus; ALP – alkaline phosphatase.

Table 3. Distribution of vitamin D level groups according to symptoms and clinical findings

		High	Sufficient (s)		Insufficient (i)		Deficient (d)		Insufficient (I) + Deficient (d)	
	n	n	n	%	n	%	n	%	n	%
Total	396	2	92	23.23	124	31.31	178	44.95	302	76.26
RF	213	2	51	23.94	71	33.33	89	41.78	160	75.12
Night sweats	213	1	41	19.25	67	31.46	104	48.83	171	80.28
RRIs	115		22	19.13	39	33.91	54	46.96	93	80.87
RF+ Night Sweats	103	1	23	22.33	30	29.13	49	47.57	79	76.70
RF+ Night Sweats + RRIs	35		7	20.00	12	34.29	16	45.71	28	80.00
O-Bain	23		4	17.39	9	39.13	9	39.13	18	78.26

RF: Rib flare, RRIs: recurrent respiratory infections.

Among 213 cases with rib flare (RF), 75.12% had low 25(OH) D levels, of which 33.33% were classified as insufficient and 41.78% as deficient. In patients experiencing night sweats, 80.28% of the 213 cases had low 25(OH) D levels, with 31.46% insufficient and 48.83% deficient. Among 115 cases with recurrent respiratory infections (RRIs), 80.87% showed low 25(OH) D levels, with 33.91% insufficient and 46.96% deficient. In 103 cases presenting both rib flare and night sweats, 76.70% had low 25(OH) D levels, with 29.13% insufficient and 47.57% deficient. Among 35 cases with the combination of rib flare, night sweats, and RRIs, 80.00% had low 25(OH) D levels, including 34.29% insufficient and 45.71% deficient. Finally, in 23 cases with genu varum (O-Bain), 78.26% exhibited low 25(OH) D levels, with 39.13% insufficient and 39.13% deficient (Table 3).

A significant difference was found between the vitamin D groups in terms of PTH, Ca, and P ($p<0.001$ for all comparisons). When the multiple comparison results were examined, the sufficient group and the deficient groups differed from each other in terms of PTH and P. The insufficient group is similar to other groups. In terms of Ca, the sufficient group has significantly higher levels than the other groups. (Table 4).

Table 4. Comparison of laboratory findings between groups according to vitamin D levels

Parameter	Deficient (≤20 ng/ml, n=178)	Insufficient (21–29 ng/ml, n=124)	Sufficient (≥30 ng/ml, n=92)	p-value
PTH (ng/L)	32.25 (3-219.30)[a]	26.40 (3-126)[ab]	24.50 (3-64.50)[b]	<0.001
Ca (mg/dL)	9.90 (6.90-11)[a]	10.10 (8.10-11.30)[a]	10.30 (8.90-11.90)[b]	<0.001
P (mg/dL)	4.53 (2.68-7.67)[a]	4.71 (3.07-6.25)[ab]	4.86 (3.73-6.43)[b]	<0.001
ALP (IU/L)	213 (30.34-562.99)	207.36 (20.90-627.13)	217.36 (30.62-1065.32)	0.771

Levels are presented as median (min–max). p-levels were calculated using Kruskal–Wallis test. The same letters show similarity between groups while different letters Show statistically significant difference between groups. Abbreviations: 25(OH)D – 25-hydroxy vitamin D; PTH – parathyroid hormone; Ca – calcium; P – phosphorus; ALP – alkaline phosphatase. Two cases with serum 25(OH)D levels >100 ng/mL (high) were excluded from this comparison.

Discussion

In our study, low Vitamin D levels were found in children with RRIs, night sweats, rib flare, and O-Bain.

The total rate of Vitamin D deficiency in the cases was higher in our region compared to the study conducted by Özhan et al.(6) (76.26% vs 63.5%), and the average Vitamin D levels were lower (24.26 + 14.29 ng/mL vs 27.21 ± 15.90 ng/mL).

It has been proposed that vitamin D plays a crucial role in natural immunity by influencing the production of antimicrobial peptides, and it is considered to be of critical importance in respiratory, skin, and intestinal infections (12, 13). Maintaining a serum Vitamin D level above 30 ng/mL is recommended for an adequate immune response (13). According to a meta-analysis conducted by Martineau et al.(14), vitamin D supplementation has been reported to prevent acute respiratory tract infection. In our study, the rate of vitamin D insufficiency + deficiency was found to be significantly low at 80.87% in individuals experiencing RRIs.

There are very few studies examining the relationship between night sweats and Vitamin D deficiency (15). In an animal experiment conducted by Nobuo Koike et al.(16), 1.25(OH)2-vitamin D3 was shown to bind to nuclear receptors in the epithelium of mouse sweat glands, myoepithelial secretory coils, and ducts in cells. In a review study by Shi-Sheng Zhou et al.(17), it was suggested that it can be concluded that the loss of sweating through the skin and the Vitamin D status may be an indicator of skin excretory function, not as a result of vitamin D deficiency. In a study by Akbaş et al.(18) involving 70 adults with hyperhidrosis, Vitamin D deficiency was found in 82.8% of cases. The sweating area was reported as craniofacial in 14.8% (<30 ng/L). Özkan et al.(19) associated head sweats with Vitamin D deficiency in infants aged 0-6 months. In our study, the rate of insufficiency and deficiency was high (80.2%) in children experiencing night sweats.

Harrison's sulcus appears as a groove at the lower end of the chest cage in small children/babies and is often associated with weak bones or chronic respiratory disease. Although the exact cause is not known, it is generally thought to result from the inward pulling of the ribs by the diaphragm (20). A complete Harrison's sulcus can be observed in severe and prolonged cases of rickets. Vitamin D has receptors in muscle cells. For genomic effects, it binds to the nuclear receptor as 1.25(OH)2D, stimulating the synthesis of certain proteins. Direct effects occur after binding to receptors on the membrane, leading to a series of enzyme activations (21). RF can be seen in children who do not develop rickets (22). In our study, the Vitamin D levels of children with RF were found to be significantly low at a rate of

75.2%. Further studies are needed to quantitatively measure this distinctiveness and explore its etiopathogenesis (21).

Infants are provided with a daily 400 IU Vitamin D3 (cholecalciferol form) supplement until the age of one in Türkiye. The European Pediatric Endocrine Society recommends a daily dose of 400 IU Vitamin D3 support up to the age of one and 600 IU for those between 1-18 years old (7).

To maximize the impact of vitamin D on Ca, bone, and muscle metabolism, a Vitamin D blood level above 30 ng/ml is recommended (7). Additionally, our study suggests that maintaining a level above 30 ng/ml may be beneficial in preventing RRIs, rib flare, and leg deformities.

When children are brought up for an examination, the child should be completely undressed and examined lying on their back for the detection of RF and O-bain. Information about night sweats and RRIs can be obtained through systemic questioning. Additionally, by checking the hospital information system or pharmacy information system, the possibility of RRIs in the patient should be investigated. Encouraging the administration of Vitamin D3 is recommended during routine examinations for infants under one year of age. If any deficiency symptoms are observed, the Vitamin D level should be measured along with other laboratory tests.

The main limitation of this study is its retrospective design. Since the data were collected from existing electronic medical records, it was not possible to control several confounding factors that may influence serum vitamin D levels, such as dietary habits, sunlight exposure, physical activity, seasonal variation, and lifestyle differences. Furthermore, due to the observational nature of the study, causal relationships between vitamin D levels and the evaluated symptoms and clinical findings could not be established. Therefore, prospective, long-term, and controlled studies are required to confirm these associations and to further clarify the clinical impact of vitamin D deficiency.

Conclusion

Vitamin D deficiency remains a widespread hypovitaminosis despite preventive treatment given up to the age of one. In a clinical setting, RRIs and complaints of night sweats should be inquired about. During physical examination, attention should be given to the presence of RF and genu varum (O-bain) findings, and if any of these are present, planning for Vitamin D testing and appropriate treatment should be warranted.

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Conflict of interest statement

There is no conflict of interest between the authors of the article.

Ethics Committee Approval

Ethical approval was obtained from the local committee (03/05/2023 no: 83). The relevant document is attached.

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