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Research

Assessment of Vitamin D Sufficiency in Different Age Groups of Children in Türkiye Using the Bhattacharya Method

Türkiye'deki Farklı Yaş Gruplarındaki Çocukların Vitamin D Yeterliliğinin Bhattacharya Yöntemi ile Değerlendirilmesi

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ABSTRACT

Objective: Vitamin D plays a role in regulating mineral balance, particularly calcium and phosphate, and in promoting bone health. It is essential to have reliable age-specific reference values for vitamin D in order to make accurate clinical decisions. This study aimed to establish reference ranges for 25-hydroxyvitamin D [25(OH)D] levels in children aged 1-19 years in Türkiye using the Bhattacharya method and to assess vitamin D status across different age groups.

Methods: Data from 243,906 [25(OH)D] measurements, collected from hospital laboratory records between 2019 and 2023, were analyzed.

Results: The reference ranges for serum [25(OH)D] levels vary by age and gender. For children aged 1-2 years, the range is 24.5-56.6 ng/mL, decreasing progressively to 16-42.9 ng/mL for ages 7-10 years. For women aged 11-15 years, the range is 14.8-35.5 ng/mL, and for ages 16-19 years, it is 15-39 ng/mL. For male patients, the ranges are slightly higher: 15.6-40.7 ng/mL for ages 11-15 years and 15.8-39.3 ng/mL for ages 16-19 years. The study revealed that the prevalence of [25(OH)D] deficiency levels below 12 ng/mL (considered deficient based on the institute of medicine criteria) increased with age among children and adolescents. This trend underscores a notable rise in deficiency rates after early childhood, peaking during adolescence.

Conclusion: The results provide significant insights into the vitamin D status of the pediatric population in Türkiye. The findings emphasise a decline in vitamin D levels with age and indicate that the highest prevalence of deficiency is seen in adolescents.

Keywords: Parathyroid hormone (PTH), vitamin D, 25-hydroxyvitamin D [25(OH)D], Bhattacharya method, indirect method, reference interval

ÖZ

Amaç: D vitamini, başta kalsiyum ve fosfat olmak üzere mineral dengesinin düzenlenmesinde ve kemik sağlığının geliştirilmesinde rol oynamaktadır. Doğru klinik kararlar verebilmek için D vitamini için yaşa özgü güvenilir referans değerlere sahip olmak önemlidir. Bu çalışmanın amacı, Bhattacharya yöntemini kullanarak Türkiye'de 1-19 yaş arası çocuklarda 25-hidroksivitamin D [25(OH)D] düzeyleri için referans aralıkları oluşturmak ve farklı yaş gruplarında D vitamini durumunu değerlendirmektir.

Gereç ve Yöntem: 2019-2023 yılları arasında hastane laboratuvar kayıtlarından toplanan 243.906 [25(OH)D] ölçümünden elde edilen veriler analiz edilmiştir.

Bulgular: Serum [25(OH)D] düzeyleri için referans aralıkları yaşa ve cinsiyete göre değişmektedir. Bir ile iki yaş arası çocuklar için bu aralık 24,5-56,6 ng/mL'dir ve 7-10 yaş arasında kademeli olarak 16-42,9 ng/mL'ye düşmektedir. On bir ile on beş yaş arası kadınlar için aralık 14,8-35,5 ng/mL ve 16-19 yaş için 15-39 ng/mL'dir. Erkek hastalar için aralıklar biraz daha yüksektir: 11-15 yaş için 15,6-40,7 ng/mL ve 16-19 yaş için 15,8-39,3 ng/mL. Çalışma, 12 ng/mL'nin altındaki (tıp enstitüsü kriterlerine göre eksik kabul edilen) [25(OH)D] eksikliği seviyelerinin yaygınlığının çocuklar ve ergenler arasında yaşla birlikte arttığını ortaya koymuştur. Bu eğilim, erken çocukluk döneminden sonra ergenlik döneminde zirve yapan eksiklik oranlarında kayda değer bir artışın altını çizmektedir.

Sonuç: Sonuçlar, Türkiye'deki pediatrik nüfusun D vitamini durumuna ilişkin önemli bilgiler sağlamaktadır. Bulgular, yaşla birlikte D vitamini seviyelerinde bir düşüş olduğunu vurgulamakta ve en yüksek eksiklik prevalansının ergenlerde görüldüğüne işaret etmektedir.

Anahtar Kelimeler: Paratiroid hormonu (PTH), vitamin D, 25-hidroksivitamin D [25(OH)D], Bhattacharya yöntemi, indirekt yöntem, referans aralığı

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INTRODUCTION

Vitamin D plays a crucial role in regulating mineral metabolism, maintaining calcium and phosphate homeostasis, and promoting bone and overall health (1). Accurate interpretation of clinical decisions requires reliable reference values for vitamin D specific to age (2,3). Parathyroid hormone (PTH) enhances calcium reabsorption and inhibits phosphate reabsorption in the kidneys. Furthermore, it enhances the synthesis of 1,25-dihydroxyvitamin D, which subsequently elevates the gastrointestinal absorption of calcium (1,4). Vitamin D is a fat-soluble vitamin that plays a role in regulating the balance of calcium and phosphorus and in enhancing bone development (5). Vitamin D deficiency increases the risk of several health problems, including rickets, bone fractures, various types of cancer, cardiovascular disease, diabetes, autoimmune and metabolic disorders, infections due to immune-system dysfunction, and even certain neuropsychiatric disorders (6). It is therefore crucial to ascertain the levels of vitamin D present in the population. The most effective laboratory test for the assessment of vitamin D levels in the body is the measurement of 25-hydroxyvitamin D [25(OH)D] (7).

Reference range studies allow us to comprehend the normal distribution and variations of laboratory test results within a population, which contributes to the evaluation of health status. This, in turn, assists in making more precise clinical decisions and diagnoses (8).

The EP28-A3C guideline proposes that population-based reference intervals can be established through either direct sampling of individuals from a healthy population (termed the direct sampling approach) or indirect sampling, also known as data mining techniques (9).

The results of data mining analysis demonstrate reference intervals that are highly comparable to those obtained from direct reference interval studies for a diverse range of analytes (10). Bhattacharya is a data mining technique that can be used to analyse large databases of patient results. It facilitates the stratification of subjects into multiple age and sex partitions without compromising statistical power, following the application of rigorous exclusion criteria (11,12).

The aim of this study was to determine reference intervals for [25(OH)D] levels in children of different age groups in Türkiye using the Bhattacharya method. Therefore, this study aims to evaluate the vitamin D status in these age groups, and provide insights into its distribution and possible deficiencies.

METHODS

Study Design and Data Collection

In our study, we calculated the reference range for [25(OH)D] (n=243,906) using hospital-based data from raw laboratory records registered in the laboratory information system (LIS) of the Central Biochemistry Laboratory of İstanbul University, İstanbul Faculty of Medicine between 2019 and 2023. The study was not designed with a specific protocol but used pre-selected patients, and specific exclusion criteria were applied for the included patients (4). Only data of participants aged between 1 and 19 years were included. The study excluded data from patients older than 19 years (n=100,102). Patients below one year old were excluded from the study due to insufficient laboratory data (n=62). Hospitalized and follow-up patients (n=51,021) were also excluded from the study. Additionally, data exceeding the detection limits of the analysis methods ([25(OH)D] levels >1000 or <5 ng/mL) were excluded from the study without statistical analysis (n=1,383). Vitamin D, calcium, and PTH measurements were requested together in the trial, and patients with calcium and PTH levels outside the reference range were excluded (n=21,170). Measurement of [25(OH)D] plasma levels was performed using a high-performance liquid chromatography (HPLC) device (Thermo Ultimate 3000, USA) with a ultraviolet detector. The vitamin D clinical research HPLC kit (Recipe Chemicals Instruments Munich, Germany) was employed for the analysis.

The [25(OH)D] test is routinely conducted in our laboratory. Prior to analysing patients' samples, an internal quality control test is performed using two samples with known specific concentrations representing normal and pathological conditions.

Ethical Approval and Informed Consent

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Approval was obtained from the İstanbul University, İstanbul Faculty of Medicine Clinical Research Ethics Committee (approval no: 13, date: 12.07.2024). As this was a retrospective study using anonymised data from the LIS, informed consent was not required.

Statistical Analysis

Visual assessment of the distribution of plasma [25(OH)D] levels was performed using a boxplot graph (Figure 1). One-way ANOVA was used for partitioning based on age and sex comparisons, with results showing $p<0.05$ being considered statistically significant (13).

The reference ranges for vitamin D hormone were calculated using the Bhattacharya method. The data were sorted into subgroups in ascending order with equal intervals. The total frequency of the data was then divided into 20 equally spaced classes, with a bin size of 2.0, for [25(OH)D]. The logarithm of the counts (represented as fi) and the Δlog (fi) values were calculated for transitions from one bin to another. To minimize the influence of random fluctuations, we utilized the 5-point Savitzky-Golay smoothing procedure, resulting in a smoother curve. Additionally, following the suggestion of Oosterhuis et al. (12), we introduced a weighting factor to improve the precision of the Bhattacharya analysis. After plotting the derivative of the frequency of the measured values against the logarithm of the concentration, we calculated the intercept (a) and slope (b) of the linear relationship. This was done only for the linear portion of the relationship where the coefficient of determination (R^2) exceeded 0.99 (as shown in Figure 2) (11,13). However, if the fit was not satisfactory, we applied a Box-Cox transformation. The parameter λ for the transformation was selected based on the best fit to the data using https://www.statology.org/box-cox-transformation-excel/ (Figure 3). The midpoint, upper, and lower reference limits (URL and LRL) were calculated and a data plot was generated using Microsoft Excel.

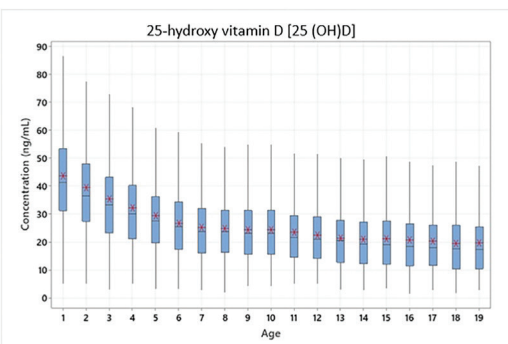

Figure 1. Distribution of serum [25(OH)D] concentration according to age. The median values are shown as horizontal lines and mean values are indicated with asterisks
[25(OH)D]: 25-hydroxyvitamin D

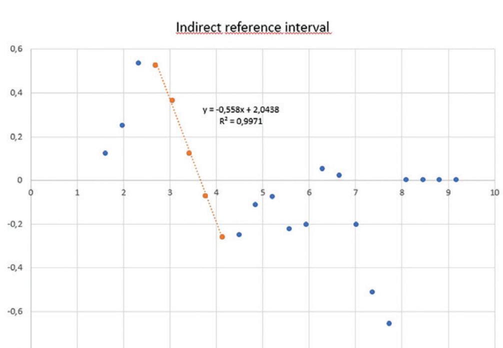

Figure 2. [25 (OH)D] Bhattagram dataset for females aged 11-14. The Gaussian component of the dataset was calculated using the slope and y-intercept of the line of best fit for these data points
[25(OH)D]: 25-hydroxyvitamin D

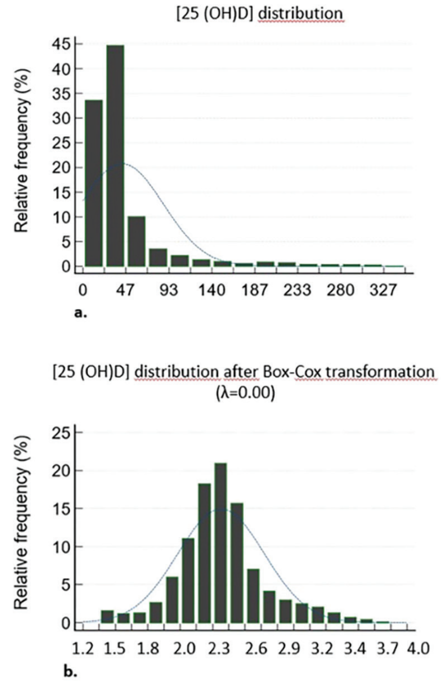

Figure 3. The distribution of a skewed [25 (OH)D] before (a) and after (b) applying the Box-Cox transformation
[25(OH)D]: 25-hydroxyvitamin D

RESULTS

After applying exclusion criteria, 70,168 vitamin D data points were included in the study. Vitamin D data from 29,921 males and 40,247 females aged 1 to 19 years were used in the study. The reference intervals for the [25(OH) D] test were calculated jointly for males and females across the first 10 age groups. For the age groups 11-14 and 15-19, separate calculations were conducted for both sexes.

The reference ranges for serum [25(OH)D] levels in children aged 1-2 years are 24.5-56.6 ng/mL and 22.4-56.3 ng/mL in spring/summer and autumn/winter, respectively. For 3-year-olds, the ranges are 21.5-55.7 ng/mL and 20.3-55.6 ng/mL; for 4-year-olds, 19.1-49.7 ng/mL and 18.6-48.2 ng/mL; for 5-year-olds, 17.0-47.3 ng/mL and 16.8-47.8 ng/mL; for 6-year-olds, 16.6-48.0 ng/mL and 16.3-46.2 ng/mL; and for children aged 7-10 years, 16.0-42.9 ng/mL and 15.7-43.0 ng/mL, respectively.

For females aged 11-15 years, the reference ranges are 14.8-35.5 ng/mL and 14.5-34.2 ng/mL in spring/summer and autumn/winter and for those aged 16-19 years, 15.0-39.0 ng/mL and 15.0-38.6 ng/mL, respectively.

In males aged 11-15 years, the ranges are 15.6-40.7 ng/mL in spring/summer and 15.4-40.3 ng/mL in autumn/winter, and for those aged 16-19 years, 15.8-39.3 ng/mL in spring/summer and 15.5-39.8 ng/mL in autumn/winter, respectively (Table 1).

Table 1. Reference ranges for the 25-hydroxyvitamin D [25 (OH)D], stratified by age, sex and season

[25(OH)D] (ng/mL)						
Age groups	**Gender**	**Season**	**n**	**Midpoint**	**Lower limit (LL)**	**Upper limit (UL)**
1-2 age	Both	Spring/Summer	1313	40.5	24.5	56.6
		Autumn/Winter	1402	39.4	22.4	56.3
3 age	Both	Spring/Summer	1232	38.6	21.5	55.7
		Autumn/Winter	1438	37.9	20.3	55.6
4 age	Both	Spring/Summer	1554	34.4	19.1	49.7
		Autumn/Winter	1611	33.4	18.6	48.2
5 age	Both	Spring/Summer	1589	32.2	17.0	47.3
		Autumn/Winter	1712	32.3	16.8	47.8
6 age	Both	Spring/Summer	1479	32.3	16.6	48
		Autumn/Winter	1519	31.25	16.3	46.2
7-10 age	Both	Spring/Summer	3810	29.5	16.0	42.9
		Autumn/Winter	4356	29.4	15.7	43.0
11-15 age	Female	Spring/Summer	3014	24.0	14.8	35.5
		Autumn/Winter	5112	24.4	14.5	34.2
11-15 age	Male	Spring/Summer	3788	28.2	15.6	40.7
		Autumn/Winter	3987	27.9	15.4	40.3
16-19 age	Female	Spring/Summer	6500	27.0	15.0	39
		Autumn/Winter	7310	26.8	15.0	38.6
16-19 age	Male	Spring/Summer	7407	27.8	15.8	39.3

DISCUSSION

This study establishes sex- and age-specific reference intervals for vitamin D in children and adolescents in Türkiye. The results show that vitamin D levels vary according to both age and sex in individuals aged 1-18 years. In addition, the study evaluates vitamin D status in the child population using the calculated reference intervals.

Vitamin D levels are influenced by several factors, including geographical location, seasonal variation, ethnicity, skin pigmentation, clothing, diet and sunscreen use (14). Vitamin D deficiency should be assessed using established cut-off values rather than reference ranges. The 2010 Institute of Medicine (IOM) guidelines define vitamin D deficiency as less than 12 ng/mL (30 nmol/L), vitamin D insufficiency as 12-19 ng/mL, and vitamin D sufficiency as 20 ng/mL (50 nmol/L) and above (15). The recommendations of the Endocrine Society Committee categorise vitamin D levels as follows: levels below 20 ng/mL are considered vitamin D deficiency, levels between 21 and 29 ng/mL indicate vitamin D insufficiency, and levels at or above 30 ng/mL are considered sufficient (with a preferred range of 40-60 ng/mL). In addition, levels above 150 ng/mL are considered indicative of vitamin D toxicity (16).

In our study, we established reference ranges for vitamin D [25(OH)D] to assess vitamin D status in the pediatric population. These reference ranges were calculated separately for each age group between 1 and 6 years. In the first three years, we found that vitamin D levels were within the IOM target range. The Turkish Ministry of Health recommends providing 400 IU/day of vitamin D to all infants to prevent deficiency and promote bone health. This recommendation, established in 2005, should be followed until the child is at least one year old, preferably extending until the age of three (17). We believe that the Ministry of Health's vitamin D supplementation program for infants has been successfully implemented in this age group. However, in our study, we found that [25(OH)D] levels decreased in children aged three and older. Our study found that vitamin D levels decrease with age, while PTH hormone levels increase. This trend may be due to increased vitamin D supplementation during early childhood. PTH and vitamin D interact in a complex feedback system to regulate calcium levels. PTH strongly stimulates the synthesis of vitamin D in the kidney, while vitamin D exerts a negative feedback effect on the release of PTH (1,18).

In particular, we observed that the reference range was lowest in the 11-15 age group. When evaluating serum

[25(OH)D] levels according to age groups, we obtained the following results: for one year, 2%; for two years, 3.3%; for three years, 5%; for four years, 7.4%; for five years, 7.8%; for six years, 10%, for 7-10 years, 13%; for 11-15 years, 25.8%; for 16-18 years, 23.9% of the results indicated [25(OH)D] levels below 12 ng/mL, which we considered deficient according to the IOM. Similarly, Yakarış et al. (19) demonstrated a decline in vitamin D levels with age in children between 2012 and 2019. The study conducted by Yeşiltepe-Mutlu et al. (20) on [25(OH)D] levels in the Turkish population found that the distribution of [25(OH)D] levels for the age range of 1-18 years is consistent with our findings. Similarly, we observed that participants aged 1-10 years had higher [25(OH)D] levels, compared to other age groups. However, in contrast to their study, we calculated separate reference ranges for each age group in the first 6 years. As in our country, vitamin D deficiency in chilhood is common worlwide (21).

In our study, we investigated the seasonal variation of [25(OH)D] levels. Our findings revealed that levels exhibited seasonal fluctuations. We observed that [25(OH)D] levels were highest within the reference range during the summer, and lowest during the winter and spring seasons (Figure 4). Previous studies have also shown seasonal variations, and these findings are consistent with our study (20,22,23).

The Bhattacharya method assumes that most patients who have come to the hospital are not actually ill, and suggests that statistical techniques can be used to distinguish between healthy and pathological data (10-12).

Baadenhuijsen and Smit (11) state that the Bhattacharya technique requires a significant amount of data to be applied effectively. This is necessary to avoid large statistical fluctuations and to identify the linear part of the distribution. The authors recommend collecting more than 1500 values for each analyte. The Bhattacharya algorithm assumes that a significant portion of an unselected general population

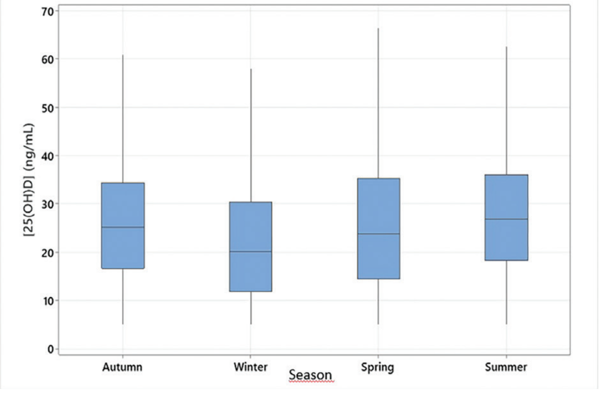

Figure 4. Seasonal distribution of [25(OH)D] levels
[25(OH)D]: 25-hydroxyvitamin D

can be regarded as "normal" It also assumes that there is only a partial overlap between the "healthy" portion and the abnormal (either high or low) portion (12).

The Bhattacharya method is a graphical technique used to determine a Gaussian distribution in a dataset. The linear segment of the obtained graph, where the R^2 value should be greater than 0.99, includes the data to be used for reference value analysis. The data between the values that establish the lower and upper limits of the curve are utilized for reference value analysis (10).

Study Limitations

This study has some limitations. Firstly, we did not use a reference population to establish reference ranges, so our study lacks comparison between reference intervals created by both direct and indirect methods. In addition, due to the unavailability of complete patient data, we could not apply all intended exclusion criteria. In particular, we did not exclude patients without a registered diagnosis from the study.

CONCLUSION

The use of the Bhattacharya method proved an effective means of calculating reliable reference intervals, distinguishing between healthy and pathological data, and identifying critical trends in [25(OH)D] levels. These findings highlight the importance of age-specific reference intervals for paediatric populations, thus enhancing the accuracy of clinical evaluations related to bone health and mineral metabolism. Furthermore, the findings of this study emphasize the necessity for effective monitoring and supplementation strategies to address vitamin D deficiency in children and adolescents.

ETHICS

Ethics Committee Approval: Approval was obtained from the İstanbul University, İstanbul Faculty of Medicine Clinical Research Ethics Committee (approval no: 13, date: 12.07.2024).

Informed Consent: Retrospective study.

FOOTNOTES

Authorship Contributions

Surgical and Medical Practices: F.H.K., S.K., A.F.A., Concept: F.H.K., S.K., A.F.A., Design: F.H.K., S.K., A.F.A., Data Collection or Processing: F.H.K., S.K., A.F.A., Analysis or Interpretation: F.H.K., S.K., A.F.A., Literature Search: F.H.K., S.K., A.F.A., Writing: F.H.K., S.K., A.F.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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