

Variations of 25-Hydroxyvitamin D Levels During COVID-19 Pandemic and its Relation to Season, Sex and Age in Children

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Abstract

We aimed to investigate variations in vitamin D levels (VDLs) during the coronavirus disease-19 pandemic and their relationship to season, sex, and age in otherwise healthy children and adolescents. We conducted a retrospective cross-sectional study at an outpatient pediatric clinic, which included 4,262 children aged 1-18 years. The study cohort was divided into three groups: Group 1 (pre-pandemic), Group 2 (pandemic), and Group 3 (post-pandemic). Vitamin D deficiency (VDD) was defined as a level below 12 ng/mL, insufficiency as a level between 12 and 20 ng/mL, and sufficiency as a serum level above 20 ng/mL. The pandemic cohort exhibited significantly lower VDLs compared with both the pre-pandemic and post-pandemic cohorts. Females had significantly lower VDLs than males. The prevalence of VDD was highest among adolescents. A significantly higher rate of VDD was observed in the pandemic group among the 6-11 and 12-18 age groups compared with both the pre-pandemic and post-pandemic groups. VDLs were significantly lower in spring and winter than in summer and autumn. Additionally, an inverse relationship was observed between age and VDLs. Our study revealed a significant prevalence of VDD in school-aged children and adolescents, with a notable decrease in VDLs observed during the pandemic compared with other time periods. Furthermore, our study highlighted the increased vulnerability of female adolescents to VDD.

Keywords: Adolescent, children, COVID-19, pandemic, vitamin D deficiency

Introduction

Vitamin D plays a critical role in regulating calcium and phosphate metabolism and exerts various effects on peripheral organs, tissues, and immune system components, demonstrating anti-inflammatory and immunomodulatory properties.¹ While it can be acquired from dietary sources, its main source is endogenously produced in the skin through ultraviolet-B exposure. Several factors, such as

skin pigmentation, race, season, body mass index, and nutrition, can influence vitamin D levels (VDLs).^{2,3} Beyond its traditional association with bone health, vitamin D regulates multiple organ systems; thus, identifying and addressing deficiencies in vitamin D are crucial. Previously linked to rickets in children, vitamin D deficiency (VDD) is now associated with significant extra-skeletal conditions like atopic and autoimmune disorders. Managing VDD



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through preventive measures and supplementation can alleviate these disorders. Hence, early detection of asymptomatic individuals who may appear healthy but have VDD is vital in preventing insufficiency.⁴

Although children generally experience mild symptoms from coronavirus disease-19 (COVID-19), the disease has led to severe complications and significant morbidity and mortality in adults.⁵ Consequently, many countries have enforced measures like social distancing, stay-at-home orders, and curfews to manage the outbreak.⁶ After the first COVID-19 case in the country, online education was switched to schools. To mitigate the spread of the virus, the Turkish government initially imposed a curfew for individuals aged 65 years and older and later extended this restriction to include those aged 20 years and older. Throughout the pandemic, various restrictions were gradually introduced and modified, culminating in the lifting of the curfew on July 1, 2021, as part of Türkiye's comprehensive pandemic control strategy. The implementation of various measures and widespread vaccination efforts have gradually diminished the spread and incidence of COVID-19. Nevertheless, the restrictions imposed during the pandemic have adversely affected children in both the short- and long-term.⁷⁻⁹ The pandemic-induced curfew has led to increased sedentary behavior and reduced sunlight exposure among children.¹⁰ There is a lack of studies investigating fluctuations in VDLs in the COVID-19 pandemic periods and the interplay of sex, age, and season in pediatric populations. Hence, we aimed to investigate the variations of VDLs in COVID-19 pandemic period and its relation to season, sex, and age in otherwise healthy children and adolescents.

Material and Method

Approval was granted by the KTO Karatay University Faculty of Medicine, Pharmaceutical and Non-Medical Device Research Ethics Committee (meeting date: 17.11.2023, decision no: 2023/021). The present study was conducted at the outpatient pediatric clinic of the University of Health Sciences Türkiye, Konya Beyhekim Training and Research Hospital from March 2018 to October 2023. The study included children whose VDLs were measured during the study period and who were between the ages of 1 and 18. Information on the dates of application, sex, age, and blood results of the participants was obtained from the hospital's computerized database. In order to measure VDLs prior to, during, and following the COVID-19 pandemic as well as to assess the effect of the pandemic's restriction measures on VDLs, the study cohort was stratified into three distinct groups: Group 1 (pre-pandemic, March 2018 to March 2020), Group 2 (pandemic, April 2020 to June 2021), and Group 3 (post-pandemic, July 2021 to October 2023). The study exclusively utilized the first recorded VDL of participants who underwent multiple

measurements. The study excluded children younger than 1 year old, those with a medical history of metabolic disorders, and individuals with conditions known to adversely affect vitamin D metabolism and levels, including chronic kidney disease, liver disease, celiac disease, and malabsorption syndromes, those receiving

corticosteroid therapy or antiepileptic medications, neurological patients who were bedridden for an extended period, and individuals diagnosed with type 1 or type 2 diabetes mellitus, as well as oncology and transplantation patients.

The participants were divided into three age groups [1-5 years old (pre-schoolers), 6-11 years old (school children), and 12-18 years old (adolescents)] to allow for comparison of VDLs. To further explore the impact of seasons on VDLs, the application seasons were categorized based on the dates that the participants submitted their

applications. Serum 25 (OH) D was measured using a fully automated immunoassay method (ADVIA Centaur XP®, Siemens, Munich, Germany). VDD was defined as a serum level below 12 ng/mL, vitamin D insufficiency (VDI) as a range between 12 and 20 ng/mL, and vitamin D sufficiency (VDS) as a serum level above 20 ng/mL.¹¹

Statistical Analysis

Categorical variables are presented as n (%). Because of their non-normal distribution, all data are presented as median (IQR). For group comparisons, the Mann-Whitney U test was used, the chi-square test or Fisher's exact test was used, and Spearman's test was used to assess correlations. Group comparisons were performed using the Kruskal-Wallis test, and multiple comparisons were adjusted using the Bonferroni corrected Mann-Whitney U test. Using stepwise multivariate linear regression analysis, it was discovered that independent determinants significantly ($p < 0.05$) explained the variance of the dependent variable. The analysis was performed using SPSS software version 21.0 for Windows, with a significance level of $p < 0.05$. GraphPad Prism 9.0 was used to create the figures.

Results

Demographic Characteristics and VDLs of the Study Groups

The study included 4262 children aged 1-18 years. The cohort's median age was 7.5 years (IQR: 8.2 years), with 2028 (47.6%) males and 2234 (52.4%) females. The median serum VDL level was 15.13 ng/mL (IQR: 10.33 ng/mL). The sex distribution was similar between the three study groups, ($p > 0.05$). In addition to having a lower median VDL than the other two groups, the pandemic group also had a younger median age (**Table 1, Figure 1**).

Only 28.4% of the participants had adequate VDLs compared with 32.1% who had VDD and 39.5% who

Highlights

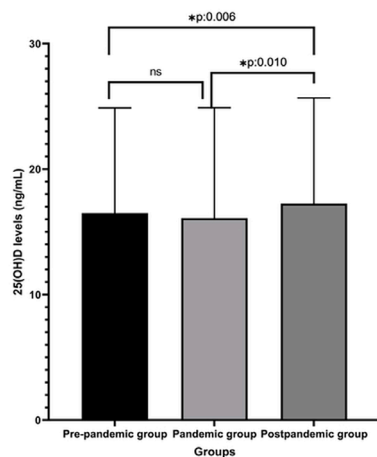
- 25-hydroxyvitamin D levels were lower in the pandemic group than in the pre-pandemic and post-pandemic groups.
- The current study highlighted the risk of 25-hydroxyvitamin D deficiency among school-aged children and adolescents, particularly female students.
- Vitamin D levels were significantly lower in spring and winter than in summer and autumn.

Table 1.
Demographic characteristics and 25(OH)D levels

Characteristics	Pre-pandemic group (n=2111)	Pandemic group (n=179)	Post-pandemic group (n=1972)	P value*	P value [§]	P value [€]	P value [€]	P value [€]
Sex								
(Males/females)	989/1112	83/96	956/1016	0.550				
(%)	(46.8/53.2)	(46.4/53.6)	(48.5/51.5)					
Age (years)	7.3 (8.4)	5.5 (8.4)	8.1 (7.90)		<0.0001	#0.004	&0.004	¥<0.0001
25(OH)D level (ng/mL)	15.01 (10.14)	14.12 (11)	15.33 (10)		0.003	#0.170	&0.006	¥0.010

Quantitative variables are presented as the median (interquartile range). Qualitative variables are expressed as numbers and percentages. Results were compared using the Kruskal-Wallis test followed by the Bonferroni-corrected Mann-Whitney U test. Significant differences were determined by $p < 0.05$ for the Kruskal-Wallis test and $p < 0.016$ ($p = 0.05/3$) for the Bonferroni correction. P values are indicated in bold. The chi-square test was performed to compare categorical variables

[§]; Kruskal-Wallis test, [€]; Mann-Whitney U test, *; Chi-square test, #; Pre-pandemic group versus pandemic group, &; Pre-pandemic group versus post-pandemic group, ¥; Pandemic group versus post-pandemic group, 25(OH)D; 25-hydroxyvitamin D

**Figure 1.** 25(OH)D values in the study groups

had VDI. A comparison of the median ages of individuals with VDD (9.9 years), VDI (7.4 years), and VDS (5.3 years) revealed a significant difference in the median age of those with VDD, with individuals in this group being notably older ($p < 0.0001$). Among all study participants, females exhibited a significantly lower median VDL than males (14 ng/mL versus 16.56 ng/mL). A significant disparity in the rate of VDD was noted when comparing age groups within the entire study cohort, with the highest occurrence observed among adolescents (**Table 2, Figure 2**).

Comparison of VDLs During the COVID-19 Pandemic According to Sex and Age

When comparing sex, regardless of age group, females had lower median VDLs than males in all three groups. Females aged 6-11 and 12-18 years showed significantly lower VDLs than males in both the pre-pandemic and post-pandemic groups. Females aged 12-18 years in the pandemic group had significantly lower VDLs than males (**Table 3**).

Comparison of VDLs During the COVID-19 Pandemic According to Age Groups

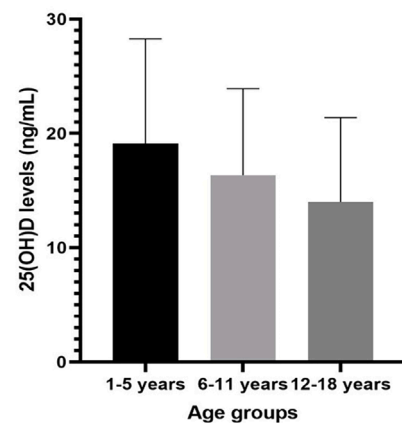
There was no obvious difference between the three groups' VDLs for children aged 1-5, whereas there was a discernible difference between the groups' median VDLs for children 6-11 and 12-18. The pandemic group showed considerably lower VDLs in these two age groups than the post-pandemic group (**Table 4**).

Table 2.
Rates of vitamin D deficiency, insufficiency, and sufficiency according to age group

Age groups	25(OH)D level (ng/mL)			Total (n, %)	P value*
	<12 (n, %)	12-20 (n, %)	>20 (n, %)		
1-5 years	373 ^a (22.3)	656 ^b (39.1)	647 ^c (38.6)	1676 (39.3)	<0.0001
6-11 years	461 ^a (31)	664 ^b (44.7)	362 ^a (24.3)	1487 (34.9)	
12-18 years	532 ^a (48.4)	365 ^b (33.2)	202 ^c (18.4)	1099 (25.8)	
Total	1366 (32.1)	1685 (39.5)	1211 (28.4)	4262	

Qualitative variables are expressed as numbers and percentages. P values are highlighted in bold

*; Chi-square test, 25(OH)D; 25-hydroxyvitamin D

**Figure 2.** 25(OH)D levels in the study population by age group

During the COVID-19 Pandemic, the Rates of VDD, VDI, and VDS by Age Group

When comparing the three groups based on age groups, a greater rate of VDD was observed in the pandemic group in the 6-11 and 12-18 age groups than in the other groups (**Table 5**).

VDLs According to Seasons

VDLs were obtained from 1149 (27%) participants in the spring, 1068 (25.1%) in the summer, 989 (23.2%) in the fall, and 1056 (24.8%) in the winter. The median VDLs were 18.97 ng/mL in summer, 17.50 ng/mL in autumn,

Table 3.
25-hydroxyvitamin D levels during the COVID-19 pandemic according to age and sex

Characteristics	Pre-pandemic group			Pandemic group			Post-pandemic group		
	Males	Females	P value [€]	Males	Females	P value [€]	Males	Females	P value [€]
1-5 years (n, %)	474 (53.9)	404 (46.1)		50 (51.5)	47 (48.4)		374 (53.3)	327 (46.6)	
25(OH)D level (ng/mL)	17.66 (10.93)	16.08 (10.53)	0.068	18.99 (10)	16.59 (11)	0.691	19.62 (10.6)	18.68 (12.68)	0.252
6-11 years (n, %)	339 (48.8)	355 (51.1)		19 (43.1)	25 (56.8)		381 (50.8)	368 (49.1)	
25(OH)D level (ng/mL)	15.71 (8.80)	14.11 (9.07)	0.002	12.78 (13)	10.60 (10)	0.538	16.82 (9.89)	15.19 (8.36)	0.001
12-18 years (n, %)	176 (32.6)	363 (67.3)		14 (36.8)	24 (63.1)		201 (38.5)	321 (61.4)	
25(OH)D level (ng/mL)	14.41 (9.58)	10.89 (7.99)	<0.0001	11.38 (4)	8.47 (5)	0.019	16 (8.83)	11.2 (7)	<0.0001
Total number of participants (%)	989 (46.8)	1122 (53.1)		83 (46.3)	96 (53.6)		956 (48.4)	1016 (51.5)	
25(OH)D level (ng/mL)	16.46 (10.22)	13.82 (9.9)	<0.0001	15.12 (12)	12.29 (9)	0.042	16.85 (10)	14.30 (10)	<0.0001

Quantitative variables are presented as the median (interquartile range). Qualitative variables are expressed as numbers and percentages. P values are indicated in bold
[€]; Mann-Whitney U test, COVID-19; Coronavirus disease-19, 25(OH)D; 25-hydroxyvitamin D

Table 4.
25-hydroxyvitamin D levels by age group during the COVID-19 pandemic

Characteristics	Pre-pandemic group	Pandemic group	Post-pandemic group	P value [§]	P value [€]		
1-5 years (n)	878	97	701				
25 (OH) D level	16.82 (11.22)	17.32 (10.70)	18.20 (10.90)	0.064			
6-11 years (n)	694	44	749				
25 (OH) D level	14.92 (9.01)	11.15 (10.90)	15.08 (8.73)	0.006	[#] 0.038	[§] 0.066	[*] 0.003
12-18 years (n)	539	38	522				
25 (OH) D level	12.20 (8.73)	9.65 (5.05)	12.79 (9.03)	0.001	[#] 0.009	[§] 0.026	[*] <0.0001

Quantitative variables are presented as the median (interquartile range). Qualitative variables are expressed as numbers. Results were compared using the Kruskal-Wallis test followed by the Bonferroni-corrected Mann-Whitney U test. Significant differences were determined by $p < 0.05$ for the Kruskal-Wallis test and $p < 0.016$ ($p = 0.05/3$) for the Bonferroni correction. P values are indicated in bold

[§]; Kruskal-Wallis test, [€]; Mann-Whitney U test, ^{*}; Pre-pandemic group versus pandemic group, [§]; Pre-pandemic group versus post-pandemic group, ^{*}; Pandemic group versus post-pandemic group, COVID-19; Coronavirus disease-19, 25(OH)D; 25-hydroxyvitamin D

Table 5.
Deficiency, insufficiency, and sufficiency rates of 25(OH)D in different age groups during the COVID-19 pandemic

Age groups	25(OH)D level (ng/mL)	Pre-pandemic group (n, %)	Pandemic group (n, %)	Post-pandemic group (n, %)	P value [*]
1-5 years	<12	204 ^a (23.2)	22 ^a (22.7)	147 ^a (21)	0.309
	12-20	354 ^a (40.3)	40 ^a (38.4)	262 ^a (37.6)	
	>20	320 ^a (36.4)	35 ^a (36.1)	292 ^a (41.7)	
6-11 years	<12	225 ^a (32.4)	23 ^b (52.3)	213 ^a (28.4)	0.012
	12-20	308 ^{a,b} (44.4)	12 ^b (27.3)	344 ^a (45.9)	
	>20	161 ^a (23.2)	9 ^a (20.5)	192 ^a (25.6)	
12-18 years	<12	265 ^a (49.2)	29 ^b (76.3)	238 ^a (45.6)	0.007
	12-20	173 ^a (32.1)	7 ^a (18.4)	185 ^a (35.4)	
	>20	101 ^a (18.7)	2 ^a (5.3)	99 ^a (19)	

Qualitative variables are expressed as numbers and percentages. P values are highlighted in bold
^{*}; Chi-square test, 25(OH)D; 25-hydroxyvitamin D

12.89 ng/mL in spring, and 12.45 ng/mL in winter. Notably, compared with summer and autumn, the median VDLs were lower in spring and winter (**Figure 3**).

Independent Variables for VDD in All Study Groups

Age and VDLs were negatively correlated ($r = -0.269$, $p < 0.0001$) in the study cohort. Furthermore, all three groups exhibited age-related correlations with VDLs, with corresponding correlation coefficients of $r =$

0.288 ($p < 0.0001$), $r = -0.500$ ($p < 0.0001$), and $r = -0.290$ ($p < 0.0001$). A multivariate regression analysis revealed that the significant independent variables for the dependent variable of VDD were winter season [odds ratio (OR): 2.725, confidence interval (CI) % 2.343-3.170, $p < 0.0001$], adolescent age group (OR: 2.520, CI% 2.173-2.923, $p < 0.0001$), pandemic group (OR: 2.170, CI% 1.579-2.983, $p < 0.0001$), and female sex (OR: 0.560, CI% 0.487-0.643, $p < 0.0001$).

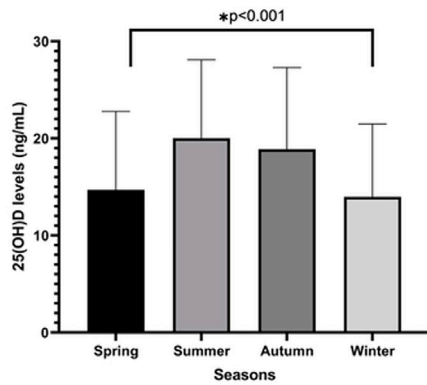


Figure 3. Season-specific 25(OH)D levels in the study populat

Discussion

This study highlights the variation in VDLs among children during different phases of the COVID-19 pandemic by providing a thorough analysis of VDLs in a pediatric cohort. Moreover, it provides insightful information about vitamin D status and VDD in relation to age, sex, and seasonal variations.

VDI and VDD are major public health issues in developing countries. This is significant because VDD is more common in the pediatric population and it has an impact on bone health during the growth period.¹² Studies continue to focus on the growing body of evidence linking VDD to non-skeletal disorders, such as inflammatory bowel disease, asthma, atopic dermatitis, type 1 diabetes mellitus, and others. Although routine vitamin D measurement is not recommended in clinical practice, it is helpful for children who may be at risk of VDD. This study assessed a pediatric cohort's vitamin D status at different points during the COVID-19 pandemic. This study clarifies how children's VDL fluctuate during these periods.

Measuring serum VDLs is necessary to determine vitamin D status because of its long half-life, which makes it unaffected by changes in children's parathyroid hormone levels.⁴ International guidelines offer varying reported reference ranges for VDD and VDI, and there is no set cut-off serum VDL that indicates VDD.¹² The outcomes of clinical research may be impacted by these differences in reference ranges. As a result, we used recently suggested consensus reference values for children in our study.¹¹

A study involving children aged 3-17 found a prevalence of 80.3% for VDD using a cut-off value of <15 ng/mL.¹³ Another study including children aged 1-18, reported a prevalence of 34.1% for VDD and 27.5% for VDI.¹⁴ Our study revealed a prevalence of 32.1% for VDD and 39.5% for VDI. These variations underscore the potential for differences in VDD prevalence not only between countries but also within different geographical regions of the same country. Even though it was conducted in a specific region, the results of our study reflecting the situation in Türkiye are quite strong because it was conducted with a significant sample size. However, the potential for limited generalizability of the results to different settings or population warrants further investigation. Exploring how regional disparities might

influence the extrapolation of these findings to other groups would be beneficial.

According to some theories, females are more likely to suffer from VDD because they spend less time outside, cover up more, and receive less sunlight exposure.¹⁵ In a study involving 331 Saudi children aged 6 to 17, it was found that girls' VDLs were substantially lower in girls than in boys. Female sex was shown to be an independent risk factor for VDD in Chinese children in another extensive study conducted by Hu et al.¹⁶ In all three COVID-19 pandemic periods, we found that females had lower VDLs than males. This finding supports the findings of previous research and highlights the significance of female sex in VDD, independent of the pandemic period. Our research revealed that female sex was an independent variable of VDD in our study groups.

According to recent studies, teenage girls are more likely than boys of the same age to have VDD and frequently have lower VDLs.¹⁴ It has been proposed that the sex effect, which is associated with variations in the levels of sex steroids released during puberty, contributes to VDD in adolescence.¹⁷ A multi-center study reported prevalence rates of 13.7% in pre-school-aged children, 18.2% in school-aged children, and 23.9% in adolescents, defined as VDD 12 ng/mL. The prevalence of VDD among teenagers was found to be 23.46% in another study.¹⁸ These rates were found to be 17.8%, 24.9%, and 42.6%, respectively, in a recent study.¹⁴ In these age groups, the prevalence of VDD was found to be, respectively, 22.3%, 31%, and 48.4% in our study. In all three periods, school-aged and adolescent females had significantly lower VDLs than males, according to our study, which also found a similar sex distribution among all the children. In addition, compared with the other age groups, adolescents had a noticeably higher prevalence of VDD. These results support those of earlier clinical studies.¹⁴ Our research indicates that children and adolescents with VDD have a higher prevalence. According to our research, adolescence is one of the independent variables associated with VDD, and the risk of the condition is roughly 2.5 times higher in this age group. This suggests that teenagers are especially susceptible to low VDLs. According to a recent study⁶, the data thus imply that vitamin D supplementation may be necessary in this age group.

According to recent research, there is a significant negative correlation between children's age and their VDL.^{19,20} According to our research, VDLs fall with advancing age. In the pre-pandemic, pandemic, and post-pandemic periods, we also discovered a negative correlation between age and VDLs.

A study conducted in Italy²¹ involved the examination of 491 children. The average VDLs were found to be lower in the post-pandemic group than in the other groups in the study. The VDLs of children in our study prior to the pandemic were comparable to those observed during the pandemic, as reported in Italy.²¹ However, our findings indicate that during the pandemic, VDLs in children were lower than those documented in earlier periods. The reduction in sun exposure and outdoor activities could be attributed to pandemic-related measures,

such as stay-at-home orders and school closures. In contrast to the other two times, children's VDLs were significantly higher in our study following the pandemic. Our research revealed that the pandemic group was an independent variable associated with VDD in our study groups. The detrimental effects of COVID-19 restrictions and extended stays home on VDLs are highlighted by these findings. Extended isolation caused by COVID-19 is probably associated with less time spent in the sun, which lowers the rate at which vitamin D is synthesized cutaneously.⁹

In a study, the impact of COVID-19 precautions on VDLs in 3600 children aged 0-6 years was examined.⁹ A comparison of 2020 with previous years demonstrated that home confinement in children aged 3-6 years not only reduced their VDLs but also increased the prevalence of VDD. Furthermore, the study found that VDLs were lower in children aged 3-6 compared to those under 3 years before and after pandemic-related home confinement, indicating an age-related decline in VDLs. In comparison with other age groups, the 1-5 age group had a lower prevalence of VDD (22.3%), according to our study. When comparing the pandemic periods, we were unable to find a significant difference in VDLs in the 1-5 age group, which may have been caused by the fact that children in this age range were exempt from pandemic restrictions. The differences in prevalence rates observed between Yu et al.⁹ study and our own study raise the possibility that different vitamin D supplementation strategies used in various nations may have an effect.

According to a recent study, year and season significantly contribute to variation in VDLs when analyzed using linear regression analysis.²¹ Regardless of the study period, we observed seasonal variations in VDLs, with winter and spring showing noticeably lower VDLs than other seasons. Our results support earlier research²¹ and show that sun exposure is essential for vitamin synthesis. All three study periods showed the expected seasonal variation, with lower VDLs in spring and winter and higher VDLs in summer. Our research also revealed that winter season was the most effective independent variable for predicting VDD in our study groups.

Study Limitations

Due to the retrospective cross-sectional study design, we were unable to access records related to factors that could influence VDLs, such as dietary intake and vitamin D supplementation. Additionally, we lack information on the exact amount of sun exposure for children and adolescents. Nevertheless, one strength of our study is the inclusion of a large number of children and adolescents.

Conclusion

Our study revealed a significant prevalence of VDD in school-aged children and adolescents, with a notable decrease in VDLs observed during the pandemic compared with other time periods. Furthermore, our study highlighted the increased vulnerability of

female adolescents to VDD. We delineated winter season, adolescence, pandemic circumstances, and female sex as significant determinants affecting VDLs independently.

Ethics

Ethical Approval: Approval was granted by the KTO Karatay University Faculty of Medicine, Pharmaceutical and Non-Medical Device Research Ethics Committee (meeting date: 17.11.2023, decision no: 2023/021).

Informed Consent: Because the study was designed retrospectively no written informed consent form was obtained from the patients.

Footnotes

Author Contributions: Sert S: Surgical and Medical Practices, Concept, Design, Data Collection or Processing, Analysis or Interpretation, Literature Search, Writing.; Taner A: Surgical and Medical Practices, Concept, Design, Data Collection or Processing, Literature Search, Writing.

Conflict of Interest: The authors declare no conflicts of interest.

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