Original article: 
Evaluation of vitamin D levels and body mass indexes of university employees

Murat Emrah MAVİŞ 1, Çiğdem YILDIRIMMAVİŞ 2, İrem ÖZAY ARANCIOĞLU 3, Hatice Kübra YILMAZ 4, Berrak ERGÜDEN 5

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 minimum 0.50 connection coefficient between 2 quantitative variables. The necessity of 30 cases was determined by using the N = [(Zα+Zβ)/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

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

1. Murat Emrah MAVİŞ, Phd, 1SEM Laboratory Instruments Marketing. Postal address: Barbaros Mah. Temmuz Street No:6 Atasehir/Istanbul TR 34746. E-mail: murat.mavis@jasem.com.tr
2. Çiğdem YILDIRIMMAVİŞ, Lecturer, Halic University, Department of Nutrition and Dietetics. Postal address: Imrahor street No:82 Beyoglu/Istanbul TR 34394. E-mail: cymavis85@gmail.com
3. İrem ÖZAY ARANCIOĞLU, Lecturer, Halic University, Department of Nutrition and Dietetics. Postal address: Imrahor street No:82 Beyoglu/Istanbul TR 34394. E-mail: iremozay@gmail.com
4. Hatice Kübra YILMAZ, Lecturer, Halic University, Department of Nutrition and Dietetics. Postal address: Imrahor street No:82 Beyoglu/Istanbul TR 34394. E-mail: dytkubrayilmaz@hotmail.com
5. Berrak ERGÜDEN, Research assistant, Halic University, Department of Nutrition and Dietetics. Postal address: Imrahor street No:82 Beyoglu/Istanbul TR 34394. E-mail: dyt.erguden@gmail.com
6. Hasan Hüsrev HATEMİ, Professor Doctor, Halic University, Department of Nutrition and Dietetics. Postal address: Imrahor street No:82 Beyoglu/Istanbul TR 34394. E-mail: husrevhatemi@yahoo.com

Correspondence to: İrem ÖZAY ARANCIOĞLU, Haliç University, Nutrition and Dietetics Department, Imrahor street No:82 Beyoglu/Istanbul TR 34394. iremozay@gmail.com
D necessity endogenously by daylight. Vitamin D in the human body, 90-95% is synthesized directly under the influence of sun rays. All types of vitamin D are connected 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]. 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. 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.

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. 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. 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. 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. 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.

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-VitD 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 Istanbul 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.

**Material and Method**

**Study Population**

After getting required ethical approval, a cross-sectional study was conducted between March-May 2018 with 87 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 was categorized according to World Health Organization guidelines for underweight (<18.5 kg/m²), normal weight (18.5–24.9 kg/m²), overweight...
(25.0–29.9 kg/m²), obese Class I (30.0–34.9 kg/m²), obese Class II (35.0–39.9 kg/m²), and obese Class III/morbidobesity (>40.0 kg/m²)13.

**Biochemical Analysis**

Blood donation from the participants was carried out by the responsible nurse working at Halic 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-VitD2 levels 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-VitD below 20 ng/mL, “vitamin D insufficiency” as a 25-OH-VitDof 21–29 ng/mL and “vitamin D adequacy” as a 25-OH-VitD of 30-100ng/mL14.
the 25-OH-VitD level of 9 (10%) was above 20 ng/mL. There were 13, 40, 23 and 11 individuals, (90%) of participants was below 20 ng/mL, while independent variables the level of significance was independent groups, and Chi-square correlation test result was used to compare two categorical participants. The total 25-OH-VitD level of 78

Table 2 summarize the characteristics of the 87 participants.

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

| Gender (n)          | Female | Male |
|---------------------|--------|------|
| n                   | 67     | 20   |
| Age (y) Mean: 27.9 SD: 8.5 |
| BMI (kg/m²) Mean: 23.7 SD: 5.6 |
| Alcohol Consumption (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 |

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/m², 18.5-24.9 kg/m², 25-29.9 kg/m² and 30-39.9 kg/m² respectively.

Table 3. Comparison of vitamin levels of 25-OH-VitD2 and 25-OH-VitD3 levels according to BMI classification

| BMI (kg/m²) | 25-OH-VitD<br>n | Mean (ng/mL) | SD | F  | p  |
|-------------|-----------------|--------------|----|----|----|
| <18.5       | 13              | 9.01         | 3.08 |
| 18.5-24.9   | 40              | 10.86        | 5.42 | 3.19 | 0.028* |
| 25-29.9     | 23              | 14.90        | 7.90 |
| 30-39.9     | 11              | 11.85        | 6.90 |
| <18.5       | 13              | 1.04         | 0.21 |
| 18.5-24.9   | 40              | 1.65         | 0.78 | 7.750 | 0.000* |
| 25-29.9     | 23              | 2.83         | 1.90 |
| 30-39.9     | 11              | 1.79         | 1.01 |

* 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) (Table 4). 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). In our study, it was found that 30% of the subjects smoked cigarettes and no significant relationship was found between smoking and 25-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-VitD2 and 25-OH-VitD3 according to gender

| 25-OH-VitD<br>Gender | n | Mean (ng/mL) | SD | t  | p   |
|-----------------------|---|--------------|----|----|-----|
| 25-OH-VitD2<br>Female  | 67 | 1.79         | 1.37 | -1.348 | 0.181 |
| 25-OH-VitD3<br>Female  | 67 | 11.04        | 6.19 | -2.022 | 0.046* |

* Independent sample t test, p<0.05
Vitamins are defined as compulsory nutrients to be taken from the outside, as distinct from, 25-OHVitD 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-OHVitD 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-OHVitD3 and 25-OHVitD2 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-OHVitD3 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-OHVitD and 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-OHVitD levels. 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-OHVitD3 synthesis in the liver8.

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-OHVitD levels 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-OHVitD levels of individuals did not differ according to gender (p>0.05) but 25-OHVitD3 levels of male subjects were found to be significantly higher than those of female 25-OHVitD3 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 sunscreen 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 Saudi 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 women17. 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 countries18. 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 cut-off 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. 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. Similarly, studies have shown an inverse relationship between serum vitamin D levels and body fat percentage, visceral adiposity and waist circumference. In a study conducted with 163 obese individuals 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/m² 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/m²) is higher than that is in underweight people (<18.5 kg/m²) (Table 2).

Studies on serum vitamin D in smokers show conflicting results. A study examined the association of smoking status with serum vitamin D in older Chinese men, taking advantage of a community-based sample with natural exposure to vitamin D. The mean (SD) of vitamin D concentration was 17.2, 15.0 and 15.4 ng/mL for ever, former and current smokers, respectively. Adjusted for Multiple Confounders, vitamin D decreased from never to former, then to current smokers (p: 0.02). Longer duration of quittingsmoking was associated with higher vitamin D concentration as compared to current smokers (p: 0.04). However, the result of the Tromso’s study is the opposite of this. The Investigation Led with 6932 individuals and found higher serum vitamin D levels in smokers. Also in our study no significant relation was found between smoking and 25-OH-VitD3 (p: 0.459). In Libon et al.’s study, fair-skinned volunteers (n=20, 4 males/16 females, mean age: 23.2 years) and black-skinned (n=11, 6 males/5 females, mean age: 23.8 years) received a single total body UVB (Ultraviolet B) exposure (0.022 J/cm²). The 25-OH-VitD levels were measured on days 0, 2 and 6. At the end of the study, the results were; on day 0, all volunteers were severely vitamin D deficient. On day 2, 25-OH-VitD levels of fair-skinned volunteer increased significantly (median: 11.9-13.3 ng/mL, p<0.0001), but not in black-skinned people (median: 8.60-8.55 nmol/L, p:0.843). Again on day 6, 25-OH-VitD levels of fair-skinned volunteers increased significantly (median: 11.9-14.3 ng/mL, p<0.0001), but not in black-skinned people (median: 8.60-9.57 ng/mL, p:0.375). The Examination Recommends That skin pigmentation adversely impacts vitamin D synthesis. In The National And Healthy Nutrition Survey Conducted in Korea, individual swere divide 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 levels were examined. Mean serum vitamin D levels of subjects in the Q1, Q2, Q3, and Q4 groups were 44.18, 48.08, 48.83, and 51.78 nmol/L, respectively. It's Found Out What Groups had significantly higher serum vitamin D levels than subjects in the Q1 group. As a result of the study, inadequate sleep duration may be associated with lower vitamin D levels in elderly adults. 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.

Acknowledgments

The authors gratefully acknowledge the volunteers who kindly participated in this study. We Also Would Like To Thank SEM Laboratory Instruments Marketing for supplying the CE-IVD certificated Jasem
vitamin D kit for LC-MS/MS and for the possibility of using automatic sample preparation system-SmArt. **Ethical clearance:** This study is approved by clinical research ethics committee of Istanbul Yenı Yüzyıl University

**Source of fund:** (if any) This research receive nospecificgrantfromany funding agency, commercialor not-for-profitsectors.

**Conflict of interest:** (alreadygiven). The Named Authors Have No Conflict of interest, financial or otherwise

**Data gathering and idea owner of this study:** The Study Made By Authors’ cooperation

**Study design:** The study made by authors’ cooperation

**Data gathering:** Murat Emrah Mavis, Hatice Kübra Yilmaz, Berrak Erguden Writing and submitting manuscript: The study made by authors’ cooperation

**Editing and approval of final draft.** Irem Ozay-Arancioglu

---

**References:**

1. Hossein-nezhad A, Holick MF. Vitamin D for Health: A Global Perspective. Mayo Clin Proc. July 2013;88(7):720-755. doi: 10.1016/j.mayocp.2013.05.011.

2. Wacker M, Holick M. Vitamin D-effects on skeletal and extraskeletal healthandtheneedfor supplementation. *Nutrients.* 2013; 5(1):111-148. doi: [10.3390/nu5010111](https://doi.org/10.3390/nu5010111).

3. Tellioğlu A, Başaran S. Vitamin D in the light of current information. *Archive Source Browsing Journal.* 2013;22(2): 259-271. doi: 10.2298/SARH1202110R

4. Engelsen O, Brustad M., Aksnes L, Lund E. Daily duration of vitamin D synthesis in human skin with relation to latitude, total ozone, altitude, ground cover, aerosols and cloud thickness. *Photochemistry and photobiology.* 2005;81(6), 1287-1290. doi: [10.1562/2004-11-19-RN-375](https://doi.org/10.1562/2004-11-19-RN-375).

5. Fidan F, Alkan BM, Tosun A. Pandemic of the Age: D Vitamin deficiency and inadequacy. *Turkish Journal of Osteoporosis.* 2014;20:71-4. doi: 10.4274/tod.94830.

6. Djennane M, Lebbah S, Roux C, Soubberbille J, Djoudi H, Cavalier E. Vitamin D status of schoolchildren in Northern Algeria, seasonal variations and determinants of vitamin D deficiency. *Osteoporos Int.* 2014; 25(5):1493-1502. doi: 10.1007/s00198-014-2623-7.

7. Sert H, Çetinkaya S, Aygın D. D Vitaminin Obesity, Diabetes Mellitus, Hypertension and Cancer. *J hum Rhythm.* 2015;1(4):143-149.

8. Çimen M, Bölgencimen Ö. Obesityand Vitamin D. *Mersin Universiteli Bilimderesi* 2016;9(2): 102-112.

9. Turkey Public Health Agency of Obesity, Diabetes and Metabolic Diseases Department. Causes of Obesity.[http://beslenme.gov.tr/index.php?lang=tr&page=41](http://beslenme.gov.tr/index.php?lang=tr&page=41)(Access date: 25.05.2018).

10. Santos P, Costa P, Santos C, Santos D. Obesity and vitamin D deficiency: A systematic review and meta-analysis. *Obes Rev.* 2015;16(4):341-349. doi: 10.1111/obr.12239.

11. González L, Ramos-Trautmann G, Díaz-Luquis GM, Pérez CM, Palacios C. Vitamin D status is inversely associated with obesity in a clinic-based sample in Puerto Rico. *Nutrition Research.* 2015; 35(4):287-293. doi: 10.1016/j.nutres.2015.02.001.

12. Gronborg IM, Lundby IM, Mølgaard C, Jakobsen J, Ovesen L, Andersen R. Association of body fat and vitamin D status and the effect of body fat on the response to vitamin D supplementation in Pakistani immigrants in Denmark. *Eur J Clin Nutr.* 2015; 69(3):405-407. doi: 10.1038/ejcn.2014.254.

13. World Health Organization guidelines for Body mass index - BMI [http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi](http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi)(Access date: 25.05.2018).

14. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *The Journal of Clinical Endocrinology & Metabolism.* 2011;96(7), 1911-1930. doi: 10.1210/jc.2011-0385.

15. Bolland MJ, Grey AB, Ames RW, Mason BH, Horne AM, Gamble GD, et al. The effects of seasonal variation of 25-hydroxyvitamin D and fat mass on a diagnosis of
vitamin D sufficiency. *Am J Clin Nutr.* 2007;86(4):959-64. doi: 10.1093/ajcn/86.4.959.

16. Giulietti A, Van Etten E, Overbergh L, Stoffels K, Bouillon R, Mathieu, C. Monocytes from type 2 diabetic patients have a pro-inflammatory profile: 1,25-Dihydroxyvitamin D3 works as anti-inflammatory. *Diabetes research and clinical practice.* 2007;77(1), 47-57. doi:10.1016/j.diabres.2006.10.007.

17. Kanan RM, Saleh A, Fakhoury HM, Adham M, Aljaser S, Tamimi W. Year-round vitamin D deficiency among Saudi female out-patients. *Public health nutrition.* 2013;16 (3), 544-548. doi: 10.1017/S1368980012002947.

18. Mittal, S., Sandhu, H., & Singh, B. (2018). Study of Vitamin D levels in patients with Chronic Kidney Disease. *Bangladesh Journal of Medical Science,* 17(4), 652-660.

19. Uçar F, Taşlıpınar MY, Soydaş AÖ, Özcan N. Ankara Etlik İhtisas Eğitim ve Araştırma Hastanesine Başvuran Hastalarda 25-OH Vitamin D Düzeyleri. *Eur J Basic Med Sci* 2012;2:12-15

20. Lagunova Z, Porojnicu AC, Lindberg F, Hxeberg S, Moan J. The dependency of vitamin D status on body mass index, gender, age and season. *Anticancer research.* 2009; 29(9), 3713-3720.

21. Kremer R, Campbell PP, Reinhardt T, Gilsanz V. Vitamin D status and its relationship to body fat, final height, and peak bone mass in young women. *The Journal of Clinical Endocrinology & Metabolism.* 2009;94(1), 67-73. doi: 10.1210/jc.2008-1575.

22. Jiang CQ, Chan YH, Xu L, Jin YL, Zhang WS, Cheng KK et al. Smoking and serum vitamin D in older Chinese people: cross-sectional analysis based on the Guangzhou Biobank Cohort Study. *BMJ Open* 2016;6:e010946. doi:10.1136/bmjopen-2015-010946.

23. Grimnes G, Almaas B, Eggen AE, Emaus N, Figenschau Y, Hopstock LA, et al. Effect of smoking on the serum levels of 25-hydroxyvitamin D depends on the assay employed. *Eur J Endocrinol.* 2010;163:339–48. doi: 10.1530/EJE-10-0150.

24. Libon F, Cavalier E, Nikkels AF. Skin Color Is Relevant to Vitamin D Synthesis. *Dermatology.* 2013;227:250–254. doi:10.1159/000354750.

25. Kim JH, Chang JH, Kim DH, Kang JW. Association Between Self-Reported Sleep Duration and Serum Vitamin D Level in Elderly Korean Adults. 2014;62:2327-2332. doi: 10.1111/jgs.13148.