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THE EFFECTS OF VDR GENE POLYMORPHISMS AND LIFESTYLE FEATURES ON VITAMIN D LEVELS OF POST MENOPAUSAL WOMEN

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Abstract

Objectives: Vitamin D deficiency is a common situation for women who are in menopause due to various reasons. This study aims to investigate the effect of VDR gene polymorphisms and lifestyle on vitamin D levels of women in menopause.

Materials and Methods: The study was planned in a cross-sectional descriptive design. Data was collected with a sociodemographic and lifestyle habits question form, and patients' blood samples were obtained for vitamin D levels and genetic tests. The data was evaluated by using SPSS 16.0 software. The logistic regression analysis model was created using the Backward elimination method, and the P-value below 0.05 was considered statistically significant.

Results: The study was carried out on 303 menopausal women. The frequency of vitamin D deficiency in patients was 71.95%. Receiving vitamin D and Omega-3 supplements and having prolonged sleep duration were found to be protective factors from vitamin D deficiency. Of the VDR gene polymorphisms, the Bsml bb genotype was found to protect from vitamin D insufficiency, while the ApaI bb genotype increased the risk of vitamin D insufficiency.

Conclusion: Vitamin D levels may be low in people who do not have sufficient sleep time. Our study found that the APA I aa genotype increased the risk of vitamin D deficiency, while the BsmI bb genotype protected from vitamin D deficiency. More studies are needed on the effects of lifestyle habits and genetic factors on serum vitamin D levels.

Keywords: Lifestyle, menopause, polymorphism, vitamin D.

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Introduction

For women who are in menopause, vitamin D deficiency is more common than the others due to reasons such as decreased amount of 7-Dehydrocholesterol in the skin, decreased renal 1-α hydroxylase activity, increased body fat mass and decreased bioavailability of vitamin D, which is a fat-soluble vitamin.[1] Vitamin D deficiency generally causes muscle weakness and muscle aches. Also, it is associated with the formation of some cancers, weak immune modulation, development of cardiovascular diseases, and impaired sexual function.[1,2]

Studies have determined that genetic variations are related to approximately 65% of vitamin D levels.[3] Many new single nucleotide polymorphisms (SNPs) have been identified for the vitamin D receptor (VDR) gene located on chromosome 12q12-14.[4] It is known that genetic variations in VDR occur in the specific regions for BsmI (rs1544410), ApaI (rs7975232), TaqI (rs731236) and FokI (rs2228570).[5] Vitamin D is an important hormone that provides calcium balance and bone mineralization in the body and acts by binding to the VDR.[6] Although VDR is mostly expressed in small intestine and osteoblasts, it is found in many human tissues.[7] FokI, BsmI, ApaI, and TaqI are the most common VDR gene polymorphisms associated with various systems such as calcium metabolism, cell proliferation and the immune system. The relation between these polymorphisms and several diseases has been reported.[5,8,9]

The basis of vitamin D production is the synthesis in the skin during exposure to sunlight.[10] Vitamin D that is taken from food sources is very limited. Therefore, it is beneficial to take it in the form of supplements.[10,11] Even though it is shown in studies that vitamin D levels can be affected by the lifestyles of people, such as exercising, smoking, and receiving multivitamins, there is not enough information in the literature about lifestyle habits that affect vitamin D levels.[12]

In this study, we aimed to investigate the effects of FokI, BsmI, ApaI, and TaqI VDR gene polymorphisms and lifestyle habits on vitamin D levels of menopausal women.

Materials and Methods

The design of the study was cross-sectional. Menopausal women between the ages of 30-70 are included in the study. The presence of menopause was determined in women who had at least one year without menstrual bleeding and had a physician-diagnosed or total abdominal hysterectomy and bilateral salpingo-oophorectomy or a history of bilateral salpingo-oophorectomy. Patients who had cancer and received vitamin D treatment within the past three months were excluded from the study. Firstly, Patients were informed about the study and informed consent was obtained. In order to collect data in the study, a data form that questioned the

sociodemographic characteristics and lifestyle habits was used, and blood samples were obtained from the patients for vitamin D levels and genetic results.

Data Collection Form

Education level (Primary school and lower, Middle school and high school or University and upper), body mass index (average (≤24.99 kg/m2), overweight (25-29.99 kg/m2) and obese (≥ 30 kg/m2)) divided into three groups; marital status (single/married) and doing sportive activity regularly (no/yes) divided into two groups. The data collection form questioned lifestyle and eating habits that may affect vitamin D levels. Information on wearing closed clothing (wearing clothing that continually covers the whole body), regular nutrition (3 meals a day at close hours), using a vitamin D preparation voluntarily beside as treatment, and using Omega-3 supplements voluntarily was coded as no/yes. Age and total sleep time in one day were coded as continuous variables. Consumption amounts (average number of days consumed in a month) of foods rich in vitamin D (milk, cheese, chicken, eggs, butter, clotted cream, cabbage, spinach, corn) were also questioned and recorded as continuous variables.

Vitamin D measurement

25-Hydroxy Vitamin (D25(OH)D3) was used to determine the vitamin D level because it had a long half-life. The active metabolite, 1.25-dihydroxy vitamin D3 (1.25(OH)2D3) levels decline only in severe deficiency and may not reflect levels in target tissues where it is generated. Accordingly, it was not used to determine the level of vitamin D. Less than 20 ng/mL D25(OH)D3 was defined as a deficiency, 20-30 ng/mL D25(OH)D3 was defined as insufficiency and higher than 30 ng/mL D25(OH)D3 was defined as sufficient. Vitamin D level was measured using the chemiluminescence microparticle immunoassay (CMIA) method in the Advia Centaur XP (Siemens, Germany) device.

Genetic study design

DNA isolation from peripheral blood was performed using the Genomic DNA Mini Kit (Invitrogen, USA). PCR amplified the obtained DNA samples. Post-PCR amplification products were run on a 1% agarose gel and visualized on a UV transilluminator. BsmI (rs1544410), TaqI (rs731236), FokI (rs2228570) and ApaI (rs7975232) VDR gene polymorphisms were detected by PCR-RFLP method. The primers required to detect BsmI polymorphism were determined based on the study of Györffy et al., TaqI, and ApaI polymorphisms were determined based on the study of Yavuz et al., and the primers required to detect FokI polymorphism were determined based on Bell et al.'s study.[13,14,15]

PCR products were discontinued according to the recommendation of the firm (Fermantas, USA), which provides restriction enzymes. The incised DNA fragments were applied electrophoresis in a 2% agarose gel and were examined under UV light. To determine the BsmI polymorphism, bands were obtained at 191 bp (base pair) for the BB genotype, 191, 115, 76 bp for the Bb genotype, and 115, 76 bp for the bb genotype. While determining TaqI polymorphism, bands were obtained at 495, 245 bp for the TT genotype, 495, 290, 245, 205 for the Tt genotype and 290, 245, 205 bp for the tt genotype. Bands were obtained at 265 bp for the FF genotype, 196, 198 bp for the Ff genotype, and 69 bp for the ff genotype to determine the FokI polymorphism. Bands in 740 bp for the AA genotype, 740,530, 210 bp for the Aa genotype and 530, 210 bp for the aa genotype were obtained to determine ApaI polymorphism. The primers required for the amplification of the VDR gene and the lengths of the amplification products are shown in Table 1.

Table 1. Primers required for amplification of VDR gene and lengths of amplification products

Name	Primer sequences	Amplification product
BsmI	5-agt gtg cag gcg att cgt ag-3	191 bp*
	5-ata ggc aga acc atc tct cag-3	
ApaI& TaqI	5-cag agc atg gac agg gag caa-3	740 bp
	5-gca act cct cat ggc tga ggt ctc-3	
FokI	5- gat gcc agc tgg ccc tgg cac tg-3	273 bp
	5- atg gaa aca cct tgc ttc ttc tcc ctc-3	

VDR: vitamin D receptor; *Bp: base pair

Statistical analysis

Statistical analysis was performed using SPSS version 16. In this study, the distribution of the data was tested with Kolmogorov-Smirnov. The statistical comparison of the mean values of two independent groups was performed using the Mann-Whitney U test. The between-group comparisons of categorical variables were performed using the Chi-square test. Independent effects of vitamin D on the different identifying factors were examined with logistic regression models. The Hosmer-Lemeshow test was used for model fit. Independent variables with a statistically significant relationship of $p \leq 0.250$ according to bivariate analysis were included in the multivariate logistic regression model with a "Backward" elimination method. A p-value lower than 0.05 was considered statistically significant.

Results

The study was carried out on 303 menopausal women. The median age (25-75p) of women participating in the study was 53 (50-57), and the frequency of vitamin D deficiency in patients was 71.95% (n = 218). In univariate analyses, those with healthy BMI and those using vitamin D and Omega-3 supplements had a higher frequency of healthy vitamin D levels (p = 0.012, p <0.001, p = 0.007, respectively) (Table 2).

Table 2. The relationship between vitamin D levels and sociodemographic characteristics

	Vitamin D Level			Statistical analysis
Sociodemographic characteristics	**Deficiency n (%) (Group I)**	**Insufficiency n (%) (Group II)**	**Normal n (%) (Group III)**	**p-value**
Body Mass Index (BMI)				
Normal (18.5-24.5)	38 (57.58)	13 (19.70)	15 (22.73)	0.012*
Overweight (25-29,9)	82 (70.69)	17 (14.66)	17 (14.66)	
Obese (30-40)	98 (80.99)	14 (11.57)	9 (7.44)	
Educational status				
Primary school and lower	149 (76.80)	23 (11.86)	22 (11.34)	0.064
Middle school and high school	47 (61.04)	14 (18.18)	16 (20.78)	
University and upper	22 (68.75)	7 (21.88)	3 (9.38)	
Regular diet				
No	62 (69.66)	13 (14.61)	14 (15.73)	0.762
Yes	156 (72.90)	31 (14.49)	27 (12.62)	
marital status				
Single	13 (56.52)	6 (26.09)	4 (17.39)	0.184
Married	205 (73.21)	38 (13.57)	37 (13.21)	
Regular sports				
No	169 (74.78)	28 (12.39)	29 (12.83)	0.129
Yes	49 (63.64)	16 (20.78)	12 (15.58)	
Take vitamin supplements				
No	202 (75.94)	39 (14.66)	25 (9.40)	<0.001*
Yes	16 (43.24)	5 (13.51)	16 (43.24)	
Take fish oil				
No	213 (72.95)	43 (14.73)	36 (12.33)	0.007*
Yes	5 (45.45)	1 (9.09)	5 (45.45)	
Wearing closed clothes				
No	100 (68.97)	24 (16.55)	21 (14.48)	0.517
Yes	118 (74.68)	20 (12.66)	20 (12.66)	
Continuous Variables	**Median (min-max)**	**Median (min-max)**	**Median (min-max)**	
Age	53 (40-71)	53.5 (43-69)	52 (40-67)	0.059
Sleep time	7 (1-10)	7 (3-10)	7 (4-10)	0.110
Milk consumption (days per month)	10 (0-30)	10 (0-30)	10 (1-30)	0.572
Cheese consumption (days a month)	30 (0-30)	30 (0-30)	30 (2-30)	0.374
Fish consumption (days a month)	4 (0-30)	4 (0-20)	4 (0-15)	0.807
Chicken consumption (days a month)	4 (0-15)	4 (0-8)	4 (0-15)	0.302
Egg consumption (days a month)	30 (0-30)	22.5 (0-30)	30 (0-30)	0.240
Butter consumption (days a month)	8 (0-30)	10 (0-30)	15 (0-30)	0.423
Clotted cream consumption (day in a month)	0 (0-30)	0 (0-30)	0 (0-4)	0.108
Corn oil consumption (day in a month)	0 (0-30)	0 (0-4)	0 (0-20)	0.102

*p value < 0.05 (row percentages have been showed.)

Considering the relationship between VDR gene polymorphisms and vitamin D levels, women with ApaI aa genotype were found to have a lower frequency of normal vitamin D levels (p = 0.029). In univariate analyses, no statistically significant difference was found between other VDR gene polymorphisms and vitamin D levels (p> 0.05) (Table 3).

Table 3. The relationship between vitamin D level and VDR gene polymorphism

	Vitamin D Level			Statistical analysis
VDR gene polymorphism	**Deficiency n (%) (Group I)**	**Insufficiency n (%) (Group II)**	**Normal n (%) (Group III)**	**p value**
Bsml				
BB	33 (78.57)	6 (14.29)	3 (7.14)	0.602
Bb	119 (73.01)	22 (13.50)	22 (13.50)	
bb	66 (67.35)	16 (16.33)	16 (16.33)	
B allele	185 (74.90)	34 (13.77)	28 (11.34)	0.159
b allele	251 (69.92)	54 (15.04)	54 (15.04)	
Taq				
TT	89 (67.94)	23 (17.56)	19 (14.50)	0.346
Tt	101 (77.10)	13 (9.92)	17 (12.98)	
tt	28 (68.29)	8 (19.51)	5 (12.20)	
T allele	279 (70.99)	59 (15.01)	55 (13.99)	0.471
t allele	157 (73.71)	29 (13.62)	27 (12.68)	
Fok1				
FF	138 (73.40)	28 (14.89)	22 (11.70)	0.657
Ff	72 (69.90)	15 (14.56)	16 (15.53)	
ff	8 (66.67)	1 (8.33)	3 (25)	
F allele	348 (72.65)	71 (14.82)	60 (12.53)	0.286
f allele	88 (69.29)	17 (13.39)	22 (17.32)	
Apa				
AA	80 (72.07)	13 (11.71)	18 (16.22)	0.029*
Aa	96 (70.59)	18 (13.24)	22 (16.18)	
aa	42 (75)	13 (23.21)	1 (1.79)	
A allele	256 (71.51)	44 (12.29)	58 (16.20)	0.180
a allele	180 (72.58)	44 (17.74)	24 (9.68)	

VDR: vitamin D receptor; *p value < 0.05

According to the logistic regression analysis based on vitamin D levels, receiving vitamin D and omega-3 supplements and prolonged sleep duration were protective factors for vitamin D deficiency (respectively; p <0.001, p = 0.020, p = 0.014). Of the VDR gene polymorphisms, the BsmI bb genotype was found to protect from vitamin D insufficiency, while the ApaI aa genotype increased the risk of vitamin D insufficiency (respectively; p = 0.013, p = 0.015) (Table 4).

Table 4. Logistic Regression Analysis on Normal Vitamin D Level

Determining factors	Odds ratio (Univariate)			Odds ratio (Multivariate)		
	β	%95 CI	p-value	β	%95 CI	p-value
Age	0.938	0.884-0.995	0.033	0.962	0.896-1.033	0.289
Education Status						
middle	2.051	1.011-4.159	0.047	0.832	0.303-2.288	0.722
high	0.809	0.227-2.877	0.743	0.267	0.048-1.480	0.131
Be married	0.723	0.233-2.244	0.575	0.495	0.102-2.396	0.382
Body mass index						
25-29,9	0.584	0.270-1.264	0.172	0.825	0.309-2.206	0.702
30-40	0.273	0.112-0.665	0.004	0.327	0.104-1.031	0.056
Regular sports	1.254	0.605-2.599	0.543	0.674	0.257-1.765	0.421
Take vitamin supplements	7.345	3.401-15.860	0<0.001	8.564	3.388-21.646	<0.001*
Take fish oil	5.926	1.720-20.417	0.005	5.947	1.330-26.599	0.020*
Sleep time	1.171	0.951-1.441	0.136	1.406	1.071-1.845	0.014*
Clotted cream consumption	0.878	0.712-1.082	0.222	0.787	0.568-1.092	0.151
Egg consumption	1.025	0.992-1.059	0.141	1.016	0.974-1.058	0.467
Corn oil consumption	0.969	0.860-1.091	0.599	0.959	0.837-1.097	0.540
Bsml						
Bb	2.028	0.577-7.132	0.270	3.301	0.684-15.931	0.137
bb	2.537	0.698-9.221	0.158	8.376	1.560-44.969	0.013*
Taq						
Tt	0.879	0.435-1.778	0.720	0.953	0.331-2.746	0.929
tt	0.819	0.285-2.350	0.710	1.587	0.303-8.314	0.585
Fok1						
Ff	1.388	0.693-2.778	0.355	1.221	0.519-2.972	0.648
ff	2.515	0.633-10.000	0.190	1.991	0.308-12.884	0.470
Apa						
Aa	0.997	0.505-1.969	0.993	0.873	0.377-2.018	0.750
aa	0.094	0.012-0.723	0.023	0.067	0.007-0.597	0.015*

β = regression coefficient, *p value < 0.05

Discussion

The study aimed to investigate the effects of lifestyle habits and VDR gene polymorphisms on vitamin D levels in menopausal women. At the end of the study, we found that receiving vitamin D supplements, Omega-3 supplements, and increased sleep duration prevented the risk of vitamin D deficiency. We concluded that the BsmI bb genotype, one of the VDR gene polymorphisms, protects from vitamin D deficiency, while the ApaI aa genotype increases the risk of vitamin D deficiency.

Our study found that taking vitamin D and Omega-3 supplements are protective factors against Vitamin D deficiency. The systemic review and meta-analysis by Alhabeeb et al. stated a significant increase in serum vitamin D levels, especially after eight weeks of omega-3 supplementation usage.[16] A randomized controlled trial by Laing et al. concluded that omega-3 and vitamin D supplements increased serum vitamin D levels.[17] Vitamin D is first hydroxylated to 25-hydroxyvitamin D and then transformed into the active form 1,25-Dihydroxy Vitamin D with the help/activation of the renal 1a-Hydroxylase enzyme. It is thought that Omega-3 fatty acid increases serum vitamin D levels by increasing external 1α-hydroxylase activity or by suppressing the 24-hydroxylase enzyme that catabolizes 1,25-Dihydroxy Vitamin D.[18] It is known that vitamin D has effects on various systems such as the cardiovascular system, hematopoietic system, and urogenital system besides its effects on bone mineral and calcium metabolism.[2,3] Therefore, serum vitamin D levels might be more critical than expected. Patients could be advised to take vitamin D and Omega-3 supplements to increase vitamin D levels.

In our study, we found that every 1-hour additional time to patients' sleep duration is protective against the risk of vitamin D deficiency. Liu et al. also concluded that sufficient sleep duration increases the levels of vitamin D.[19] Studies have also reported a positive relationship between increased sleep quality and high serum vitamin D levels.[20] The literature data are insufficient to explain sleep and serum vitamin D levels. The positive effect of vitamin D on melatonin, known as the sleep hormone, or the presence of VDR in the hypothalamus region where sleep is regulated, may be responsible for the relationship between sleep duration and quality and serum vitamin D level. More studies are needed to explain the direction and mechanism of action between vitamin D level and sleep duration and quality.[20,21] More studies are needed to explain the direction and mechanism of action between vitamin D level and sleep duration and quality.

As a result of our study found that ApaI aa genotype increases the risk of vitamin D deficiency, and the BsmI bb genotype is protective against vitamin D deficiency. When we look at the literature, it was concluded that the BsmI bb genotype protects against vitamin D deficiency in the studies of Divanoglou et al., similar to our study.[22] Likewise, the studies of Sinharay et al. in India also support the conclusion that the BsmI bb genotype protects against vitamin D deficiency.[23] Although they found lower vitamin D levels in women with bb genotype in their

study by Ahmad et al., They did not find a statistically significant difference between BB and Bb genotypes.[24] Also, we found that FokI and TaqI gene polymorphisms did not affect serum vitamin D levels. However, Ma et al. have found that vitamin D levels may change depending on FokI and TaqI VDR polymorphisms.[25]

When we look at the studies conducted in Turkey, there was no significant difference between BsmI, ApaI, TaqI and FokI polymorphisms and serum vitamin D levels in the studies of Albas et al. and Korucu et al.[26,27] In the study of Elkama et al., serum vitamin D level was found to be higher in patients with FokI ff, BsmI Bb, TaqI Tt and ApaI AA genotypes.[28] Our study concluded that people with the Apa AA genotype had higher serum vitamin D than those with the aa genotype. These differences between genetic polymorphisms and vitamin D levels may exist due to ethnic and geographic reasons, lifestyle differences, or laboratory differences between studies. Based on the current literature data, it is impossible to explain the relationship between VDR gene polymorphisms and vitamin D levels.

One of the study's limitations may be due to/caused by the patients selected only from a tertiary healthcare institution. Similar studies need to be done in other health institutions. Another limitation of the study is that the patient's responses to the questions about their lifestyle habits are based on self-report and not standardizing the vitamin D and Omega 3 supplements they use.

In conclusion, vitamin D and Omega 3 supplements can protect patients from vitamin D deficiency. It could be recommended for all patients to use supplement preparations in a certain way by measuring vitamin D levels periodically to protect patients from hypervitaminosis. It should be kept in mind that vitamin D levels may be low in people who do not have enough sleep duration. Our study found that the ApaI aa genotype increases the risk of vitamin D deficiency, while the BsmI bb genotype was protective against vitamin D deficiency. Considering that vitamin D deficiency may cause serious effects, therefore more studies are essential on this subject.

Ethical Considerations: The ethical approval of this study was received with decision no.295 (date 20.12.2017) from the Izmir Katip Çelebi University Faculty of Medicine Ethics Committee.

Conflict of Interest: Izmir Katip Celebi University Scientific Research Projects Unit covered the financial fees required for the study. The authors declare no conflict of interest.

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