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BLOOD SAMPLING SEASONALITY AS AN IMPORTANT PREANALYTICAL FACTOR FOR ASSESSMENT OF VITAMIN D STATUS

UTICAJ SEZONSKOG UZORKOVANJA KRVI KAO VAŽAN PREANALITIČKI FAKTOR ZA PROCENU STATUSA VITAMINA D

Patrizia Bonelli, Ruggero Buonocore, Rosalia Aloe, Giuseppe Lippi

Laboratory of Clinical Chemistry and Hematology, Academic Hospital of Parma, Parma, Italy

Summary

Background: The measurement of vitamin D is now commonplace for preventing osteoporosis and restoring an appropriate concentration that would be effective to counteract the occurrence of other human disorders. The aim of this study was to establish whether blood sampling seasonality may influence total vitamin D concentration in a general population of Italian unselected outpatients.

Methods: We performed a retrospective search in the laboratory information system of the University Hospital of Parma (Italy, temperate climate), to identify the values of total serum vitamin D (25-hydroxyvitamin D) measured in outpatients aged 18 years and older, who were referred for routine health check-up during the entire year 2014.

Results: The study population consisted in 11,150 outpatients (median age 62 years; 8592 women and 2558 men). The concentration of vitamin D was consistently lower in samples collected in Winter than in the other three seasons. The frequency of subjects with vitamin D deficiency was approximately double in samples drawn in Winter and Spring than in Summer and Autumn. In the multivariate analysis, the concentration of total vitamin D was found to be independently associated with sex and season of blood testing, but not with the age of the patients.

Conclusions: According to these findings, blood sampling seasonality should be regarded as an important preanalytical factor in vitamin D assessment. It is also reasonable to suggest that the amount of total vitamin D synthesized during the summer should be high enough to maintain the levels > 50 nmol/L throughout the remaining part of the year.

Keywords: vitamin D, preanalytical variability, biological variability, seasons

Kratak sadržaj

Uvod: Merenje vitamina D danas se uobičajeno obavlja radi prevencije osteoporoze i uspostavljanja odgovarajuće koncentracije koja bi efikasno sprečila nastanak drugih ljudskih bolesti. Cilj ove studije bio je da se ustanovi da li sezona, odnosno godišnje doba u kom se uzorkovanje vrši može uticati na koncentraciju ukupnog vitamina D, u opštoj populaciji neselektovanih italijanskih pacijenata.

Metode: Sproveli smo retrospektivnu pretragu u laboratorijskom informacionom sistemu Univerzitetske bolnice u Parmi (Italija, umerena klima), kako bismo utvrdili koje su vrednosti ukupnog vitamina D (25-hidroksivitamin D) u serumu izmerene kod nehospitalizovanih pacijenata starih 18 godina i više, upućivanih na rutinske zdravstvene preglede tokom čitave 2014. godine.

Rezultati: Ispitivana populacija sastojala se od 11 150 nehospitalizovanih pacijenata (prosek godina 62; 8 592 žena i 2 558 muškaraca). Koncentracija vitamina D bila je dosledno niža u uzorcima uzetim tokom zime nego tokom ostala tri godišnja doba. Učestalost ispitanika sa nedostatkom vitamina D bila je otprilike duplo veća u uzorcima uzetim u zimu i proleće nego u leto i jesen. Multivarijaciona analiza pokazala je da je koncentracija ukupnog vitamina D bila nezavisno povezana s polom i sezonom testiranja krvi, ali ne sa starošću pacijenata.

Zaključak: Prema ovim nalazima, sezonu uzrokovanja krvi treba posmatrati kao važan preanalitički faktor u proceni vitamina D. Takođe, ima smisla pretpostaviti da bi količina ukupnog vitamina D koji se sintetiše tokom leta trebalo da bude dovoljna da se održe nivoi > 50 nmol/L tokom preostalog dela godine.

Ključne reči: vitamin D, preanalitička varijabilnost, biološka varijabilnost, godišnja doba

Address for correspondence:

Prof. Giuseppe Lippi
U.O. Diagnostica Ematochimica,
Azienda Ospedaliero-Universitaria di Parma,
Via Gramsci, 14, 43126 – Parma, Italy
Tel. 0039-0521-703050
Tel. 0039-0521-703791
e-mail: glippi@ao.pr.it, ulippi@tin.it

Introduction

Vitamin D, also known as 25-hydroxyvitamin-D, is a fat-soluble secosteroid which plays an essential role in regulating the metabolism of calcium, iron, magnesium, phosphate and zinc. Recent evidence attests that this vitamin exerts a variety of pleiotropic effects, since it influences the biology and functioning of the brain, gonads, skin, vascular smooth muscle and immune cells (1). Vitamin D deficiency, which is conventionally defined as a concentration lower than 50 nmol/L (i.e., < 20 ng/mL) (2), is now regarded as a risk factor for the development and progression of a number of human disorders, including cancer, diabetes and cardiovascular disease (3). It has also been associated with a larger prevalence of hyponatremia in elderly patients (4), thus reinforcing its multifaceted and still partially uncovered biological role. Due to the important biological link with the health status, the screening of patients at risk of developing vitamin D deficiency has been recommended, not only for preventing osteoporosis, but also for restoring an appropriate concentration of vitamin D that would be effective to counteract the development of other and more severe pathological conditions (5).

The routine measurement of vitamin D presents several challenges, which are mostly attributable to the use of heterogeneous assay technologies and the lack of standardization against the reference material (6). At variance with other laboratory tests (7), however, the impact of preanalytical variability on measured vitamin D values is apparently lower, in that the concentration of this compound has been reported to be »solid as a rock« at different temperature of storage and under the common preanalytical conditions experienced in medical laboratories (8, 9). Even the common sources of biological variability, such as postprandial changes (10) and physical exercise (11) exert a negligible effect on the actual vitamin D concentration.

At variance with this evidence, some earlier studies based in New Zealand and the US showed that the seasonal variation is an important determinant of vitamin D status, wherein a lower concentration of this compound has been observed during the months of the year with the shortest daylight period (12–14). Although the intensity of UV radiation is not enough for continuous synthesis of vitamin D in the skin throughout the year at certain latitudes, no previous studies have clearly identified seasonality as a substantial source of preanalytical variability in vitamin D testing. Therefore, the aim of this cross-sectional study was to assess whether the concentration of total vitamin D may be influenced by the season of blood sampling in an Italian resident population.

Materials and Methods

We performed a retrospective search in the laboratory information systems of the University Hospital of Parma (North-West Italy), to identify all values of total serum vitamin D (25-hydroxyvitamin D) which were measured in outpatients aged 18 years and older, who were referred for routine health check-up during the entire year 2014. In accord with previous investigations (12–14), no specific exclusion criteria were applied since we planned to assess vitamin D status in the largest possible number of subjects from an unselected general population. The laboratory service of the University Hospital of Parma serves an area with an estimated population of 444,450 inhabitants. The climate in the province of Parma is typical of central-southern Pianura Padana (the Po river valley), i.e., temperate, with 777 mm precipitation per year, hot and humid Summers (peak temperatures up to 32–35 °C) and cold Winters (lowest temperatures approximating –10 °C).

The concentration of 25-hydroxyvitamin-D was measured using the same competitive chemiluminescence assay (Liaison, DiaSorin, Saluggia, Italy). Quality and consistency of results were validated throughout the study period by means of internal quality controls (IQCs) and participation in an external assessment scheme (EQA). According to a recent evaluation, the total imprecision of this assay was comprised between 2.9–5.5% (15), whereas the correlation with reference chromatographic assays ranged between 0.95 and 0.96 (15, 16). Results of measurements were finally reported as median and interquartile range (IQR). The 25-hydroxyvitamin D deficiency was defined as a serum concentration of 25-hydroxyvitamin-D < 50 nmol/L (i.e., < 20 ng/mL), in agreement with the current recommendations of the International Osteoporosis Foundation (IOF) (2). Seasonality was defined according to equinoxes and solstices of the year 2014 (i.e., Spring: March 20 to June 20; Summer: June 21 to September 22; Autumn: September 23 to December 20; Winter: January 1 to March 19 and December 21 to December 31). The differences of values were analyzed using ANOVA (for continuous variables) and Pearson's χ^2 test with Yates' correction (for categorical variables). Univariate (Spearman's rank correlation coefficient) and multivariate analyses were also performed using Analyse.it (Analyse-it Software Ltd, Leeds, UK). The study was performed in accordance with the Declaration of Helsinki, under the terms of relevant local legislation.

Results

The final study population consisted in 11,150 outpatients (median age 62 years and IQR, 49–73 years; 8 592 women and 2 558 men). Vitamin D values were globally higher in women (62 nmol/L; 38–86 nmol/L) than in men (56 nmol/L; IQR, 33–77 nmol/L; $p<0.001$). The main results of this study are shown in *Table I*. Although no significant difference was observed in the age of the patients across differ-

Table I Age, sex and values of total vitamin D (median and interquartile range) according to the season of blood testing in an unselected population of Italian outpatients.

	Winter	Spring	Summer	Autumn	p
n	2381	3048	2371	3350	
Age (years)	62 (49–73)	62 (49–73)	62 (49–74)	62 (50–74)	0.628
Sex (Women; %)	1885 (79%)	2411 (79%)	1820 (77%)	2476 (74%)	<0.001
Total vitamin D values (nmol/L)	50 (30–70)	56 (33–80)	68 (47–89)	68 (44–89)	<0.001
Total vitamin D values <50 nmol/L (%)	1116 (47%)	1319 (43%)	612 (26%)	962 (29%)	<0.001

Table II Values of total vitamin D (median and interquartile range) according to the season of blood testing in subjects aged <65 years and in those aged 65 years or older.

	Winter	Spring	Summer	Autumn	p
Age < 65 years, n	1322	1686	1304	1851	
Total vitamin D (nmol/L)	50 (33–71)	56 (36–77)	71 (53–90)	65 (47–86)	<0.001
Age ≥ 65 years, n	1059	1362	1067	1499	
Total vitamin D (nmol/L)	55 (27–80)	56 (27–80)	65 (38–89)	68 (41–92)	<0.001

Table III Univariate (Spearman's correlation) and multivariate analysis between total vitamin D values and age, sex and season of blood testing in an unselected population of Italian outpatients.

Parameter	Univariate	Multivariate
Age	r=–0.023; p=0.013	β=0.0001; p=0.998
Sex	r=–0.076; p<0.001	β=–6.43; p<0.001
Season of testing	r=0.180, p<0.001	β=5.35; p<0.001

ent seasons of blood testing, the prevalence of women was slightly but significantly higher in Winter and Spring compared to Summer and Autumn. The median concentration of total vitamin D was found to be consistently lower in samples collected in Winter than in other seasons. Compared to samples collected in Winter, vitamin D values were 35% higher when measured in Summer and Autumn, but also 12% higher when measured in Spring. The frequency of subjects with vitamin D deficiency (i.e., vitamin D < 50 nmol/L) was also approximately double in samples collected in Winter and Spring than in Summer and Autumn (*Table I*). Accordingly, the odds ratios (ORs) and 95% confidence interval (95% CI) of vitamin D deficiency were substantially lower in patients who had their blood analyzed 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 who had their blood tested in Winter. Interestingly, the OR of vitamin D deficiency was also lower in samples collected in Summer (OR, 0.46; 95% CI, 0.41–0.5; p<0.001) and Autumn (OR, 0.52; 95% CI, 0.48–0.59; p<0.001) compared to Spring. Interestingly, the results were unchanged in a subanalysis of patients aged less than 65 years and 65 years or older (*Table II*), since the median concentration of total vitamin D in both populations remained lower in samples collected in Winter than in other seasons.

In the univariate analysis, the concentration of total vitamin D was found to be associated with age, sex and season of blood testing (*Table III*). In the multivariate analysis, in which vitamin D concentration was entered as a dependent variable whereas age, sex and season of blood testing were entered as independent variables, the concentration of total vitamin D was found to be independently associated with sex and season of blood testing, but not with the age of the patient (*Table III*).

Discussion

The sunlight is an essential determinant of vitamin D status, since it stimulates the production of this vitamin in the skin. The direct effect of sunlight on vitamin D metabolism is hence dependent upon direct sunlight irradiation, which is expectedly higher in Summer and lower in Winter, but is also influenced by age and skin pigmentation of the subject, as well as by outdoor habits, clothing style and sunscreen use (17). Previous studies showed that seasonal variations may have an effect on the diagnosis of vitamin D deficiency. Bolland et al. (13) published two consecutive studies based in New Zealand, concluding that seasonal-adjusted thresholds for diagnosing vitamin D deficiency may be advisable, thus setting diagnostic cutoffs approximately 20–50% higher during summertime (12, 13). Similar results were recently published by Rosecrans et al. (14), who studied 148,821 serum samples during a 2-year period in a northern-latitude city in the US, and concluded that vitamin D deficiency and insufficiency were the highest during the Winter season. Another small Italian study investigated the effect of seasonal sunlight changes in 13 subjects who had their blood drawn in different months of the year, concluding that vitamin D values were 27% higher in samples obtained in September/October compared to those collected in February/March (18).

Taken together, the results of our study support these previous findings. Compared to samples collected in the Winter season, the concentration of total vitamin D in our population of Italian residents was found to be 35% higher when measured in Summer and Autumn, but also 12% higher when measured in Spring (*Table I*). The prevalence of vitamin D deficiency was hence nearly 2-times higher in samples collected in Winter and Spring compared to Summer and Autumn. Despite the fact that older subjects usually have lower values of total vitamin D because the synthesis in skin decreases with age, the trend of lower levels in Winter than in other seasons was confirmed in subjects aged < 65 years as well as in those aged 65 years or older (*Table II*). The innovative value of these findings lies in the fact that some differences can be identified with previous epidemiological observations. At variance with previous studies based in the US (where food fortification with vitamin D is mandatory) and New Zealand (where food fortification is voluntary, but a large number of alimentary products are sourced from Australia, where fortification with vitamin D is mandatory), our analysis was settled in a Nation without a policy of vitamin D fortification, as for most European Countries. Another important aspect is the fact that the temperate climate in the Parma province (i.e., North-West Italy) is rather different from that of the US and New Zealand, but is similar to Central European Countries located between 40 and 60 north latitude such as Serbia, Croatia, Germany, France and Spain among others. It is hence reasonable to suggest that our findings may be broadened to a vast area of the European continent.

Due to the high prevalence in the general population (19) and its association with a number of human disorders (1), vitamin D deficiency remains an important health care issue. Although widespread screening is unadvisable, current recommendations suggest that vitamin D testing should be performed in all patients at risk for deficiency, in order to timely restore an appropriate concentration of this essential vitamin (5). To achieve a correct diagnosis of vitamin D deficiency, however, blood sampling seasonality should now be regarded as an important preanalytical factor. Although the retrospective design that characterizes this study as well as those previously published (12–14) may be seen as a limitation (i.e., specific patient values could not be longitudinally followed throughout the different seasons of the year), it seems reasonable to conclude that, in the absence of supplementation, the amount of total vitamin D synthesized during the summer should be high enough to maintain the levels of this vitamin > 50 nmol/L throughout the remaining part of the year.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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