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Determination of 25 hydroxyvitamin D reference ranges in Hatay region by indirect method

Hamdi Oğuzman[1], Serdar Doğan[1]

[1] Hatay Mustafa Kemal University Tayfur Ata Sökmen Faculty of Medicine, Department of Medical Biochemistry, Hatay, Türkiye

Abstract

Objective: The aim of this study is to determine the reference ranges of 25 hydroxyvitamin D (25(OH)D) levels from the serum 25(OH)D results of the patients and to investigate variations across age and gender groups.

Methods: The patients (n=26829) who applied to Hatay Mustafa Kemal University Hospital between January 2018 and December 2019 and whose 25(OH)D levels were studied were included in this retrospective study. Serum 25(OH)D levels were studied by the chemiluminescence immunoassay method. The hospital information management system was used to obtain the test results and patient data. The indirect method was used to determine the reference ranges.

Results: The prevalence of 25(OH)D deficiency in the patients participating in this study was 60.9% in the general population, 54.9% in men, and 67.5% in women. It has been observed that 25(OH)D deficiency is more common in women than in men. The reference ranges determined in this study were lower than the reference ranges provided by the manufacturer.

Conclusion: In this study, 25(OH)D levels and reference ranges of Hatay Region were determined in a very large population. It can be said that the results of our region will contribute to the studies to be carried out on the determination of 25(OH)D levels and reference ranges throughout the country.

Keywords: Vitamin D, Vitamin D Deficiency, Laboratory Tests

INTRODUCTION

Vitamin D is a vitamin in steroid-structured, belonging to the group of fat-soluble vitamins (1). This vitamin is taken with food at a rate of 10-20%, and 80-90% is synthesized in the skin under the influence of UVB rays. Vitamin D plays a very crucial role in maintaining calcium and phosphorus balance and bone mineralization (2). Furthermore, studies have shown that vitamin D is associated with chronic diseases such as diabetes and cardiovascular diseases, psychiatric and sleep disorders, some malignancies, osteoporosis, infectious diseases, autoimmune diseases, and hypertension (3). The best indicator of the status of vitamin D synthesized in the human body is serum 25 hydroxyvitamin D (25(OH)D) level, but there is no consensus in the literature regarding the optimal level of 25(OH) D. In most of the published guidelines, serum 25(OH)D levels above 20 ng/mL are considered sufficient, between 10-20 ng/mL as insufficient and below 10 ng/mL as deficiency (4).

The reference range is the interval wherein the reference values, determined through statistical methods for a given test determined in the population of reference individuals are defined (5). In determining the reference range, direct or indirect methods are used in the selection of the reference individual from the reference population.

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Corresponding Author: Hamdi Oğuzman, Hatay Mustafa Kemal University Tayfur Ata Sökmen Faculty of Medicine, Department of Medical Biochemistry, Hatay, Türkiye
Email: hamdioguzman@gmail.com
ORCID iD: 0000-0003-1412-7689

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The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommends the use of the direct method for reference range determination. However, there are various studies in the literature in which the indirect method was used as a reference (6-9). There are reference ranges determined by the relevant manufacturer for each test studied in clinical laboratories, but it is recommended that each clinical laboratory determine its own reference range due to differences between populations and clinical laboratories (5,10). The objective of this study is to ascertain the reference ranges for serum 25(OH)D levels based on patient data and to investigate variations among age and gender groups.

METHODS

Patients who applied to Hatay Mustafa Kemal University Hospital between January 2018 and December 2019 and whose 25(OH)D levels were studied were included in this retrospective study. Serum 25(OH)D levels were analyzed by the chemiluminescence immunoassay method using a Siemens kit and calibrators on the ADVIA Centaur XP (Siemens, Germany) autoanalyzer in Hatay Mustafa Kemal University Hospital Central Laboratory, Department of Biochemistry. Demographic data of the patients and 25(OH)D vitamin results were obtained from the Hospital Information Management System (HIS). The 25(OH)D results of the patients followed up with the diagnosis of hyperparathyroidism, cancer, celiac disease, chronic liver disease, and stage III, IV, and V chronic kidney disease was excluded from the study. Furthermore, patients who had more than one 25(OH)D test result had their initial result accepted for the research, and the remaining findings were excluded. To obtain serum, blood samples were centrifuged at 1500*g for 10 minutes using gel-coated biochemistry tubes. During the period of the study, two different levels of internal quality control samples and an external quality control program were used in the laboratory, and quality control follow-up was carried out. In addition, during the study, all tests were studied on serum samples taken into the same brand biochemistry tubes, using autoanalyzers, kits, and calibrators from the same manufacturer.

Statistical analysis

Analyzes were performed after removing outliers with the SPSS software program version 23.0 (IBM Corporation, Armonk, NY, USA). Patient data were defined as a number, percentage, and mean±SD. Kolmogorov-Smirnov test, Shapiro-Wilk test, and histograms were used to evaluate the normal distribution of test results. Student-t test was used for comparisons between groups. Comparisons between age groups were made with the ANOVA test, and post-hoc with the Tukey test.

The non-parametric (percentage estimation method) method was used to determine the lower and upper limits of the reference ranges. In the distribution of the test results of the reference population, the results within the 95% section in the center were accepted as the reference range, and the results between 2.5% and 97.5% for men and women were determined according to the formula below.

Lower limit value=0.025 x (n+1)

Upper limit value= 0.975 x (n+1)

n represents the number of data. $p<0.05$ was considered statistically significant.

RESULTS

At first stage, 25(OH)D results of 28240 patients were evaluated. In these results, extreme values were removed by using the statistical program. Afterward 25(OH)D reference ranges were determined as a result of the test of 26829 patients. The 25(OH)D data for the general population and by gender are shown in Table 1 before and after the exclusion of extreme values, respectively. The 25(OH)D reference ranges for which 2.5 percentile and 97.5 percentile values were determined

Table 1. General and sex based vitamin D statistics before and after exclusion of extreme values

VITD (ng/mL)	n	Mean	SD	Median	CI (%95)		Var	Range	Min	Max	IQR
					Lower Limit	Upper Limit					
Before extreme are removed	28240	15.44	9.56	13.79	15.33	15.55	91.55	143.89	4.2	148.09	10.53
After extreme are removed	26829	14.77	7.02	13.79	14.69	14.69	49.31	33.33	4.2	37.53	9.94
Females	19006	14.45	7.03	13.42	14.35	14.35	49.47	33.33	4.2	37.53	10.04
Males	7823	15.53	6.93	14.63	15.38	15.38	48.11	33.32	4.2	37.52	9.67

VITD: 25(OH) Vitamin D, SD: Standard Deviation, CI: Confidence Interval, Var: Variance, Min: Minimum, Max: Maximum, IQR: Interquartile Range

by the indirect method are given in Table 2. The mean age of 19006 female patients participating in the study was 43.99±8.17 years, and the mean age of 7823 male patients was 44.39±21.41 years. There was no statistical difference in the mean age of men and women (p>0.05).

After eliminating the extreme values, the 25(OH)D level was found to be 14.77±7.02 ng/mL in the general population, 14.45±7.03 ng/mL in female patients, and 15.53±6.93 ng/mL in male patients (Table 1). The 25(OH)D reference range was found to be 4.50-31.25 ng/mL in the general population, 4.35-30.95 ng/mL in women, and 4.97-31.63 ng/mL in men (Table 2).

Table 2. Reference ranges determined according to 25(OH) Vitamin D values observed in the 2.5th and 97.5th percentiles.

VITD (ng/mL)	n	Age (Mean±SD)	Percentile	
			2.5.	97.5.
General	26829	44.11±19.17	4.5	31.25
Females	19006	43.99±18.17	4.35	30.95
Males	7823	44.39±21.41	4.97	31.63

VITD: 25(OH) Vitamin D

Figure 1 shows that 25(OH)D data according to age groups are given. Serum 25(OH)D level is 13.72±6.68 ng/mL in the 18-30 age group, 14.4±6.88 in the 31-40 age group, 14.74±6.95 ng/mL in the 41-50 age group mL is 14.93±6.94 ng/mL in the 51-60 age group, 14.89 ±6.96 ng/mL in the 61-70 age group, and 15.38±7.41 ng/mL in those over 70 years of age.

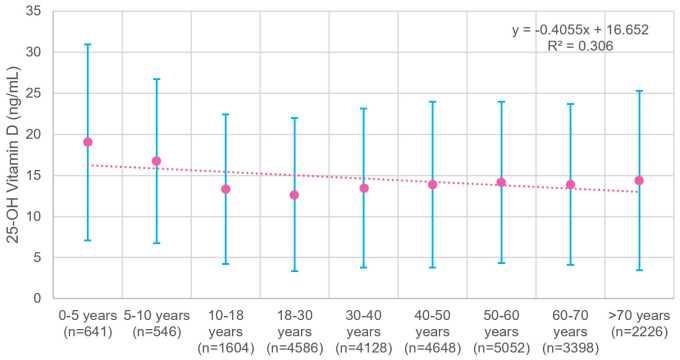

Figure 1. 25(OH) Vitamin D values according to age groups after removing extreme values.

In this study, the prevalence of 25(OH)D insufficiency (<20 ng/mL considered as insufficiency) was 76.8% in the general population, 73.7% in men, and 78.3% in women. 25(OH)D deficiency (<10 ng/mL was considered as the deficiency) was found in 60.9% of the general population, 54.9% in men, and 67.5% of women.

DISCUSSION

In the present study, the reference ranges of 25(OH) D levels in different age and gender groups were determined and the differences between the groups were showed. It has been found that the reference ranges for both men and women in the Hatay Region are lower than the current reference ranges proposed by the manufacturer.

Reference ranges play an important role in the evaluation of test results studied by clinical biochemistry laboratories by clinicians. It is essential to determine the reference population correctly in determining the reference range. Among the methods used here, the direct method is a laborious and costly method. For this reason, the indirect method, which is an easy-to-apply and inexpensive method, can be preferred in determining the reference population. In this method, patient results are obtained via HIS to determine the reference range with percentile distribution. Results are ordered from smallest to largest, and then 2.5% of the lower and upper limits are eliminated. Thus, the 95% part in the center is determined as the reference range. The high number of results in determining the reference range is an important factor that increases the reliability of the results. In this method, the results of at least 120 patients are required to obtain a healthy result (5-7). In the current study, a reference range study was performed on the test results of 26829 patients using the method mentioned above. This number of patients is sufficient for reference range studies.

The manufacturer has reported the reference values for 25(OH)D levels as 7.4-44.0 ng/mL for adult individuals. However, the reference ranges for 25(OH)D in the central laboratory of our hospital are given as 15.7-60.3 ng/mL for summer and 8.8-46.3 ng/mL for winter in patient results. In this study, the 25(OH)D reference range was found to be 4.50-31.25 ng/mL in the general population, 4.35-30.95 ng/mL in women, and 4.97-31.63 ng/mL in men. These values are lower than the values of the manufacturer. With the present study, 25(OH)D reference ranges of Hatay Region were evaluated for the first time in a very large population by using the HIS data using the indirect method. These results obtained from Hatay Region may contribute to the studies to be carried out on the determination of 25(OH)D levels and reference ranges throughout the country. In addition, it was aimed that the reference ranges determined in this study can

be used by clinical laboratories in our region, so that the 25(OH)D results of the patients will be evaluated more accurately.

There was no significant difference in mean age between male and female patients included in the study. The mean ages of female and male patients were found to be similar to previous vitamin D reference range studies in our country (8,9,11).

In studies conducted in Turkey, the prevalence of 25(OH)D deficiency was reported to be 63% (the lowest 34%, the highest 91%) in adult patients (12-14), 39.5% in male patients, and 64.7% in female patients (14). In a study conducted in Adana, which is a neighbor of Hatay Region, the rate of 25(OH)D deficiency was found to be 60.6% (15). In this study, the prevalence of 25(OH) D deficiency was found to be 60.9% in the general population, 54.9% in men, and 67.5% in women. In this study, deficiency rates were found similar to previous studies in our country. In addition, the results of studies conducted in our country and around the world have shown that the prevalence of 25(OH)D deficiency is higher in women (14). The reasons why vitamin D deficiency is more common in women than in men are biological differences, behavioral differences, and clothing style. In the current study, vitamin D deficiency was found to be higher in women, which is consistent with the literature.

Many studies have shown that 25(OH)D levels are affected by various factors such as geography, seasons, race, chronic kidney and liver diseases, obesity, gender, insufficient exposure to sunlight, and age (14, 16). However, in various studies examining 25(OH)D levels according to age groups, it was seen that there was no significant difference between the groups ($p>0.05$). There was no statistically significant difference in terms of age groups in the studies conducted by Durmaz et al. in the Amasya region (17), by Hekimsoy et al. in the Manisa region (18), by Alpdemir and Alpdemir in the Bilecik region (9), and by Uçar et al. in the Ankara region (19). Similar to the previous studies no difference was found between age groups in terms of 25(OH)D levels.

CONCLUSION

In conclusion, in this study, serum 25(OH)D levels and reference ranges of Hatay Region were determined in a very large population. It can be said that the results will contribute to the studies to be carried out on the determination of 25(OH)D levels and reference ranges throughout the country and that the reference ranges determined in this study can be used by our institution and the clinical laboratories of Hatay Region, so that the serum 25(OH)D results of the patients will be evaluated more accurately.

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Conflict of Interest

The authors declare that they have no conflict of interests regarding content of this article..

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Ethical Declaration

Ethical permission was obtained from the Hatay Mustafa Kemal University, Medical Faculty Clinical Research Ethics Committee for this study with date 23/05/2019 and number 22, and Helsinki Declaration rules were followed to conduct this study.

Authorship Contributions

Concept: HO, SD, Design: HO, Supervising: HO, SD, Financing and equipment: SD, HO, Data collection and entry: SD, HO, Analysis and interpretation: SD, HO, Literature search: SD, Writing: HO,SD, Critical review: SD, HO

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