Original article:

Evaluation of vitamin D levels and body mass indexes of university employees

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Abstract:

Objective: Vitamin D is known to have important effects on human health. The existence of a relationship between obesity and vitamin D levels, which have been shown to cause a number of health problems with various studies, is certain. In this study, the relationship between vitamin D levels and body mass indexes(BMI) of individuals was evaluated. The study is unique for being the only research on literature that carried out with university employees. ***Materials and methods:*** Blood samples, anthropometric measurement (BMI) was obtained from 87 volunteer working at Haliç University. The questionnaire form covering the basic questions applied by means of face-to-face method. Quantification of the analytes for 25-hydroxyvitamin-D was carried out using LC-MS/MS. The relationship between 25-hydroxyvitamin-D values and BMI was evaluated with Tukey HSD multiple comparison test. 95% confidence and 80% power sampling were applied to the predictions of a minimum0.50 connection coefficient between 2 quantitative variables.The necessity of 30 cases was determined by using the $N = [(Z_{\alpha}+Z_{\beta})/C]^2 + 3$formula.Considering the losses, the work will be carried out over 80 people. ***Results and Discussion:*** There's significant difference between 25-hydroxyvitamin-D2 and 25-hydroxyvitamin-D3 and the BMI levels of the subjects(p:0.028<0.05-p:0.000<0.05). The average 25-hydroxyvitamin-D3 and 25-hydroxyvitamin-D2 levels of overweight participants were significantly higher than the mean levels of underweight participants'. No significant relationship was found between 25-hydroxyvitamin-D3 and 25-hydroxyvitamin-D2 levels and the time spent outdoors during summer and winter. **Conclusion:** To prevent vitamin D deficiency and insufficiency is to obtain some sensible sun exposure, ingest foods that contain vitamin D, and take supplement.

Keywords: Body Mass Index, Nutrition, Obesity, Vitamin D, LC-MS/MS

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Introduction

Vitamin D (D represents D2, D3, or both) is a secosterol produced endogenously in the skin from sun exposure or obtained from foods that naturally contain vitamin D, counting cod liver oil and fatty fish (eg, salmon, mackerel, and tuna); UV-irradiated mushrooms; foods fortified with vitamin D; and supplements [1.] Vitamin D consists of two forms. One of them is ergocalciferol (Vitamin D2) from plants and the other is cholecalciferol, which is taken from animal source foods and is occurs with exposing of the 7-Dehydrocholesterol present in the skin of the individual to the sun rays (ultraviolet B)[2]. Humans meet the greater part of the vitamin

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D necessity endogenously by daylight[3]. Vitamin D in the human body, 90-95% is synthesized directly under the influence of sun rays. All types of vitamin D areconnected to vitamin D restricting protein in the serum, and to a lesser extent albumin. It undergoes successive hydroxylation in the liver and kidney, transforming into the active forms 25-hydroxyvitamin D (25-OH-VitD) and 1,25-dihydroxyvitamin D [1,25-(OH)2- VitD][3,4]. The liver, fish, egg yolk, salmon, mackerel, milk, broccoli, green onions are a group of foods rich in vitamin D [3-5]. In recent years, lack of vitamin D is a global health problem. Vitamin D deficiency is widely seen in industrialized countries, especially in the northern regions[5,6]. Until recently, it was stated that vitamin D is only effective on bone, kidney and intestine. But today it is reported that vitamin D affects many organs including brain, prostate, breast, colon, pancreas, immunocytes, muscles, endothelium and myocardium. Late investigations have demonstrated that inadequacy of vitamin D is a risk factor for some illnesses, for example, insulin resistance, metabolic syndrome, increased risk of cardiovascular disease, obesity, cancer, Type 1, Type 2 Diabetus Mellitus and hypertension[7,8].

Obesity emerges as a public health problem in both developed and developing countries. Although the factors causing obesity are not fully known, inadequate physical activity, excessive and wrong nutrition as well as genetic, environmental, neurological, physiological, biochemical, sociocultural and psychological problems or a combination of several of the problems may be said to be obesity factors[9].

Late investigations show that obesity is related with vitamin D insufficiency. In a meta-analysis study of 23 researches, it was found that obese subjects had vitamin D deficiency by 35% compared to those with normal weight, 24% vitamin D deficiency compared to those who were overweight, there is a significant relationship between body mass index and vitamin D deficiency[10].Studies conducted to determine the relationship between serum 25-OH-VitD and obesity have shown a significant negative correlation between serum 25-OH-VitD levels and obesity[7].In a study conducted by Gonzalez et al., a low vitamin D level was found in individuals with high body mass index, waist circumference, waist and height ratio[11]. Many studies indicate that vitamin D deficiency is associated with obesity, but also there are studies that indicate no association. Grooborg and colleagues found that there was no relationship between vitamin D level and body fat ratio and body fat ratio did not respond to vitamin D supplementation[12].

Most of the studies investigating the relationship between vitamin D and obesity are cross-sectional. The quantity of studies have demonstrated that obesity occurs more frequently in individuals with low vitamin D levels.In this association, vitamin D is a vitamin that is soluble in fat, so this molecule may have been pulled back from the circulation by being kept in fat tissue, which is increased in individuals with high body mass index and that the increased levels of 1,25-(OH)2-VitD3 due to high parathormone suppress the synthesis of 25-OH-VitD3 in the liver. This study was aimed to determine the frequency of vitamin D deficiency by examining vitamin D levels in individuals aged 18-65 years who are employees of a university located in İstanbul and to investigate whether there is a significant difference between vitamin D levels and body mass indexes of individuals. In the literature, due to the lack of research on university employees and that they are away from sunlight because of working indoor, low vitamin D levels are predicted.

MaterialandMethod

Studypopulation

Aftergettingrequiredethicalapproval, a cross-secti onalstudywasconductedbetween March-May 2018 with87 individuals ages 18 years to 65 years who were the employees of Haliç University and voluntary to participate in the study. According to the sample formulation in the descriptive statistics, it has been calculated that 80 individuals should be included in the study. More individuals have been studied with losses taken into account. Volunteers with chronic illness (drug users for metabolic bone disease, liver and kidney disease, surgical interventions in the gastrointestinal system, malabsorption syndromes, tuberculosis treatment and anticonvulsant use), receive vitamin D supplementation and pregnant or lactating individuals were not included in the study.

Demographic Data

The questionnaire form prepared by the researchers covering the basic questions such as demographic characteristics, physical activity, amount of sunbathing, nutritional status, smoking and alcohol use, skin color characteristics, daily sleep hour were applied by means of face-to-face method.

Anthropometry

Participants' Body Mass Indexes (BMI) were determined using the Tanita BC418 MA and BMI wascategorizedaccordingto World HealthOrganizationguidelinesforunderweight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight

(25.0–29.9 kg/m^2), obese Class I (30.0– 34.9 kg/m^2), obese Class II (35.0–39.9 kg/m^2), andobese Class III/ morbidobesity (>40.0 kg/m^2)[13].

Biochemical Analysis

Blood donation from the participants was carried out by the responsible nurse working at Haliç University. In the scope of the study, blood samples taken on 8 hour fasting from volunteers were divided into sera and 25-OH-VitD3, 25-OH-VitD2levels in the relevant serum were determined. The analysis of 25-OH-VitD3, 25-OH-VitD2 were conducted using Agilent Infinity 1290 HPLC(High Performance Liquid Chromatography) system consisting of a binary pump, degasser and autosampler coupled with 6460 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). For the measurement of the 25-OH-VitD3 and D2 concentrations, samples were prepared by automatic sample preparation system-MsArt (Sem Laboratory Systems and Solutions, Istanbul, TURKEY) which had been adapted for utilizing CE-IVD(In vitro diagnostic) certificated Jasem vitamin D kit for LC-MS/MS (Sem Laboratory Systems and Solutions, Istanbul, TURKEY). The kit includes four serum-based standards (calibrants) used for creating calibration curve, labelled stable isotope-*d6*-25-OH-VitD3 as the internal standard (IS), bi-level quality control serum samples (QCs), mobile phases (mobile phase A and B), reagents (reagent-1 and 2), chromatographic and mass detection parameters of the method. Positive electronic spray ionization (+ ESI) in multiple-reaction monitoring (MRM) mode was implemented for the MS/MS detection of the analytes.

Automated kit sample preparation procedure was as follows; 50 µL of calibrants/QC/specimen was transferred into a 96 deep-well plate. After that, 50 µL of reagent-1 was added for dilution before swirling for 5 sec. Subsequent to the dilution step, 25 µL of the IS was added then agitated for 5 sec. After that, 225 µL of reagent-2 transferred in order to perform protein precipitation. After shaking 5 sec, the 96 deep-well plate was automatically transported to the centrifuge which was equipped within MsArt to centrifuge at 2000 g force for 5 min. At the end of the procedure, the 96 deep-well plate was manually located autosampler of LC-MS/MS system prior to injection. HPLC system was operated to inject 10 µL of prepared calibrants/QCs/samples into the Jasem analytical column-specified for vitamin D analysis which was maintained at 20 °C. Chromatographic separation was carried out using mobile phase A and B with gradient elution at a flow rate of 0.7 mL/ min. The HPLC elution was as follows: the initial LC gradient of 22% A was held for 3.8 min. Then, the gradient was immediately converted to 2% A and maintained for 2.5 min. Finally, the column was equilibrated at 22% A for 2.2 min. The total running time was 8.5 min. Mass spectrometric(MS) detection was performed on Agilent 6460 triple quadrupole MS equipped with an ESI source in the positive ion mode. The optimal MS detector settings were as follows: drying gas temperature 150 °C, drying gas flow 11 L/min, nebulizer pressure 40 psi, sheath gas temperature 300 °C, sheath gas flow 11 L/min and capillary voltage of 3000 V (+). The positive ESI mode was operated for the detection of 25-OH-VitD3 and D2 and IS as precursor ions resulting in protonated form (m/z = $[M+1]^+$) without loss of water. The MS/MS detection was achieved with two product ion transitions generated collision-induced dissociation (CID) of corresponding precursor ion with exception of the IS. Single mass transition was selected as the product ion for the MS/MS detection of IS. MRM transitions of the 25-OH Vitamin D3, D2 corresponding IS were monitored at optimum fragmentation voltages (FV) and optimum collision energies (CE) (Table 1). The peak area ratio of the 25-OH-VitD3 and D2 to the assigned IS was evaluated for quantification of the analytes concentration. Calibrations were based on the matrix-matched curves, for 25-OH-VitD3 it was linear over a concentration range of 2.25–74.4 µg/mL with a 0.999 correlation coefficient and for 25-OH-VitD2 it was linear over a concentration range of 1.44–78.8 µg/mL with a 0.999 correlation coefficient. The recovery results obtained for two levels of QC were ranged from 92.9 % to 117.1 %. Relative standard deviation (RSD %) results were ranged from 1.98 % to 3.15 % applying two levels of QC. The limit of quantitation (LOQ) and the limit of detection (LOD) obtained for 25-OH-VitD3 were 0.026 and 0.008 µg/ mL, respectively. The limit of quantitation (LOQ) values obtained for 25-OH-VitD3 and 25-OH-VitD2 were 1.52 and 1.44 µg/mL, respectively.

"Vitamin D deficiency" is defined as a 25-OH-VitDbelow 20 ng/mL, "vitamin D insufficiency" as a 25-OH-VitDof 21–29 ng/mL and "vitamin D adequacy" as a 25-OH-VitDof 30-100ng/mL[14].

Table 1. MRM transitions and MS/MS conditions of 25-OH Vitamin D3, D2 and IS

Compound Name	Precursor Ion	Product Ion	FV	CE	Polarity
25-OH-VitD2	413.3	395.3*	110	2	Positive
25-OH-VitD2	413.3	355.3	110	2	Positive
25-OH-VitD3	401.3	383.3*	120	2	Positive
25-OH-VitD3	401.3	365.3	120	4	Positive
d_6-25-OH-VitD3 (IS)	407.3	389.3	120	2	Positive

* Assigned as quantitative ion

Statistical analyses

SPSS 22.0 program was used for statistical evaluation of all data. The mean ± standard deviation (SD), frequency and percentage was used for descriptive analysis. Independent sample t-test was used to compare independent 2-group averages, One-way ANOVA test was used to compare two or more independent groups, and Chi-square correlation test result was used to compare two categorical independent variablesThelevel of significancewas set at p<0.05.

Results and discussion

Table 2 summarize the characteristics of the 87 participants. The total 25-OH-VitD level of 78 (90%) of participants was below 20 ng/mL, while the 25-OH-VitD level of 9 (10%) was above 20 ng/mL. There were 13, 40, 23 and 11 individuals which were in the range of BMI levels that below 18.5 kg/m^2, 18.5-24.9 kg/m^2, 25-29.9 kg/m^2 and 30-39.9 kg/m^2 respectively.

Table 2. Descriptive characteristics of the participants (n=87)

Gender (n)	
Female	67
Male	20
Age (y)	Mean: 27,9 SD: 8,5
BMI (kg/m^2)	Mean: 23,7 SD: 5,6
AlcoholConsumption (n)	
Yes	11
No	76
Smokers (n)	
Yes	27
No	60
Maritalstatus (n)	
Married	22
Single	63
Widow / Divorced	2
EducationStatus (n)	
Primary School	7
Middle School	5
High School	31
University	21
MS / PhD	23
Physical Activity (weekly)	
30-60 min	16
60-120 min	12
120-150 min	8
150 >	11

There was a significant difference between the 25-OH-VitD2 and 25-OH-VitD3 levels of the subjects and the BMI levels (F:3.190; p:0.028<0.05-F:7.750; p:0.000<0.05). When BMI levels were assessed by Tukey HSD multiple comparison test; the average 25-OH-VitD3 and 25-OH-VitD2 levels of overweight participants (25-29.9) were significantly higher than the mean 25-OH-VitD3 and 25-OH-VitD2 levels of underweight participants (<18.5) (Table 3).

Table 3. Comparison of 25-OH-vitamin D3 and D2 levels according to BMI classification

25-OH-VitD	BMI[1] (kg/m^2)	n	Mean (ng/mL)	SD	F	p
25-OH-VitD3	<18.5	13	9.01	3.08	3.19	0.028*
	18.5-24.9	40	10.86	5.42		
	25-29.9	23	14.90	7.90		
	30-39.9	11	11.85	6.90		
25-OH-VitD2	<18.5	13	1.04	0.21	7.750	0.000*
	18.5-24.9	40	1.65	0.78		
	25-29.9	23	2.83	1.90		
	30-39.9	11	1.79	1.01		

[1]One-way ANOVA test, *p<0.05
BMI: Body Mass Index

The 25-OH-VitD2 levels of the individuals did not differ between male and female subjects (p>0.05) while the average 25-OH-VitD3 level of male subjects was significantly higher than the average 25-OH-VitD3 level of female subjects (t:-2.022; p:0.046<0.05) (Table4). 30 of the subjects in our study were found to have less than 6 hours of sleep, 35 of them were 7 hours, 19 of them were 8 hours and 3 of them were sleeping for 9 hours. There was no significant relationship between sleep duration and 25-OH-VitD3 (p:0.425). Inourstudy, it wasfoundthat 30% of thesubjectssmokescigarettesandnosignificantrelationwasfoundbetweensmokingand25-OH-VitD3 (p: 0.459). Also there wasn't significant difference between 25-OH-VitD2 and 25-OH-VitD3 levels of the individuals and sun-cream usage and skin color properties (p>0.05). No significant relationship was found between 25-OH-VitD3 and 25-OH-VitD2 levels of the participants and the time spent outdoors during summer and winter months (p>0.05).

Table 4. Comparison of vitamin levels of 25-OH-vitamin D3 and D2 according to gender

25-OH-VitD	Gender	n	Mean (ng/mL)	SD	t[1]	p
25-OH-VitD2	Female	67	1.79	1.37	-1.348	0.181
	Male	20	2.23	1.02		
25-OH-VitD3	Female	67	11.04	6.19	-2.022	0.046*
	Male	20	14.25	6.41		

[1]Independent sample t test, p<0.05

Vitamins are defined as compulsory nutrients to be taken from the outside, as distinct from, 25-OH-VitD is considered to be a steroid hormone because it is produced in one tissue and given to the blood circulation, has effects on other tissues and this effect of 25-OH-VitD is regulated by feedback mechanisms [3]. For accurate assessment of a patient's vitamin D status, serum levels of the major circulating and clinically relevant human vitamin D metabolites 25-OH-VitD3 and 25-OH-VitD2 should be measured individually as the indicators using a reliable assay. Conventionally, the determination of total concentration of vitamin D metabolites has been conducted utilizing immunoassays. Thanks to the technique's inherent analytical specificity and sensitivity compared to immunoassays, LC-MS/MS is regarded as the gold-standard technique for accurate and simultaneous measurement of these indicators. From the perspective of rapid and precise sample preparation implementing on large number of specimens, performing with automated liquid handling system has significant role in obtaining reliable results. JasemMsArt installed with straightforward sample preparation protocol including only dilution and protein precipitation steps provided precision by preventing operator-related error throughout the manually sample preparation process. Furthermore, quantification of the analytes was carried out using LC-MS/MS supporting by stable isotope labelled 25-OH-VitD3 as internal standard for both. Thus, applying CE-IVD certificated and validated LC-MS/MS kit with automated sample preparation system ensured to acquire accurate vitamin D profile of the patient. Most of the studies investigating the relationship between 25-OH-VitDand obesity are cross-sectional. The results of the investigations are contradictory, since standardization of the obtained data is difficult. The number of studies showing that obesity occurs more frequently in individuals with low 25-OH-VitDlevels. In this connection, it is emphasized that vitamin D is a fat soluble vitamin, so that this molecule may be withdrawn from the circulation by keeping the increased fat in obese individuals and that increased 1,25-(OH)2-VitD3 levels due to high parathormone, 1,25-(OH)2- Vit D suppress the 25-OH-VitD3 synthesis in the liver[8].

Hekimsoy et al. examined the vitamin D levels of 391 patients over 20 years of age and living in rural areas during winter. In general, they found an average of 16.9±13.09 ng/mL of vitamin D levels. They found that 25-OH-VitDlevels of females were more deficient (78.7%) than males (66.4%). Bolland et al. evaluated 1606 healthy postmenopausal women and 378 middle-aged and elderly male patients living in New Zealand, found that 73% of women and 39% of men with vitamin D levels <20 ng/mL [15]. In our study, 25-OH-VitD2 levels of individuals did not differ according to gender ($p>0.05$) but 25-OH-VitD3 levels of male subjects were found to be significantly higher than those of female 25-OH-VitD3 levels (Table 3).

Factors affecting vitamin D production can be classified into two groups as external and personal factors. Latitude, sea level, seasons, time of day, clouds and aerosols can be counted as external factors and skin color, age, weight, clothing style, use of sun-cream as personal factors. Zone 2 (between 23° 27 '-66° 33' latitudes), our country also includes, does not receive enough ultraviolet for vitamin D synthesis for at least 1 month per year [16]. In a study conducted by Kanan et al., the seasonal changes in vitamin D levels in Suudi 1556 pre- and post-menopausal women were investigated and high-dose vitamin D deficiency was detected despite the routine implementation of 10-20 µg vitamin D3 in post-menopausal women[17]. Our study was conducted in İstanbul at the latitude of 41,00527' and there was no significant relationship between duration of outdoors and vitamin D levels in winter and summer ($p>0.05$). Vitamin D levels have been investigated in many countries[18]. In order to make cross-country comparisons more valid, standardized measurement results are used with data from a central laboratory system or with different methods such as liquid chromatography, mass spectrometry (LC-MS/MS). Studies show that vitamin D deficiency has a high prevalence in many countries like China, Turkey, India, even in tropical countries such as Iran and Saudi Arabia have high rates of vitamin D deficiency. In a study conducted by Mansoor et al., the average vitamin D level was 16.44 ng/mL. The mean cutoff value for vitamin D deficiency was 20 ng/mL and through this value it's

found out that 69.9% for vitamin D deficiency and 21.1% for vitamin D inadequacy[19]. Studies done with obesity and serum 25-OH-VitD3 are generally evaluated according to BMI.In a study conducted with 2126 participants, a negative correlation was found between the BMI values of individuals and serum 25-OH-VitD3 levels[20]. Similarly, studies have shown an inverse relationship between serum vitamin D levels and body fat percentage, visceral adiposity and waist circumference[21]. In a study conducted with 163 obese individiulas in Thailand in 2014, the prevalence of vitamin D deficiency (<20 ng/mL) and vitamin D inadequacy (<30 ng/mL) were 49 (30.1%) and 148 (90.8%), respectively. In all, 98% of the individuals with body mass index >35 kg/m^2 had vitamin D inadequacy and serum 25-OH-VitD concentrations were negatively associated with percent body fat. The results of our study indicates that the average 25-OH-VitD3 and 25-OH-VitD2 levels in overweight people (25-29.9 kg/m^2) is higher than that is in underweight people (<18.5 kg/m^2) (Table 2).

Studies on serum vitamin D in smokers show conflicting results. A study examined the association of smokingstatuswith serum vitamin D in olderChinese men, takingadvantage of a communitybasedsamplewithnaturalexposureto vitamin D. Themean (SD) of vitamin D concentration was 17.2, 15.0 and 15.4 ng/mL fornever, formerandcurrentsmokers, respectively. Adjusted For Multiple Confounders, vitamin D decreased fromnevertoformer, thentocurrentsmokers (p: 0.02). Longerduration of quittingsmokingwas associated with higher vitamin D concentration as pertocurrentsmokers (p: 0.04)[22]. However, theresult of theTromso'sstudy is theopposite of this. The Investigation Ledwith 6932 individuals and found higher serum vitamin D levels in smokers[23]. Also in our study no significant relation was found between smoking and 25-OH-VitD3 (p: 0.459). InLibon et al.'sstudy, fair-skinnedvolunteers (n=20, 4 males/16 females, meanage: 23.2 years) andblack-skinned (n=11, 6 males/5 females, meanage: 23.8 years) received a single total body UVB(Ultraviolet B) exposure (0.022 J/cm^2). The 25-OH-VitD levelsweremeasured on days 0, 2 and 6. At theEnd of thestudytheresultswere; on day 0, allvolunteerswereseverely vitamin D deficient. On day 2, 25-OH-VitD levels of fair-skinnedvolunteer sincreasedsignificantly (median: 11.9-13.3 ng/mL, p<0.0001), but not in black-skinnedpeople (median: 8.60-8.55 ng/mL, p:0.843). Again on day 6, 25-OH-VitD levels of fair-skinnedvolunteersincreasedsignificantly (median: 11.9-14.3 ng/mL, p<0.0001), but not in black-skinnedpeople (median: 8.60-9.57 ng/mL, p:0.375). The Examination Recommends That skin pigmentation adversely impacts vitamin D synthesis[24]. In The National And Healthy Nutrition Survey Conducted in Korea, individual sweredivide into 4 groups according to the sleeping periods [Q1 (≤4 hours), Q2 (5-6 hours), Q3 (7-8 hours) and Q4 (≥9 hours)] and 25-OH-VitD levelswereexamined. Mean serum vitamin D levels of subjects in the Q1, Q2, Q3, and Q4 groupswere 44.18, 48.08, 48.83, and 51.78 nmol/L, respectively. It's Found Out What Groups had significantly higher serum vitamin D levelsthansubjects in the Q1 group. As a result of thestudy, inadequatesleepdurationmay be associatedwithlower vitamin D levels in elderly adults[25]. According the results of our study there was no significant relationship between sleep duration and 25-OH-VitD3 (p: 0.425).

Conclusion

In conclusion, it was found that there is vitamin D deficiency on participants of our study (20 ng/mL). There is potentially a great upside (in terms of improving overall health and well-being) to increasing serum 25-OH-VitD levels above 20 ng/mL. An effective strategy to prevent vitamin D deficiency and insufficiency is to obtain some sensible sun exposure, ingest foods that contain vitamin D, and take a vitamin D supplement. This study has some limitations that the study has been conducted with a specific region and a small number of individuals. With the studies to be conducted with a large number of individuals and covering all regions of Turkey can be obtained greater information.

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