TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2016) 46: 1512-1517

doi:10.3906/sag-1504-19

Determination of reference range with the indirect method of the 25-hydroxyvitamin D_3 test in the Balıkesir region, Turkey

Medine ALPDEMİR*, Mehmet Fatih ALPDEMİR
Department of Medical Biochemistry, Balıkesir State Hospital, Balıkesir, Turkey

Received: 07.04.2015 • **Accepted/Published Online:** 06.12.2015 • **Final Version:** 17.11.2016

Background/aim: The aim of this study is to determine the reference ranges of 25-hydroxyvitamin D3 (25(OH)vitD_3) by an indirect method using data obtained from patients over the course of 3 years.

Materials and methods: The 25(OH)vitD_3 test results of the patients who applied to the medical biochemistry laboratory of Balıkesir State Hospital between the years of 2010 and 2014 were analyzed. Patient data were retrospectively taken from the laboratory information system. The 25(OH)vitD_3 levels of patients were examined after exclusion of outliers. Patients were divided into subgroups according to season, sex, and age. The central 95% reference intervals were calculated using a nonparametric method.

Results: Calculated reference intervals showed lower values than the recommended reference values of the manufacturer. In our study, 25(OH)vitD_3 test results obtained for the reference values were 6.43–30.0 ng/mL for the percentile range of 2.5–97.5. For 25(OH)vitD_3, the determined reference interval for our data was significantly different from the data provided by the manufacturer.

Conclusion: This work should also be carried out in a healthy population. Data obtained from this study can be combined with the reference range determination studies for other regions in Turkey, and therefore it can contribute to the determination of the reference ranges of the Turkish community. This study is important for verification of the reference range recommended by the manufacturer.

Key words: Reference values, 25(OH)vitD_3, Balıkesir, Turkey

1. Introduction

A reference interval is defined as the interval that determines the reference values for clinical diagnostic laboratory tests, provided from the sample reference distribution of the values obtained from a well-defined healthy population using certain statistical methods (1). The International Federation of Clinical Chemistry (IFCC) and Clinical and Laboratory Standards Institute (CLSI) both advise that each laboratory should determine its own reference intervals (2,3). Due to regional and laboratory differences based on population, diet, technical equipment used, and selection of the reference group, it is extremely important for each laboratory to determine its own reference intervals (4).

The calculation of a reference interval and especially the determination of the estimation limits is a matter of debate. Increases in the variety of analysis techniques and methods, due to improvements in technology, have led to the necessity of practical and reliable calculation methods. The most widely used methods considered by clinical laboratories in calculating reference intervals are those proposed by the CLSI (nonparametric) and the IFCC (parametric and nonparametric) (2,5,6).

In determining the reference interval, direct and indirect methods are used to select the reference subjects that are most representative of the population. The direct method is the selection of the subjects from the main population based on predefined criteria, in which questionnaires prepared based on these criteria are first completed and then laboratory analyses of the subjects are performed (2,5). The indirect method is the selection of test results according to certain rules from a database in which the analysis of the results are recorded regardless of individuals (7).

Vitamin D has two forms: cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2). The efficacy of vitamin D_2 is less than one-third that of vitamin D_3. The main supply of vitamin D_3 comes from 7-dehydrocholesterol. It is converted to cholecalciferol in the skin with the effect of UV light. Vitamin D_3 forms 25-hydroxycholecalciferol (25(OH)vitD_3) in the liver with the effect of 25-hydroxylase. 25(OH)vitD_3 is converted to 1,25-dihydroxyvitamin D_3 in

* Correspondence: bitigic@hotmail.com

the kidneys with the effect of 1-alpha-hydroxylase, which is the biologically active form. Vitamin D exists in the circulation primarily as 25(OH)vitD_3 (8).

Large-scale studies have proven that vitamin D deficiency may be a risk factor for many public health disorders. Vitamin D deficiency increases the risk of various types of cancer, cardiovascular diseases, diabetes, autoimmune and metabolic disorders, infectious diseases arising from immune deficiency, and even some neuropsychiatric disorders (9–14). It is therefore important to determine the vitamin D levels of the population.

The present study aimed to evaluate the vitamin D levels of the population of the province of Balıkesir and to determine the population-based reference values of the 25(OH)vitD_3 test using the indirect method.

2. Materials and methods

The study included the results of 25(OH)vitD_3 tests performed between 2010 and 2014 in the clinical biochemistry laboratory at Balıkesir State Hospital. Permission to use the data was granted by the Balıkesir General Secretary of the Union of Public Hospitals (approval number and date: 3178 and 26/02/2014). The results were obtained retrospectively from the laboratory information system. Female (n = 6739) and male (n = 1225) patients between 18 and 70 years old were initially selected. Extreme values were excluded by using SPSS 16.0, and the results of 5853 (84.1%) female and 1104 (15.9%) male patients were then evaluated. The mean ages of female and male patients were 46 and 48 years, respectively. In consideration of age, the patient group was separated into five subgroups (18–30 years, 31–40 years, 41–50 years, 51–60 years, and 61–70 years). Patients were also grouped according to the seasons: winter (December-January-February), spring (March-April-May), summer (June-July-August), and fall (September-October-November).

Patient results from nephrology and oncology clinics were not included in the study. Results from other clinics of patients with vitamin D deficiency and hyperparathyroidism were also eliminated. In order to avoid patient repetition and involvement of patients taking vitamin D, only the initial vitamin D values of the subjects were included. 25(OH)vitD_3 test results showing the upper and lower limits of the reference range (4–100 ng/mL) were also not included.

Serum 25(OH)vitD_3 measurements were performed on a Cobas E411 instrument using the electrochemiluminescence immunoassay method (Roche Diagnostics GmbH, Mannheim, Germany). Control material PreciControl Bone (Level 1-2-3) was used for internal quality control. Intraassay precision values of the test for the concentrations of 22.7, 44.9, and 74.2 ng/mL were 4.8%, 4.0%, and 4.1%, respectively, while interassay precision values for the same concentrations were 8.6%, 7.7%, and 6.6%, respectively.

2.1. Statistical methods and calculations

The nonparametric method was used to calculate the reference interval. Extreme values were excluded using SPSS 16.0. Upper and lower limit values of the reference interval were calculated using the nonparametric method (percentile estimation method), and points corresponding to 95% of the distribution were sought. Related formulas are given below:

Lower limit value = $0.025 \times (n + 1)$,

Upper limit value = $0.975 \times (n + 1)$,

'n' = the number of data [2].

Results were evaluated using SPSS 16.0. The Kolmogorov–Smirnov Z-test was used as a normal distribution test. The Mann–Whitney U-test was used for comparisons between two groups, while a one-way variance analysis was applied for comparisons of age subgroups and season groups. For multiple comparisons of groups that showed differences in variance analysis, the Tukey test was used for groups that showed homogeneous variance, and the Tamhane test was used for groups that did not show homogeneous variance. $P < 0.05$ was accepted as statistically significant.

3. Results

Statistics of all patients' data before and after the elimination of extreme values, including their descriptive statistical data, are presented in Table 1. Descriptive statistical data for sex and age groups after the elimination of extreme values are presented in Table 2, and for season groups they are given in Table 3.

The reference interval calculated for 25(OH)vitD_3 levels was notably lower than those proposed by the producing company (health-based reference values were 20–32 ng/mL and population-based reference values were 24–60 ng/mL) (Table 4). In the current study, a 95% reference interval for 25(OH)vitD_3 calculated using the indirect method was 6.43–30.0 ng/mL. Comparison between the sexes showed a significant difference ($P < 0.05$). The reference intervals calculated for the female and male patients were 6.3–29.05 ng/mL and 8.65–34.86 ng/mL, respectively. Age groups did not show statistically significant differences ($P > 0.05$, Table 2). However, differences were significant between the season groups. Patients in the summer group showed significant differences when compared to the patients in the other season groups. The highest mean 25(OH)vitD_3 levels were detected in summer and fall, whereas the lowest mean levels were detected in winter and spring (Table 3).

When the cut-off value for 25(OH)vitD_3 deficiency was accepted to be <20 ng/mL, we determined vitamin D deficiency in 71.23% (4956 patients) of all the patients

Table 1. Statistical calculations before and after discarding the extreme values.

N	Before discarding the extreme values	After discarding the extreme values
	7964	6957
Mean (ng/mL)	17.32	16.00
SD	10.74	7.42
Median	14.62	14.43
Variance	115.44	55.10
Range	75.0	34.46
Minimum	4.0	5.47
Maximum	79	39.93
Interquartile range (IQR)	13.09	11.03

Table 2. Statistical calculations of sex and age data after discarding extreme values.

N (%)	Female	Male	18–30 years	31–40 years	41–50 years	51–60 years	61–70 years
	5853 (84.1%)	1104 (15.9%)	889 (12.8%)	1253 (18%)	1871 (26.9%)	1824 (26.2%)	1120 (16.1%)
Mean (ng/mL)	15.17	20.39	16.76	15.79	15.70	16.29	15.82
SD	7.05	7.77	7.43	7.51	7.45	7.52	7.32
Median	13.62	20.0	15.70	14.0	14.0	14.64	14.16
Variance	49.79	60.44	55.28	52.60	55.58	56.67	53.70
Range	30.53	34.33	33.47	33.51	33.63	34.43	34.32
Minimum	5.47	5.60	5.53	5.49	5.49	5.50	5.47
Maximum	30.53	39.93	39.0	39.0	39.12	39.93	39.79
IQR	10.48	11.88	11.44	10.84	11.07	11.48	11.23

Table 3. Statistical calculations of season data after discarding the extreme values.

N	Winter	Spring	Summer	Autumn
	851 (12.2%)	2323 (33.4%)	2442 (35.1%)	1341 (19.3%)
Mean (ng/mL)	13.38	13.89	17.60	18.43
SD	6.06	6.06	7.84	8.01
Median	12.11	12.54	16.48	17.65
Variance	36.72	36.71	61.59	64.20
Range	24.94	25.53	34.44	34.35
Minimum	5.51	5.47	5.49	5.5
Maximum	30.35	31.0	39.93	39.85
IQR	8.80	9.0	11.92	12.81

Table 4. Determined reference values by indirect method.

Total reference values	95% reference values	Lower 90% CI	Upper 90% CI
	6.43–30.0 ng/mL	6.26–6.6	29.83–30.17
Reference values for female patients	6.31–29.05 ng/mL	6.25–6.61	28.87–29.03
Reference values for male patients	8.65–34.86 ng/mL	8.2–9.1	34.41–35.31
Reference values for season			
Winter	6.18–26.31 ng/mL	6.03–6.33	25.65–26.97
Spring	6.18–25.65 ng/mL	6.03–6.33	20.08–26.29
Summer	6.73–32.04 ng/mL	6.56–6.90	31.24–32.84
Fall	7.19–32.45 ng/mL	7.01–7.37	31.64–33.26

CI: Confidence interval.

studied; 49.54% (547 patients) of the male patients and 75.32% (4409 patients) of the female patients were vitamin D-deficient. Rates of vitamin D deficiency were 84.84% (722 patients) in winter, 81.27% (1888 patients) in spring, 63.63% (1554 patients) in summer, and 59.06% (792 patients) in fall.

4. Discussion

Serum 25(OH)vitD_3 determination is a good indicator of general vitamin D status. Vitamin D_2 and vitamin D_3 are two important forms of vitamin D; however, the potency and duration of activity of vitamin D_2 are less than those of vitamin D_3. Vitamin D levels are generally evaluated by the measurement of serum 25(OH)vitD_3 concentration (15). The measurement of 25(OH)vitD_3 is important as a clinical indicator of nutritional vitamin D deficiency. When 25(OH)vitD_3 levels are <20 ng/mL, 20–30 ng/mL, and >30 ng/mL, it is accepted to indicate vitamin D deficiency, vitamin D insufficiency, and normal vitamin D, respectively (16–19). Though cut-off values are proposed by some institutions to determine vitamin D deficiency and insufficiency, clinical correlations for these cut-off levels are not so evident (20). For this reason, we aimed to determine the reference interval of the 25(OH)vitD_3 test with the indirect method and explore its relation to age, sex, and season by using the patient data of this test obtained within approximately 4 years.

The use of the reference interval in evaluating laboratory test results has indispensable importance. It is essential for clinicians to decide the clinical diagnosis and therapy. Additionally, the IFCC and CLSI propose that every laboratory determine its own reference intervals. For the selection of reference individuals that optimally represent the reference population, the direct and indirect methods are used. In the direct method, individuals are sampled from the main population based on defined criteria and in this method questionnaires prepared based on these criteria are first completed, then laboratory analyses are performed. At least 120 healthy subjects are needed for each group, which is difficult to achieve. In the indirect method, tests results are selected based on certain rules from a database in which analysis results are recorded regardless of individuals (2,3). When factors such as application difficulties and expenses are considered, the indirect method is more advantageous for laboratories in determining their own reference intervals.

In the current study, we calculated the 95% 25(OH) vitD_3 reference interval within a 90% confidence interval. The reference interval was determined to be notably lower than the lower limit reference value that the manufacturing company proposed (reference values based on health status and population). We found significant differences in group comparisons related to sex, and the reference interval values were also different (Table 4). According to these results, though the reference interval lower limit values that we found were much lower than the cut-off value proposed for vitamin D deficiency, it would be accurate to determine the 25(OH)vitD_3 reference interval in relation to sex. In the present study, 25(OH)vitD_3 levels did not differ significantly between the subgroups of age. In a study performed in the United States, the prevalence of vitamin D deficiency was the lowest in patients who were 1–8 years old, and it then increased to a considerable degree until 30 years of age in men and until 18 years of age in women. It did not change considerably in later years (21). Group comparisons that we made in relation to seasons showed significant differences. In both sexes and in age subgroups, serum vitamin D concentrations were

lower in winter, and these levels increased in summer and fall. Bhattoa et al. determined the lowest level in winter and the highest level in summer (22). In many studies, vitamin D level was affected by factors including seasons, geographic area, ethnicity, kidney diseases, malabsorption syndromes, genetics, obesity, sex, age, inadequate sun exposure, and regular use of sun-protective agents (23,24).

In this study, a large proportion of the patients were determined to have 25(OH)vitD$_3$ deficiency when the cut-off value was accepted as <20 ng/mL for the deficiency of 25(OH)vitD$_3$. Vitamin D deficiency was considerably frequent in female patients. The rate of 25(OH)vitD$_3$ deficiency was very high in winter, whereas it decreased as fall was approaching. These results are in accordance with those of studies performed in many countries, and these studies determined that 25(OH)vitD$_3$ deficiency is commonly seen worldwide (25,26). Vitamin D insufficiency/deficiency leads to calcium malabsorption, secondary hyperparathyroidism, muscle weakness, and/or osteoporosis or osteomalacia. In addition, low vitamin D status was related to severe diabetes mellitus type 1, cardiovascular diseases, some types of cancer, decreased cognition, pregnancy complications, autoimmune diseases, and the risk of allergies (27).

While sampling the patients, factors that may affect vitamin D levels were excluded in our study; however, we detected vitamin D deficiency in most of the patients. The reference interval that we determined with the indirect method from the 25(OH)vitD$_3$ results of the hospital information system was not completely compatible with the healthy population. An evaluation of the reference interval by using patient test results is a relatively simple and inexpensive method. The disadvantages of this method are a lack of compatibility of the results with the healthy population defined by the IFCC and CLSI, and insufficiency of preanalytical and analytical controls while obtaining the results (2,3,5). This study must therefore be performed in a healthy population as well. Our data can be evaluated with the reference interval results obtained from other regions of Turkey and may also contribute to the determination of 25(OH)vitD$_3$ reference intervals and vitamin D levels in the Turkish population. Our study is important for the comparison of the reference interval proposed by the producing (manufacturing) company, and also for the indication of vitamin D levels in the population. It would also be accurate to use the direct method in determining the reference interval of the vitamin D test, and to compare the two results.

References

1. Laleli Y. Determination of reference interval. Turk J Biochem 2003; 28: 225-227.
2. CLSI. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition. CLSI Document C28-A3. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2008.
3. Solberg HE. International Federation of Clinical Chemistry (IFCC), Scientific Committee, Clinical Section, Expert Panel on Theory of Reference Values, and International Committee for Standardization in Haematology (ICSH), Standing Committee on Reference Values. Approved recommendation (1986) on the theory of reference values. Part 1. The concept of reference values. J Clin Chem Clin Biochem 1987; 25: 337-342.
4. Buldu S, Halıcı C, Figen N, Elmalı F. Determination of pediatric reference ıntervals for three coagulation tests in widespread use for city of Kayseri population. Turk J Biochem 2012; 37: 362-367.
5. CLSI. How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline-Second Edition. CLSI document C28-A2. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2000.
6. Solberg HE. Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. J Clin Chem Clin Biochem 1987; 25: 645-656.
7. Laleli Y, Akbay A. Tıbbi laboratuvarlarda standartdizasyon ve kalite yöntemi kitabı. In: Taga Y, Aslan D, Güner G, Kutay FZ, editors. Laboratuvar verilerinin istatistiksel değerlendirmesi. 1st ed. Ankara, Turkey: Mart Yayıncılık; 2000. pp. 124-137.
8. Turpeinen U, Hohenthal U, Stenman UH. Determination of 25-hydroxyvitamin D in serum by HPLC and immunoassay. Clin Chem 2003; 49: 1521-1524.
9. Pludowski P, Holick MF, Pilz S, Wagner CL, Hollis BW, Grant WB, Shoenfeld Y, Lerchbaum E, Llewellyn DJ, Kienreich K et al. Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality—A review of recent evidence. Autoimmun Rev 2013; 12: 976-989.
10. Pludowski P, Karczmarewicz E, Bayer M. Practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europe-recommended vitamin D intakes in the general population and groups at risk of vitamin D deficiency. Endokrynol Pol 2013; 64: 319-327.
11. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? J Steroid Biochem Mol Biol 2014; 144: 138-145.
12. Anderson JL, May HT, Horne BD. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. Am J Cardiol 2010; 106: 963-968.

13. Sabetta JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA, Landry ML. Serum 25-hydroxyvitamin D and the incidence of acute viral respiratory tract infections in healthy adults. PLoS One 2010; 5: e11088.

14. Harms LR, Burne THJ, Eyles DW, McGrath JJ. Vitamin D and the brain. Best Pract Res Clin Endocrinol Metab 2011; 25: 657-669.

15. Leino A, Turpeinen U, Koskinen P. Automated measurement of 25-OH vitamin D3 on the Roche Modular E170 analyzer. Clin Chem 2008; 54: 2059-2062.

16. Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 2006; 81: 353-373.

17. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006; 84: 18-28.

18. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. Lancet 1998; 351: 805-806.

19. Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, Vamvakas EC, Dick IM, Prince RL, Finkelstein JS. Hypovitaminosis D in medical inpatients. N Engl J Med 1998; 338: 777-783.

20. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2011; 96: 1911-1930.

21. Looker AC, Johnson CL, Lacher DA, Pfeiffer CM, Schleicher RL, Sempos CT. Vitamin D status: United States, 2001-2006. NCHS Data Brief 2011; 59: 1-8.

22. Bhattoa HP, Nagy E, More C, Kappelmayer J, Balogh A, Kalina E, Antal-Szalmas P. Prevalence and seasonal variation of hypovitaminosis D and its relationship to bone metabolism in healthy Hungarian men over 50 years of age: the HunMen Study. Osteoporos Int 2013; 24: 179-186.

23. Shin SY, Kwon MJ, Song J, Park H, Woo HY. Measurement of serum total vitamin D (25-OH) using automated immunoassay in comparison (corrected) with liquid chromatography tandem-mass spectrometry. J Clin Lab Anal 2013; 27: 284-289.

24. Pludowski P, Grant WB, Bhattoa HP, Bayer M, Povoroznyuk V, Rudenka E, Ramanau H, Varbiro S, Rudenka A, Karczmarewicz E et al. Vitamin D status in Central Europe. Int J Endocrinol 2014; 2014: 1-12.

25. Van Schoor NM, Lips P. Worldwide vitamin D status. Best Pract Res Clin Endocrinol Metab 2011; 25: 671-680.

26. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. J Clin Endocrinol Metab 2012; 97: 1153-1158.

27. Hossein-Nezhad A, Holick MF. Vitamin D for health: a global perspective. Mayo Clinic Proc 2013; 88: 720-755.