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Evaluation of Vitamin D Status in Seasonal Serum Samples

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Abstract

***Introduction:** Today, the measurement of vitamin D is commonly used for the diagnosis of osteoporosis and for establishing optimal serum levels that may help prevent various other diseases. The aim of this study was to investigate whether the season—specifically the time of year when the blood sample is collected—affects total serum vitamin D concentration in the population of the Dukagjini Plain in the Republic of Kosovo.*

***Materials and Methods:** A retrospective study was conducted at two laboratories: the "Biolab- Zafi-F" Medical Laboratory in Peja and the "Biolab-Zafi" Polyclinic Laboratory in Klina. Serum levels of total 25-hydroxyvitamin D were analyzed in non-hospitalized patients aged over 58 years, during the period from January 1, 2022, to August 31, 2024. Vitamin D measurements were performed using the cobas e 411 analyzer, a fully automated system based on ElectroChemiLuminescence (ECL) technology.*

***Results:** A total of 863 outpatient serum samples were analyzed. Vitamin D concentrations were consistently lower in samples collected during the winter season compared to the other three seasons. Multivariate analysis revealed that total vitamin D concentration was independently associated with sex and season of sample collection, but not with patient age.*

***Conclusion:** The season in which blood samples are collected should be considered an important preanalytical variable in vitamin D testing. The amount of vitamin D synthesized during the summer appears sufficient to maintain serum levels above 45–55 nmol/L throughout the rest of the year.*

Keywords: Vitamin D, Preanalytical Variability, Seasonality

Introduction

Vitamin D is metabolized in the liver to form 25-hydroxyvitamin D (25(OH)D), while the kidneys further convert it into its active form, 1,25-dihydroxyvitamin D [1]. Among these, 25(OH)D is the predominant circulating form and is widely accepted as the most reliable indicator of vitamin D status [2]. The two main forms relevant to human health are vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Vitamin D functions as a pro-hormone and plays a vital role in calcium and phosphorus regulation, as well as bone mineralization [3]. Deficiency

in vitamin D is typically defined as a serum concentration of less than 20 ng/mL [4]. Hypovitaminosis D can contribute to osteoporosis by reducing calcium absorption, inducing secondary hyperparathyroidism, and increasing bone resorption. Consequently, low vitamin D levels are often associated with elevated parathyroid hormone (PTH) levels.Recent research has revealed the presence of vitamin D receptors in various cell types, suggesting biological functions beyond mineral metabolism [5]. Several studies have identified hypovitaminosis D as a potential risk factor for numerous conditions, including cancer, diabetes, and cardiovascular diseases [6]. The most reliable method for assessing vitamin D status is the measurement of serum 25(OH) D concentrations. Of the two types, 25(OH)D3 is considered more biologically significant, as it maintains higher serum levels compared to 25(OH)D2, which is derived primarily from dietary sources or supplementation [7]. However, routine measurement of vitamin D poses certain challenges, largely due to variability in assay methods and lack of standardization against reference materials [8]. Pre-analytical variability, such as differences in sample storage temperature and handling, appears to have a relatively minor impact on vitamin D measurement [9]. Additionally, common sources of biological variability, including postprandial status, have been shown to exert negligible influence on 25(OH)D levels [10]. Nevertheless, several studies from New Zealand and the USA have identified seasonal variation as a major determinant of vitamin D status, with lower concentrations typically observed during months with reduced daylight hours [11,12,13]. Given that UV radiation levels are insufficient for continuous cutaneous vitamin D synthesis throughout the year, the aim of this study is to assess whether seasonality, as a significant source of pre-analytical variability, affects total vitamin D concentrations in the population of the Dukagjini Plain in the Republic of Kosovo.

Statistical Analysis

Differences in values were analyzed using ANOVA (for continuous variables) and the Pearson X2 test with Yates' correction (for categorical variables). Univariate analyzes (Spearman's rank correlation coefficient) and multivariate analyzes were also performed using Analyse-it Software Ltd, Leeds, UK.

Materials and Methods

A retrospective study was conducted in two laboratories: the Medical Laboratory "Biolab-Zafi-F" in the city of Peja and the laboratory of the Polyclinic "Biolab-Zafi" in Klina.A total of 863 outpatient serum samples were analyzed. Vitamin D concentrations were consistently lower in samples collected during the winter season compared to the other three seasons. The study aimed to evaluate serum levels of total vitamin D (25-hydroxyvitamin D) in non-hospitalized patients over the age of 58, during the period from January 1, 2022, to August 31, 2024.Vitamin D concentration was measured using the Cobas e 411 analyzer, a fully automated system that employs ElectroChemiLuminescence (ECL) technology for detection.

Results

Table 1: Age, gender and total vitamin D values according to blood analysis season.

Parameters	Spring	Summer	Autumn	Winter	p-values
Number of patients n= 863	227 (26.3%)	203 (23.5%)	251(29.8%)	182 (21.8%)	
Average age (years) n= 58-70	66	64	61	63	0.598
Gender (female) n= 468 (%)	112 (24%)	103 (22.0%).	117 (25.0%)	136 (29.0%)	<0.001
Gender (men) n=395 (%)	91 (23.0%)	87 (22.0%)	107 (27.0%)	110 (28%)	<0.001
Women: vitamin D (nmol/L)	57 (25- 61)	71(28-86)	69 (24-72)	46 (21-65)	<0.001
Men: vitamin D (nmol/L)	62 (28-71)	78 (29-82)	65 (28-80)	57 (26-68)	<0.001
Women: vitamin D <30 nmol/L (%)	n= 46 (41)	n= 35 (34)	n= 52 (44.4)	n=58 (42.6)	<0.001
Men: vitamin D <30 nmol/L (%)	n= 41 (45.0)	n= 29 (37.1)	n=56 (52.3)	n=58 (52.7)	<0.001

p-values <0.001 significant

Table 1 presents the findings of the study, which included a selected older outpatient population comprising 863 individuals (median age: 63.5 years; interquartile range [IQR]: 58–70 years; 468 women and 395 men). Overall, vitamin D levels were significantly higher in men (median: 65.5 nmol/L; IQR: 27.8–75.3 nmol/L) compared to women (median: 60.7 nmol/L; IQR: 24.5–71.2 nmol/L; $p < 0.001$). There was no statistically significant difference in the age distribution of patients across the different seasons of blood sample collection ($p = 0.598$). However, the proportion of women was significantly higher in samples col-

lected during winter and autumn compared to those collected in summer and spring.The mean concentration of total vitamin D was consistently lower in samples collected during winter than in other seasons. Compared to winter values, vitamin D levels were 33% higher in samples collected during summer and autumn, and 18% higher in those collected during spring.The prevalence of vitamin D deficiency (defined as serum 25(OH)D < 30 nmol/L) was notably greater in samples collected in winter and autumn compared to summer and spring (see Table 1).Furthermore, the odds ratios (ORs) and 95% confidence intervals (CIs) for vitamin D deficiency were significantly lower in patients whose blood was collected in spring (OR: 0.86; 95% CI: 0.78–0.96; p = 0.008), summer (OR: 0.39; 95% CI: 0.35–0.45; p < 0.001), and autumn (OR: 0.46; 95% CI: 0.41–0.51; p < 0.001), compared to those tested in winter.Notably, the odds of vitamin D deficiency were also lower in samples collected in spring (OR: 0.46; 95% CI: 0.41–0.50; p < 0.001) and autumn (OR: 0.52; 95% CI: 0.48–0.59; p < 0.001), when compared to those collected in summer.

Table 2: Vitamin D values according to the season of blood analysis in subjects aged <63 years and in those aged >63-70 years.

Parameters	Spring	Summer	Autumn	Winter	p-values
Age with<63. Vitamin D concentration (nmol/L)	48 (35-68)	64 (52-88)	61 (47-79)	51 (32-64)	0.001
Age >63-70 years, n (%). Vitamin D concentration (nmol/L)	41 (27- 64)	57 (34-67)	48 (31-63)	38 (24-58)	0.001

p-values <0.001 significant

The results presented in Table 2 remained consistent in the subgroup analysis of patients aged <63 years and those aged 63–70 years. In both age groups, the mean concentration of total vitamin D continued to be lower in samples collected during winter compared to other seasons.

Table 3: Univariate (Spearman's correlation) and multivariate analysis between total vitamin D values and age, gender and season of blood tests in outpatients.

Parameters	Univariant	Multivariate
Age	r=–0.025; p=0.014	b=0.0001; p=0.998
Gender	r=–0.074; p<0.001	b=–5.98; p<0.001
Test season	r=0.178, p<0.001	b=5.24; p<0.001

In the univariate analysis, total vitamin D concentration was significantly associated with age, sex, and the season in which the blood sample was collected (Table 3).In the multivariate analysis, where vitamin D concentration was treated as the dependent variable and age, sex, and season of blood collection were included as independent variables, total vitamin D concentration was found to be independently associated with sex and season, but not with patient age.

Discussion

Sunlight is a crucial determinant of vitamin D status, as it stimulates endogenous synthesis in the skin. The extent of this production depends on the intensity of solar UVB radiation, which is generally higher in summer and lower in winter. Additional factors that influence vitamin D synthesis include age, skin pigmentation, clothing style, use of sunscreen, and lifestyle habits [14]. Previous studies have demonstrated that seasonal variations significantly affect the diagnosis of vitamin D deficiency. For example, Bolland et al. [13] conducted two consecutive studies in New Zealand and concluded that seasonally adjusted diagnostic thresholds may be advisable. Their findings suggest that cut-off values for vitamin D deficiency should be approximately 20–50% higher during the summer months [11,12]. Similar results were reported by Rosecrans et al. [13], who analyzed 148,821 serum samples over a two-year period in a northern U.S. city and found that vitamin D deficiency and insufficiency peaked during winter. A smaller Italian study involving 13 individuals reported that serum vitamin D levels were 27% higher in samples collected in September/October compared to those collected in February/March [15]. The results of our study align with these prior observations. In the selected elderly population from the Dukagjini Plain region, total serum vitamin D concentrations were 33% higher in samples collected during summer and autumn, and 18% higher in spring, compared to those collected in winter (Table 1). Furthermore, the prevalence of vitamin D deficiency was nearly twice as high in samples taken in winter and autumn compared to summer and spring.Although older individuals typically exhibit lower vitamin D levels due to decreased cutaneous synthesis with aging, our study confirmed the same seasonal pattern among both younger (<63 years) and older (>63–70 years) subgroups (Table 2). This consistency reinforces the seasonal impact on vitamin D levels regardless of age group.The innovative contribution of our study lies in its context. Unlike previous research conducted in countries with mandatory (USA) or widespread voluntary (New Zealand) food fortification policies, our study was performed in a setting with-

out any vitamin D fortification regulations, as is the case in most European countries. Additionally, the climate in the Dukagjini Plain (Kosovo) is temperate and more comparable to Central European regions than to the climates of the USA or New Zealand. Therefore, our findings may have broader applicability to similar European settings.Given the high prevalence of vitamin D deficiency in the general population [16] and its established association with a range of health conditions [1], this remains a significant public health concern. While routine population-wide screening is not recommended, current clinical guidelines emphasize the importance of vitamin D testing in individuals at risk for deficiency, enabling timely intervention and restoration of adequate levels [5].

Conclusion and Recommendations

Based on the findings of this study, the season in which blood samples are collected should be considered a significant pre-analytical factor when evaluating serum vitamin D levels. The amount of vitamin D synthesized during the summer months appears to be sufficient to maintain concentrations above 45–50 nmol/L throughout the remainder of the year.It is recommended that individuals—particularly those with bone-related health conditions—receive prophylactic vitamin D supplementation, especially during periods of low sun exposure. Additionally, in both age groups examined, strategies such as vitamin D fortification of food products and safe sun exposure during peak UV hours may help maintain adequate vitamin D levels year-round.

References

1. Holick, M. F., Chen, T. C. (2008). Vitamin D deficiency: A worldwide problem with health consequences. American Journal of Clinical Nutrition, 87(l), 1080-1086.
2. DeLuca, H. F. (2004). Overview of general physiologic features and functions of vitamin D. American Journal of Clinical Nutrition, 80(l), 1689-1696.
3. Holick, M. F. (2013). The D-lightful vitamin D for health. Journal of Medical Biochemistry, 32, 1–10.
4. Dawson-Hughes, B., Mithal, A., Bonjour, J. P., Boonen, S., Burckhardt, P., Fuleihan, G., E. (2010). IOF position statement: Vitamin D recommendations for older adults. Osteoporosis International, 21, 1151-1154.
5. Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. Journal of Clinical Endocrinology & Metabolism, 96(7), 1911-1930.
6. Targher, G., Pichiri, I., Lippi, G. (2012). Vitamin D, thrombosis, and hemostasis: More than skin deep. Seminars in Thrombosis and Hemostasis, 38, 114-124.
7. Roth, H. J., Schmidt-Gayk, H., Weber, H., Niederau, C. (2008). Accuracy and clinical implications of seven 25-hydroxyvitamin D methods compared with liquid chromatography–tandem mass spectrometry as a reference. Annals of Clinical Biochemistry, 45(Pt 2), 153-159.
8. Serteser, M., Coskun, A., Inal, T. C., Unsal, I. (2012). Challenges in vitamin D analysis. Journal of Medical Biochemistry, 31, 326-332.
9. Colak, A., Toprak, B., Dogan, N., Ustuner, F. (2013). Effect of sample type, centrifugation, and storage conditions on vitamin D concentration. Biochemia Medica, 23, 321-325.
10. Mochizuki, A., Kodera, Y., Saito, T., Satoh, M., Sogawa, K., Nishimura, M. (2013). Preanalytical evaluation of serum 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2 measurements using LC-MS/MS. Clinica Chimica Acta, 420, 114-120.
11. Bolland, M. J., Grey, A. B., Ames, R. W., Mason, B. H., Horne, A. M., Gamble, G. D. (2007). The effects of seasonal variation of 25-hydroxyvitamin D and fat mass on a diagnosis of vitamin D sufficiency. American Journal of Clinical Nutrition, 86, 959-964.
12. Bolland, M. J., Chiu, W. W., Davidson, J. S., Grey, A., Bacon, C., Gamble, G. D. (2008). The effects of seasonal variation of 25-hydroxyvitamin D on diagnosis of vitamin D insufficiency. New Zealand Medical Journal, 121, 63-74.
13. Rosecrans, R., Dohnal, J. C. (2014). Seasonal vitamin D changes and the impact on health risk assessment. Clinical Biochemistry, 47, 670-672.
14. Lips, P., van Schoor, N. M., de Jongh, R. T. (2014). Diet, sun, and lifestyle as determinants of vitamin D status. Annals of the New York Academy of Sciences, 1317, 92-98.
15. Bianchi, S., Maffei, S., Prontera, C., Battaglia, D., Vassalle, C. (2012). Preanalytical, analytical (DiaSorin LIAISON), and clinical variables potentially affecting the 25-OH vitamin D estimation. Clinical Biochemistry, 45, 1652-1657.
16. Lippi, G., Nouvenne, A., Ticinesi, A., Bonelli, P., Salvagno, G. L., Cervellin, G. (2015). The burden of vitamin D deficiency in a Mediterranean country without a policy of food fortification. Acta Biomedica, 86, 59-62.