Original article

Vitamin D Deficiency Among Medical Students in Osijek, Croatia

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Abstract

Aim: This study aimed to evaluate serum levels of 25-OH D3 (calcidiol) among students of the Faculty of Medicine Osijek, Croatia, thereby determining to what extent vitamin D deficiency is present.

Methods: The present cross-sectional analysis was based on data collected from 60 participants. Blood sampling was done in March 2021. Concentrations of 25-OH D3 were measured using LC/MS-MS procedure on Shimadzu LCMS 8050 and RECIPE kit for serum levels of 25-OH-D3.

Results: The study was conducted on a sample of 60 respondents aged 19 to 28, of whom 16 were men and 44 were women. All subjects had a 25-OH D3 deficiency (<20 ng/ml), while 11 had an extreme deficiency. Mean 25-OH D3 concentration was 11.36 ng/ml, ranging from 7.70 ng/ml to 16.70 ng/ml. There was no statistical significance of 25-OH D3 concentration levels between the sexes (Student's t-test, P>0.05).

Conclusion: Vitamin D deficiency was detected in all subjects. In addition to the results of several other studies conducted worldwide that evaluated vitamin D status among medical students, this study further highlights the problem affecting this student subgroup.

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Introduction

Over the past decade, there has been a growing number of studies focusing on vitamin D and its roles in human health and disease. Initially, it was thought to be only essential for rickettsial disease prophylaxis; however, contemporary research suggests the importance of vitamin D in maintaining individuals' well-being and overall health (1,2).

The active form of vitamin D, 1,25-dihydroxycholecalciferol (1,25-OH D3), has a pivotal role in maintaining mineral homeostasis and enhancing absorption of calcium and phosphate into the extracellular fluid by acting on the intestines, kidneys, and bones. Therefore, it regulates bone growth and reorganization by interfering with the activity of osteoclasts and osteoblasts (3).

Vitamin D receptors are expressed in almost all cell types, which may explain the multiple actions of vitamin D on different tissues (4). Although the proportion of the role of vitamin D and its effects on non-skeletal health is still debatable, it is associated with regulation of the innate and adaptive immune systems, reduction of inflammation, cell proliferation and differentiation, insulin and glucose secretion regulation, preventive effects on cardiovascular and neurodegenerative diseases, and even antiaging effects (5–9).

For most people, the primary source of vitamin D is skin exposure to ultraviolet radiation, specifically UVB rays, where 7-dehydrocholesterol is converted to pre-vitamin D3 in the skin during exposure to UVB rays (10). However, it can also be obtained through diet and dietary supplements (11).

Vitamin D testing has increased significantly in recent years, establishing the presence of vitamin D deficiency on a pandemic scale worldwide (12). It is estimated that more than a billion people worldwide have low vitamin D levels, with extensive observational data indicating that 40% of the European population and 24% of the US population have 25-OH D3 below 20 ng/ml (13).

Vitamin D deficiency can be diagnosed by measuring serum levels of 25-hydroxyvitamin D3 (14,15). Although there is no consensus on optimal levels of 25 OH-D3, most guidelines suggest that concentrations below 20 ng/mL indicate vitamin D deficiency (16). (Table 1).

Table 1. Recommended serum levels of 25-OH D3 for verification of deficiency and adequate levels of vitamin D; according to Croatian Guidelines for the Prevention, Detection and Therapy of Vitamin D Deficiency in Adults (11).

25-OH D3 (ng/mL)	Interpretation
<10 ng/mL	Extreme deficiency of vitamin D
<20 ng/mL	Deficiency of vitamin D
<30 ng/mL	Insufficiency of vitamin D
≥30 ng/mL	Adequate level of vitamin D
>100 ng/mL	Excess
>150 ng/mL	Intoxication

In 2016, Guidelines for Preventing, Detecting and Treating Vitamin D Deficiency in Adults were issued in Croatia, according to which recommended levels of 25-OH D3 are between 30 and 100 ng/ml (17).

Numerous conditions are associated with vitamin D deficiency, such as chronic diseases, obesity, and malabsorption syndromes. It also occurs during pregnancy. In addition, skin pigmentation, clothing style, diet, sunscreen

use, the season of the year, and latitude can also affect vitamin D synthesis and its serum levels, potentially leading to its deficiency, which increases the risk of developing skeletal and non-skeletal diseases (11,18).

This study was aimed at evaluating serum levels of 25-hydroxyvitamin D3 among medical students of the Faculty of Medicine Osijek in March 2021, thereby also determining to what extent vitamin D deficiency might be present among future healthcare professionals. They are potentially at a greater risk of developing hypovitaminosis D due to their work-related lifestyle, which could be even aggravated by the currently present COVID-19 pandemic.

Material and methods

The present cross-sectional analysis was based on data from 60 participants aged 19 to 28. Blood sampling was done in March 2021 at the Faculty of Medicine in Osijek and then analyzed at the Clinical Hospital Center Osijek, Croatia. Informed consent was obtained from all study participants. The research was approved by the Ethics Committee of the Faculty of Medicine Osijek, University of Osijek (Class: 602-04/22-08/02. No: 2158-61-46-22-149).

Subjects' blood was drawn into round bottom, polystyrene test tubes, 14 mL, 17 x 100 mm (Becton Dickinson, Croatia) to separate serum from cells. The blood was centrifuged at 3000 rpm for 10 minutes. After centrifugation, the serum was separated into other tubes using disposable tubes, which were stored in a refrigerator at -20 ˚ C until analysis. According to the manufacturer's instructions (Shimadzu Corp.), the sample was analyzed on an LCMS-8050 analyzer. The LCMS-8050 analytical system (Shimadzu, Japan) itself consists of NEXERA X2 HPLC (high-performance liquid chromatography) system connected to a mass spectrometer (MS). The LC-MS / MS method is characterized by high sensitivity, specificity, and the possibility of simultaneous analysis of multiple analytes. It is considered the gold standard for determining vitamin D concentration in serum.

Statistical analysis

Numerical data was described by arithmetic mean and standard deviation. Normality of distribution of numerical variables was determined by using the D'Agostino-Pearson test. Differences in normally distributed numerical variables between two independent groups were assessed by using the Student's t-test. All P values were two-sided. The significance level was set to Alpha = 0.05. The MedCalc Statistical Software version 20.106 was used for statistical analysis (https://www.medcalc.org/).

Table 2. Sample age, gender and 25-OH D3 concentrations (ng/ml) (N=number, SD=standard deviation).

Total number of participants	60
Male	16
Female	44
Male: female (ratio)	1:2.75
Age (males), mean(SD)	21.9(1.2)
Age (females), mean(SD)	21.7(2.2)
Age (years), mean(SD)	21.9(1.2)
25-OH D3 levels (ng/ml)	
N	60
Mean ±SD	11.37(1.59)
Minimum	7.67
Maximum	16.68
25-OH D3 levels; males (ng/ml)	
N	16
Mean (SD)	11.13(1.69)
Minimum	7.67
Maximum	13.78
25-OH D3 levels; females (ng/ml)	
N	44
Mean (SD)	11.46(1.53)
Minimum	8.29
Maximum	16.68

Results

The study was conducted on a sample of 60 respondents aged 19 to 28, of whom 44 (73.33%) were women and 16 (26.67%) were men. All subjects had a 25-OH D3 deficiency (<20 ng/ml), with 11 (18.33%) of them exhibiting an extreme deficiency according to recommended serum levels of 25-OH D3 (17). The mean 25-OH D3 concentration was 11.36 ng/ml, ranging from 7.70 ng/ml to 16.70 ng/ml.

The mean 25-OH D3 concentration in women was 11.45 ng /ml, while in men it was 11.13 ng/ml (Table 2). We examined differences in 25-OH D3 concentration levels with respect to gender. The results showed no statistical significance of 25-OH D3 concentration levels between the sexes (Student's t-test, P>0.05). Pearson's correlation coefficient suggests that the relationship between age and 25 OH-D3 concentration is not significant (r=0.06; P>0.05)..

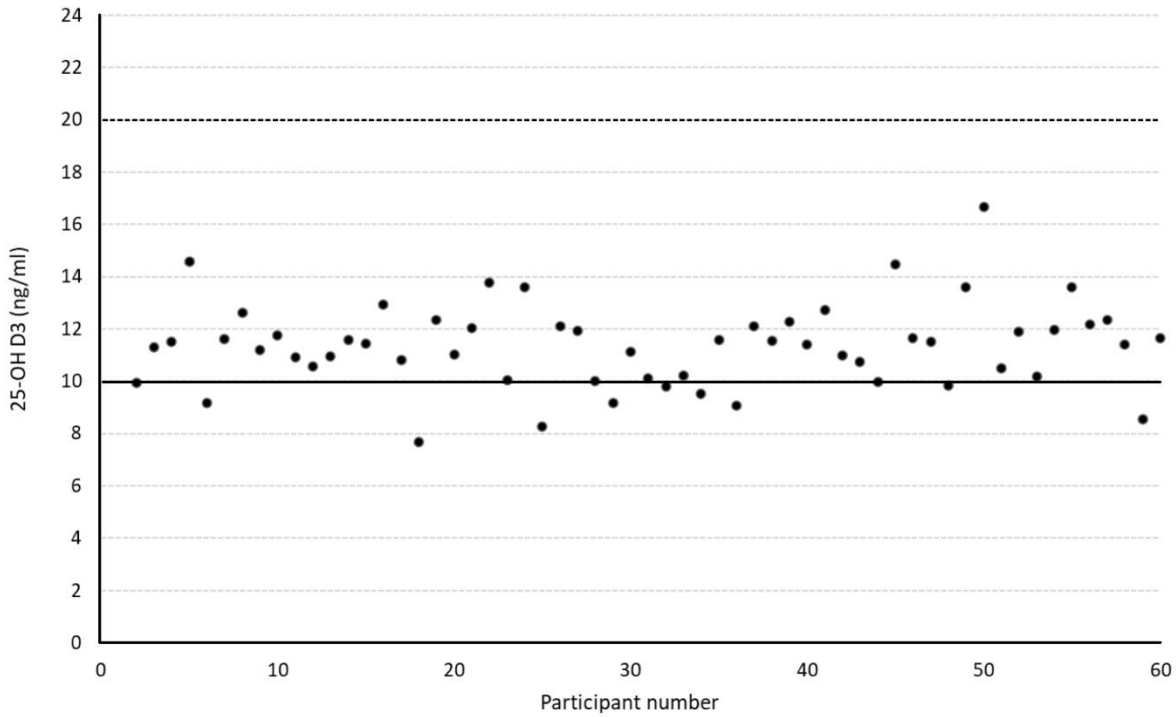

Figure 1. This scatter graph shows the 25-OH D3 status in the examined sample of 60 participants; expressed in ng/ml. The dashed black line indicates the threshold of vitamin D deficiency, while the solid black line indicates the threshold of extreme vitamin D deficiency. All subjects were vitamin D deficient (<20 ng/ml), where 11 (18.33%) of them had an extreme deficiency (<10 ng/ml).

Discussion

According to our knowledge, this is the second study in Croatia examining vitamin D levels among medical students. A study conducted in Rijeka, Croatia established a deficiency in half of the respondents, while we found hypovitaminosis among all of the participants (19). The results raise the question about the underlying cause and about the necessity of implementing prophylactic measures for this population in particular.

The incidence of hypovitaminosis D increased in certain subpopulations due to various risk factors, such as lack of sun exposure, use of sunscreen, long hours indoors, latitude, diet, age, and various chronic and acute diseases (11). A 2017 meta-analysis emphasized the presence of an occupational risk among healthcare professionals, specifically medical students and residents (20).

Several studies worldwide involved measuring vitamin D levels among medical students and found a significant prevalence of vitamin D deficiency. In a study conducted in Spain in 2011, 62% of students had lower than recommended levels of vitamin D (21).

Kardelen et al. determined vitamin D deficiency in 92% of medical students from Turkey, while Nadeem et al. found that only 13.6 % of healthy young medical students in Pakistan had vitamin D levels within the normal range (22, 23). A study conducted in Boston, USA in 1999 found deficiency among 34% of medical students (24).

Contrary to those findings, Leary et al. found a low presence of hypovitaminosis among medical students in the US; only 5% of the subjects were deficient in Florida and 13% in Pennsylvania (25).

In 2018, a study was also conducted in Osijek, Croatia, in the same period of the year as the current study. It also involved the measuring of vitamin D levels among young people aged 19 to 28. As in our study, a high incidence of hypovitaminosis was found, with 72.2% of the subjects having lower levels than 20 ng/mL. Judging by those findings, a high incidence of hypovitaminosis was present. However, a deficiency was significantly less present than in our study, where all of the subjects were medical students (26).

Circumstances that may have affected vitamin D levels

Sampling was carried out in March, when vitamin D levels are lower due to insufficient sun exposure and seasonal variation in the intensity of solar UV light (27). Research indicates that vitamin D concentrations for all age groups in Central Europe begin to decrease in early September and increase in late May (28). In this context, it has been found that medical students in Croatia do not get enough sunlight during the day (29).

A diet poor in vitamin D is another risk factor that may contribute to its low levels. A study from the UK found that students do not get enough vitamin D through their diet, where only 14% of respondents met recommended intake levels (30). A similar trend was noticed among students in China (31). In addition, a 2019 study found that medical students in Rijeka, Croatia, get only a fifth of the recommended vitamin D intake through diet (29).

The period of the COVID-19 pandemic is another circumstance that may affect vitamin D levels in this sample. The Croatian government, like many others, has implemented a series of measures to reduce the number of people affected by COVID-19, including social distancing and recommendations to stay at home, which could potentially limit sun exposure and in turn contribute to lowering vitamin D levels. Yamaguchi et al. found a significant increase in hypovitaminosis D among healthcare workers during the COVID-19 pandemic, where about 90% of the sample was deficient in vitamin D (32). This is essential, assuming that participants had a similar lifestyle in that period as our study sample, considering the duration of indoor activities in medical care and daily life routines.

Also, all study respondents live in the area around 45˚ latitude. Latitude is considered a statistically significant risk factor for vitamin D deficiency in the sense that it is inversely proportional to the level of circulating 25-OH D3 (10). Leary et al. found that medical students at higher latitudes had lower vitamin D levels. In that study, subjects (also medical students) were located at a similar latitude (42˚) as in our sample; but compared to our results, the prevalence of hypovitaminosis was significantly lower — 13 % of participants were vitamin D deficient (25).

Vitamin D supplementation

A 2018 study found that 30.5% of Croatian students practice some form of supplementation, among which medical students used almost twice as much as others (33). Jovanović et al., established that very few of their subjects (4.7%) used vitamin D supplementation (19). Cholecalciferol, ergocalciferol, calcidiol, and calcitriol are used; they are acceptable and straightforward replacements for lack of sun exposure and insufficient intake through diet. Considering

different pharmacokinetic characteristics, metabolism, and points of regulation, cholecalciferol, known as vitamin D3, is most often recommended and used to prevent and treat deficiency (34).

Although much has been said about the importance of preventive use and optimal vitamin D supplementation in health and disease, there is no uniform consensus on the recommended daily intake. The Institute of Medicine and the Endocrine Practice Guideline Committee defined specific guidelines for the general population, where recommended intake was 600 IU/day for people aged 1 to 70, with the upper tolerable intake limit of 4000 IU/day (16).

In 2016, Croatian Guidelines for the Prevention, Detection and Therapy of Vitamin D Deficiency in Adults defined "Vitamin D therapy for verified deficiency, depending on age and comorbidities"(17). According to the guidelines, vitamin D deficient people over 18 years of age without other comorbidities should take vitamin D supplementation of 6000 IU/day for eight weeks, then maintain with 1500-2000 IU/day. Considering that all subjects were deficient, following the previously mentioned recommendations could be one of the effective ways of optimizing vitamin D levels in the serum.

Conclusion

In addition to the results of several other studies conducted worldwide that evaluated vitamin D status among medical students, this study further highlights the problem affecting this student subgroup.

Also, it would be helpful to examine vitamin D levels among medical doctors who, through education, should have gained insight into the concept of a healthy lifestyle and proper prevention methods of various conditions, including hypovitaminosis.

Study limitations

The limitation of this study is that it was conducted on a small sample. Also, testing was done only once in the winter period. Moreover, there is a lack of assessment of participants' lifestyles correlated with vitamin D status, medical history of vitamin D-related diseases, and vitamin D supplementation.

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Competing interests. None to declare.

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