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Özgün Araştırma / Original Article

Analysis of vitamin D Receptor Polymorphisms in Turkish Patients with Obstructive Sleep Apnea Syndrome

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Abstract

Objective: Numerous studies investigating Vitamin D receptor (VDR) polymorphisms in different populations have been present in current literature. For this reason, we designed a study to investigate the role of three known common VDR genetic polymorphisms (Apa-I/rs7975232, Bsm-I/ rs1544410 and Taq-I/rs731236) in Turkish individuals affected by Obstructive sleep apnea syndrome (OSAS).

Methods: The study was carried out on a total of 175 consecutive subjects, including 80 OSAS patients and 95 healthy participants. Single Nucleotide Polymorphism (SNP) Detection was performed with the iPLEX® Assay and the MassARRAY® System for detection of Apa-I (rs7975232), Bsm-I (rs1544410), and Taq-I (rs731236) polymorphisms.

Results: Fifty-seven C alleles (71.3%) and 59 T alleles (73.8%) were detected in the group of 80 OSAS patients in terms of rs1544410 polymorphism. When the patient and control groups were evaluated statistically at allelic level, it was observed that the T allele increased the risk of disease and this increase was statistically significant (Odds Ratio (OR) = 1.549 [Confidence Interval (CI): 1.012-2.371], (p=0.043). However, there were no significant differences between other VDR polymorphisms (rs7975232 and rs731236) and OSAS clinical data (p=0.6 and p=0.9, respectively).

Discussion: As a conclusion, no statistically significant relationship was found between all three VDR polymorphisms and OSAS patients' clinical features. Further studies should be performed by creating a large sampling group. Finally, population studies should be given importance considering the variability of polymorphism according to ethnic origin.

Keywords: Obstructive sleep apnea syndrome, Apnea-hypopnea index, vitamin D, VDR polymorphisms

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Obstrüktif Uyku Apne Sendromuna Sahip Türk Hastalarda Vitamin D Reseptör Polimorfizmlerinin İncelenmesi

Öz

Giriş: Mevcut literatürde, farklı popülasyonlarda Vitamin D reseptörü (VDR) polimorfizmlerinin analiz edildiği birçok çalışma bulunmaktadır. Bu sebepten, Obstrüktif uyku apne sendromu (OUAS)'tan muzdarip Türk bireylerde en çok bilinen 3 VDR genetik polimorfizmin (Apa-I/rs7975232, Bsm-I/ rs1544410 ve Taq-I/rs731236) araştırılmasına yönelik bir çalışma dizayn ettik.

Yöntemler: Bu çalışmada, 80 OUAS hastası ve 95 sağlıklı gönüllü olmak üzere toplam 175 katılımcı yer almıştır. Apa-I (rs7975232), Bsm-I (rs1544410), ve Taq-I (rs731236) gen bölgelerine ait Tek nükleotid polimorfizmleri (SNP), iPLEX® Assay ve the MassARRAY® System ile gerçekleştirilmiştir.

Bulgular: Çalışma sonucunda, 80 OSAS hastasından oluşan grupta rs1544410 polimorfizmi açısından, 57 adet C alleli (%71,3) ve 59 adet T alleli (%73,8) olduğu tespit edildi. Hasta ve kontrol grubu allellik seviyede istatistiksel olarak değerlendirildiğinde T allelinin hastalık riskini artırdığı ve bu artışın istatistiksel olarak anlamlı olduğu gözlenmiştir (OR=1.549 [CI:1.012-2.371], p=0.043). Ancak, diğer 2 VDR polimorfizmleri (rs7975232 ve rs731236) ile OSAS klinik verileri karşılaştırıldığında, istatistiksel olarak anlamlı bir farklılık tespit edilmemiştir.

Tartışma: Sonuç olarak tüm klinik veriler ve VDR polimorfizmleri istatistiksel olarak değerlendirildiğinde; OUAS hasta grubunun VDR polimorfizmleri ile klinik verileri arasında istatistiksel olarak anlamlı bir ilişki bulunamadı. Gelecek çalışmalarda, hasta grubu oluşturulurken daha büyük örneklem alınmalı ve son olarak etnik kökene göre polimorfizm değişkenliği göz önünde bulundurularak populasyon çalışmalarına önem verilmelidir.

Anahtar kelimeler: Obstrüktif uyku apne sendromu, apne-hipopne indeksi, D vitamin, VDR poliformizmi.

INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is the most common sleep disorder all around the world and it is defined by repeating episodes of upper airway obstruction (apnea/no airflow and hypopnea/partially obstructed airflow) related with oxygen desaturation, sleep fragmentation, snoring and excessive daytime sleepiness[1,2]. The pathogenesis of OSAS is still unclear. However, it is probably a multifactorial disorder, including the mechanisms of oxidative stress, lipid metabolism and inflammation[3,4]. Additionally, several risk factors are known for OSAS, such as obesity and diabetes as well as age, male gender, ethnicity, smoking, alcohol consumption, and medical comorbidity[5].

OSAS, a clinical disease, probably results from multiple interacting genetic and environmental factors, and it has been involved in several complex pathogenic pathways, including obesity, craniofacial morphology, muscle and connective tissue factors, and control of ventilation[6]. Obesity is the most known characteristic of OSAS in adults and is most commonly diagnosed with an increased Body Mass Index (BMI). On the other hand, Apnoea-Hypopnoea Index (AHI) is simply a count of the number of apneas and hypopneas per hour of sleep. Increasing AHI values indicate increasing disease severity in OSAS. At that point, the relationship between BMI and AHI is uncertain[7,8]. By the way, the relationship between OSAS and serum concentrations of vitamin D has been the subject of numerous scientific studies[4,9]. On the other hand, oxygen saturation (SpO2) is another important factor for OSAS patients. When the OSAS is aggravated, an important drop oxygen saturation is seen. This may result with an abnormal breathing in patients[4,6].

Vitamin D is a steroid pro-hormone that is mainly taken with dietary or synthesized by sunlight. Additionally, VD is converted to its

enzymatically active form (1,25 (OH)2D3') in kidney and liver. This form of VD specifically binds to its specific nuclear receptor (VDR) and the Retinoid X receptor which makes heterodimers with the VDR. Then, it regulates the expression of the related gene by binding to the VitD3 sensitive regions in the promoters of different genes. Thus, VD has a crucial role in a wide variety of human physiology, including calcium homeostasis, bone formation, cellular growth, proliferation, apoptosis and immune homeostasis[10-12].

In humans, the VDR gene (over 100 kb) is located at chromosome 12q13.11 and contains 12 exons (www.ncbi.nlm.nih.gov/gene, Gene ID; 7421 update 23 Dec. 2018). It is expressed in many tissues, especially small intestine, duodenum and colon. Additionally, VDRs have been identified in several brain areas, including the hypothalamus which regulates the changes in the sleep–wake cycle and its polymorphisms are associated with a variety of neuropsychiatric disorders[10]. In recent years, several single nucleotide polymorphisms (SNPs) of the VDR gene which vary markedly in different ethnic groups have been reported to be associated with susceptibility to many diseases including OSAS. Some authors hypothesized that the SNP at Apa-I (rs7975232), Bsm-I (rs1544410) and Taq-I (rs731236), located in the region of intron 8/exon 9 of the vitamin D receptor gene, are effective in mRNA stability[11,12]. Recent studies have been shown that vitamin D may also contribute to the development of OSAS through a mechanism which vitamin D receptors (VDR) are involved[2].

Numerous studies investigating VDR polymorphisms in different populations have been present in current literature. For this reason, we designed a study to investigate the role of three known common VDR genetic polymorphisms (Apa-I/rs7975232, Bsm-I/ rs1544410 and Taq-I/rs731236) in Turkish individuals affected by OSAS.

METHODS

Subjects and Ethical Approval

The study was carried out on a total of 175 consecutive subjects, including 80 OSAS patients who were diagnosed at Neurology polyclinic and sleep laboratory of Kafkas University Research Hospital and 95 healthy participants. The patient and control groups were determined according to the clinical findings such as snoring, witnessed apnea and daytime sleepiness with Epworth insomnia scale[13].

Polysomnographic recordings of OSAS-diagnosed patients were performed with the Embla N7000 system (Medcare-Embla, Reykjavik, Iceland). Related parameters such as electroencephalography, electrocardiography, electrooculography, submental and anterior tibialis muscle electromyography, nasal pressure, oronasal airflow with thermal sensor, snoring, oxygen saturation with finger oximetry and thoracic and abdominal inductance pethismography were recorded, and sleep disorder respiratory events were scored manually by the clinician according to AASM (American Academy of Sleep Medicine) criteria[14]. Height, weight, and body mass index (BMI) [weight (kg)/height2 (m)] were measured using a standardized protocol. In addition, information about CAD (Coronary Artery Disease), hypertension and diabetes of OSAS patients was recorded.

The ethical approval was obtained from Kafkas University, Faculty of Medicine, and Clinical Research Ethics Committee (Approval date and number: 10.07.2015/06). The study was carried out in accordance with the Helsinki Declaration of Human Rights, as revised in 2013 and patients gave their informed consent.

Blood Sample Preparation

At first, the blood samples were taken to 2 ml EDTA tubes from the subjects, and stored at -200C until DNA isolation was performed. In the

DNA extraction, Taigen Lab Turbo-24 Nucleic Acid isolation device (Taigen-TAIWAN) and Lab Turbo mini DNA isolation kit (Catalog No: LGD 480-220) were used with Membrane-column method. The purity and concentration of DNA samples were checked using a Nano-Drop 8000 spectrophotometer (Thermo Fisher Scientific Inc, Wilmington, U.S.A.). Genomic DNA samples were diluted with elution solution to obtain a final concentration (5 ng/µL). DNA samples were stored at -800C until SNP genotyping was performed.

Single Nucleotide Polymorphism Detection

Single Nucleotide Polymorphism Detection was performed with the iPLEX® Assay and the MassARRAY® System. VDR single-nucleotide polymorphism (SNP) genotyping was performed using the MassARRAY system (Agena Bioscience, San Diego, California) using the matrix-assisted desorption/ionization time of flight mass spectrometry (MALDI-ToF-MS, Sequenom, San Diego, USA) method according to the manufacturer's instructions. For the detection of Apa-I (rs7975232), Bsm-I (rs1544410), and Taq-I (rs731236) polymorphisms, primer pairs specific to the target regions in the genomic DNA and elongation probes for the iPLEX Gold reaction (single base elongation reaction) were designed using Agena Bioscience's Assay Designer software. Primers for VDR gene polymorphisms are listed in Table 1.

Table I: The list of PCR and iPLEX Extension Primers

SNP ID	2nd-PCRP	1st-PCRP	AMP_LEN	UEP_SEQ
rs1544410	ACGTTGGATGAGTGTGCAGGCGATTCGTAG	ACGTTGGATGAGAGCAGAGCCTGAGTATTG	122	cccaaTGGGGCCACAGACAGGCCTGC
rs731236	ACGTTGGATGTGCCTTCTTCTCTATCCCCG	ACGTTGGATGTGCAGTGTGTTGGACAGGC	138	gGGACGCCGCGCTGAT
rs7975232	ACGTTGGATGTCACCGGTCAGCAGTCATAG	ACGTTGGATGAGAAGGCACAGGAGCTCTCA	116	GGATTGAGCAGTGAGG

The target region in the DNA was amplified by multiplex Polymerase Chain Reaction (PCR) using the specially designed primer pairs. The thermal cycler conditions for amplification PCR is; 95°C for 2 min (initial), 95°C for 30 sec, 56°C for 30 sec 72°C for 1 m (45 cycles), and 72°C for 3 min (final).

The resulting amplicons were subjected to the Shrimp Alkaline Phosphatase (SAP) reaction, and then the probes constructed with the IPLEX Gold reaction were hybridized with the target site and the single mass-modified nucleotide elongation was performed. The PCR conditions of SAP step was as following; 100°C for 30 sec, 37°C for 40 min, and 85° C for 5 min (1 cycle).

The iPLEX reaction products were treated with a cationic exchange resin to remove ions such as Na+, K+, Mg+, and to minimize the background contamination. The products obtained after this step were transferred to the matrix chip by nanodispenser and simultaneous detection was carried out after ionization by MassARRAY® mass spectrometry. MassARRAY® TYPER 4.0 genotyping software was used to obtain spectroscopic images and allele-specific peaks and analyze the data obtained after laser pulse (iPLEX SpectroCHIP® II analysis).

Statistical Analysis

The demographic information of both groups and genotyping results obtained by MassARRAY genotyping were analyzed the Statistical Package for the Social Sciences (SPSS, version 15.0, IBM, Armonk, New York 10504, NY, USA). Genotypes and alleles were

expressed as “numbers” and “percentages”. For each SNP, allele and genotype frequencies (Pearson χ2 statistics), O.R., and p values, as well as dominant and recessive genetic models, were analyzed using FINETTI program provided as an online source (http://ihg.gsf.de/cgi-bin/hw/hwa1.p). Hardy-Weinberg equilibrium of tested groups and Armitage’s trend test (ATT) were also calculated using FINETTI. ATT takes into account genotypes rather than alleles, avoiding a possible bias due to doubling of sample size[15]. It assumes additive (or codominant) disease model where all disease allele are independent and have the same contribution to the disease risk. In the patient group; genotype/continuous data (as mean and STD) and genotype/categorical data analysis were performed. All statistical tests were conducted at a significance level of 0.05.

RESULTS

Study Groups

In this study, we enrolled 175 subjects (80 OSAS patients and 95 healthy volunteers) with a mean age of 53.81±12.1 and 47.26±13.41 years, respectively (p = 0.21). Of the 80 OSAS patients, 28 (35%) were female and 52 (65%) were male while 42 (44.2%) were female and 53 (55.8%) were male of 95 healthy volunteers (p = 0.21). Demographic variables of two groups were showed in Table 2.

Table II: Demographic variables of OSAS and healthy groups

Groups	**Patients (n=80)**	**Controls (n=80)**	**P value**
Gender			
Male	52 (%65)	53 (%56.25)	0.21
Female	28 (%35)	42 (%43.75)	
Age (years) (Mean±SD)	53.81 ± 12.1	47.26 ± 13.41	0.21

Distribution of VDR polymorphism in groups:

The distribution of genotypic and allelic frequencies of the VDR gene polymorphisms, the analysis of VDR polymorphisms based on dominant and recessive genetic models, Hardy-Weinberg equilibrium and Armitage's trend test (ATT) results for each SNP (rs1544410, rs7975232, rs731236) were given in Table 3, Table 4 and Table 5, respectively.

As a result of the study, 57 C alleles (71.3%) and 59 T alleles (73.8%) were detected in the group of 80 OSAS patients in terms of rs1544410 polymorphism. In the control group, 81 C allele (86.2%) and 63 T allele (67%) were detected. When the patient and control groups were evaluated statistically at allelic level, it was observed that the T allele increased the risk of disease and this increase was statistically significant (OR = 1.549 [CI: 1.012-2.371], p = 0.043).

In genotypically examination, rs1544410 polymorphism was observed in 73% of the patients and 63% in the control group. When the genotype frequencies of rs1544410 polymorphism were statistically evaluated, the odds ratio values for Recessive (CC+CT vs TT), Homozygous (CC vs TT) and Dominant (CC vs CT+TT) genetic models were calculated as 1.382 (CI: 0.716-2.669), 2.612 (CI: 1.086-6.279), and 0.398 (CI: 0.186-0.85), respectively. Positive correlations were detected in homozygous (p=0.03) and dominant (p=0.015) genetic models. According to the Armitage’s trend test, the common odd ratio was found as OR= 1.585 and p = 0.043 according to the T risk allele. According to the obtained statistical data, it was found that the T variant was associated with the risk of disease at allelic and genotypic level.

As a result of the study, 62 A alleles (77.6%) and 49 G alleles (61.3%) were detected in the group of 80 OSAS patients in terms of rs731236 polymorphism. In the control group, 84 A allele (88.4%) and 59 G allele (62.1%) were detected

Table III: Genotype and allele frequencies of VDR rs1544410 polymorphisms.

Polymorphism	Groups	Genotypes					HWE *(p value)*	MAF	Association analyses	Genetic model (OR [95% CI]/ P-value)				Armittage's trend test
										Allele	Genotype (frequency)			
		CC	CT	TT	Total C alleles	Total T alleles				C vs T	CC vs TT	CC vs (CT + TT)	(CC+ CT) vs TT	Common odds ratio and p value
rs1544410	Case (n=80)	21 (0.26)	36 (0.45)	23 (0.28)	57 (71.3%)	59 (73.8%)	0.37	0.49	Case vs Control	1.549 [1.012-2.371] ***p=0.043***	2.612 [1.086-6.279] **p=0.03**	1.382 [0.716-2.669] p=0.33	0.398 [0.186-0.850] ***p=0.015***	1.585 ***p=0.043***
	Control (n=95)	31 (0.33)	50 (0.53)	13 (0.13)	81 (86.2%)	63 (67%)	0.39	0.60						

Statistically significant values are displayed in bold letters (HWE Hardy-Weinberg equilibrium; MAF minor allele frequency; OR odds ratio; 95% confidence interval).

Table IV: Genotype and allele frequencies of VDR rs731236 polymorphisms.

Polymorphism	Groups	Genotypes					HWE *(p value)*	MAF	Association analyses	Genetic model (OR [95% CI]/ P-value)				Armittage's trend test
										Allele	Genotype (frequency)			
		AA	GA	GG	Total A alleles	Total G alleles				A vs G	AA vs GG	AA vs (GA + GG)	(AA+ GA) vs GG	Common odds ratio and p value
rs731236	Case (n=80)	31 (0.38)	31 (0.38)	18 (0.22)	62 (77.6%)	49 (61.3%)	0.07	0.58	Case vs Control	1.235 [0.803-1.9] p=0.3	1.9 [0.78—4.63] p=0.15	0.96 [0.523-1.778] p=0.9	0.45 [0.199-1.023] p=0.052	1.295 p=0.34
	Control (n=95)	36 (0.38)	48 (0.45)	11 (017)	84 (88.4%)	59 (62.1%)	0.5	0.63						

Statistically significant values are displayed in bold letters (HWE Hardy-Weinberg equilibrium; MAF minor allele frequency; OR odds ratio; 95% confidence interval).

Table V: Genotype and allele frequencies of VDR rs7975232 polymorphisms.

Polymorphism	Groups	Genotypes					HWE *(p value)*	MAF	Association analyses	Genetic model (OR [95% CI]/ P-value)				Armittage's trend test
										Allele	Genotype (frequency)			
		AA	CA	CC	Total A alleles	Total C alleles				A vs C	AA vs CC	AA vs (CA + CC)	(AA + CA) Vs CC	Common odds ratio and p value
rs7975232	Case (n=80)	60 (0.76)	7 (0.9)	12 (0.15)	67 (84%)	19 (24%)	**7.72e-09**	0.72	Case vs Control	0.875 [0.519—1.477] p=0.6	0.534 [0.275—1.03] p=0.06	0.38 [0.136—1.067] p=0.058	0.372 [0.125—1.112] p=0.0588	1.114 p=0.66
	Control (n=95)	59 (0.63)	29 (0.31)	6 (0.06)	88 (93%)	35 (37%)	1.00	0.37						

Statistically significant values are displayed in bold letters (HWE Hardy-Weinberg equilibrium; MAF minor allele frequency; OR odds ratio; 95% confidence interval).

When the patient and control groups were evaluated statistically at allelic level, the results were as following; G allele OR=1.235. [CI:0.803-1.9] (p = 0.3) and A allele OR= 0.81 [CI:0.526-1.246] (p = 0.9). As a result, both A and G alleles had no effect on the disease In genotypically examination, rs731236 polymorphism was observed in 61.3% of the patients and 62.1% in the control group. According to the Armitage's trend test, the common odd ratio was found as OR= 1.295 and p = 0.34 according to the G risk allele. According to the obtained statistical data,

no relation was found between A or G variant of rs731236 polymorphism at allelic or genotypic level.

As a result of the study, 67 A alleles (84%) and 19 C alleles (24%) were detected in the group of 80 OSAS patients in terms of rs7975232 polymorphism. In the control group, 88 A allele (93%) and 35 C allele (37%) were detected. When the patient and control groups were evaluated statistically at allelic level, the results were as following; C allele OR= 1.143, CI: [0.677-1.929] (p = 0.6) and A allele OR= 0.875, CI: [0.519-1.477] (p = 0.6).

In genotypically examination, rs7975232 polymorphism was observed in 24% of the patients and 37% in the control group. According to the Armitage's trend test, the common odd ratio was found as OR= 1.114 ve p= 0.66 according to the C risk allele. According to the obtained statistical data, no relation was found between A or C variant of rs7975232 polymorphism at allelic or genotypic level.

Association of VDR polymorphisms with clinical characteristics of OSAS patients:

The mean and standard deviation values obtained by Chi-square analysis of the BMI (kg/m2), minimum peripheral oxygen saturation (MinSpO2) and AHI values of OSAS patients (n= 80) were shown in Table 6 for each VDR SNP (rs1544410, rs7975232, rs731236) polymorphism.

Table VI: Clinical characteristics of OSAS patients-1

Clinical data	Mean±SD	rs1544410 (p value)	rs7975232 (p value)	rs731236 (p value)
BMI (kg/m²)	31.62 ± 5.65	0.2	0.23	0.18
MinSpO₂	72.19 ± 12.22	**0.06**	0.59	0.35
Apnea–hypopnea index	42.75 ± 31.78	0.39	0.49	0.39

The Chi-square analysis results of the information of Diabetes, CAD (Coronary Artery Disease), Hypertension of OSAS patients (n= 80) were shown in Table 7 for each VDR SNP (rs1544410, rs7975232, rs731236) polymorphism.

In conclusion, when all clinical data and VDR polymorphisms were statistically evaluated, no significant relationship was found between VDR polymorphisms and clinical data of OSAS patient group.

DISCUSSION

"VDR gene polymorphism, which has a genomic and non-genomic function, has an effect on the occurrence of OSAS" was the hypothesis of this study. On the other hand, we aimed to investigate the role of three known common VDR genetic polymorphisms (Apa-I/rs7975232, Bsm-I/ rs1544410 and Taq-I/rs731236) in Turkish individuals affected by OSAS.

OSAS is a common disorder, with prevalence estimated at 4% for men and 2% for women among adults in the Western countries[3]. We enrolled a total of 175 participants, including 80 OSAS patients (patient group) who had 53.81±12.1 mean age and 95 healthy participants (control group) who had 47.26±13.41 mean age and were determined regarding Epworth insomnia scale. Of the 80 OSAS patients, 28 (35%) were female and 52 (65%) were male while 42 (44.2%) were female and 53 (55.8%) were male of 95 healthy volunteers. In our study, there was no statistically significant difference between the patient and control groups in terms of gender and age.

A meta-analysis study performed by Cindy Lee et al included 14 scientific articles which were investigating the serum level of vitamin D in OSAS disease. The authors declared that there was a relative insufficiency in serum vitamin D levels among OSAS patients compared to control patients, which was incrementally exacerbated with increasing severity of sleep apnea[9]. In another meta-analysis study performed by Archontogeorgis et al., the authors reported that a limited number of studies have assessed the association between lower serum concentrations of vitamin D and

Table VII: Clinical characteristics of OSAS patients-2

Clinical data	rs1544410/ Genotype			p value	rs7975232/ Genotype			p value	rs731236/ Genotype			p value
	CC	CT	TT		AA	GA	GG		AA	CA	CC	
Diabetes NO	16	30	18		50	5	8		24	26	14	
YES	5	5	4	0.66	8	2	4	0.21	7	4	3	0.189
CAD NO	16	26	17		44	6	8		21	24	14	
YES	5	9	5	0.96	14	1	4	0.63	10	6	3	0.41
Hypertension NO	5	15	8		23	3	2		8	12	8	
YES	16	20	14	0.35	35	4	10	0.3	23	18	9	0.28

OSAS, and contradictory results have been produced by these studies. Low levels of serum vitamin D in OSAS patients was detected in three of 4 Turkish studies, and no association between OSAS and serum vitamin D levels in the remaining one study[4]. This study had also showed that the relationship between serum vitamin D levels and OSAS clinical parameters such as AHI, BMI and minimum oxygen saturation was varied in different populations. Two studies performed in Turkish population reported that there was no statistically significant relationship between serum vitamin D levels and BMI, AHI, MinSpO2 in both control and patient groups. However, one study detected that BMI was found to be slightly increased in patients with low level of serum vitamin D.

Considering the present meta-analysis study, it was accepted that there was a relationship between OSAS and serum vitamin D levels; so, serum vitamin D levels were not measured in this study. This is one of the limitations of our study.

The nuclear receptor VDR belongs to a unique transcription factor superfamily, whose members are directly activated by small

lipophilic compounds. Accordingly, the biologically most active vitamin D compound, 1,25 (OH) 2D3, is the specific high-affinity ligand of VDR. The most studied VDR polymorphisms in the literature (Bsm-I/rs1544410), Taq-I/rs731236 and Apa-I/rs7975232) in different groups of patients can be reached through the NCBI SNP consortium or Celera database. The VDR Bsm-I (rs1544410) and Apa-I (rs7975232) polymorphisms are located in intron 8 near the 3' end of the VDR gene, while Taq-I (rs731236) A/G polymorphism is located at codon 352 in exon 9 and leads to silent codon change for isoleucine. In particular, Bsm-I and Apa-I cause a change in mRNA stability, leading to a decrease in mRNA level. Ragia et al[2] detected that there was no significant relationship between OSAS and Taq-I and Apa-I polymorphisms. Similarly, in studies performed in Turkish population reported no association

between these VDR polymorphisms and the elevated risk of OSAS. Ragia et al[2] have also been reported that VDR Fok-I CC genotype may contribute to the formation of OSAS. Unlike their study results, we detected that Bsm-I (rs1544410) C/T polymorphism increased the risk of OSAS at allelic and genotypic levels.

The polymorphism in Fok-1 CC genotype causes decrease in serum vitamin D level in OSAS patient group according to the data obtained from the only study in the current literature which investigates the relationship between them. In addition, it was stated that the presence of this polymorphism had a significant relationship with BMI scores and minimum oxygen saturation levels[2]. In our study, all OSAS patients were in obese category according to World Health Organization BMI criteria and no significant relationship was found between BMIs and VDR polymorphisms. On the other hand, no significant relationship was detected between the VDR polymorphisms and AHI scores and minimum oxygen saturations of OSAS patients according to the 2008 AASM criteria.

In the literature, VDR polymorphism has been studied frequently in many different disease groups such as CAD, hypertension and diabetes. We took and recorded the information about these diseases of OSAS patients by orally. No correlation was detected between CAD and VDR polymorphisms according to the meta-analysis study performed by Alizadeh et al. in 2017[16]. Similarly, we could not detect a correlation between the risk of CAD and VDR polymorphisms in OSAS patients. Another meta-analysis study performed by Fei Yu et al. reported that VDR polymorphism in Taq-I allele was associated with Type-2 diabetes[17]. Unlike this result, we could not detect any relationship between VDR polymorphisms and Type-2 diabetes in our OSAS patients. A prospective study by Lu Wang et al in 2013 determined that a significant relationship was detected between VDR Bsm-1 and Fok-I polymorphisms and hypertension risk[18]. Finally, none of the VDR polymorphisms in our study were associated with hypertension in OSAS patients.

CONCLUSION

As a conclusion, T allele increased the risk of OSAS disease in Bsm-I (rs1544410) C/T polymorphism in VDR gene at both allelic and genotypic levels. However, no statistically significant relationship was found between all three VDR polymorphisms and OSAS patients' clinical features. Further studies should be performed by creating a large sampling group. Finally, population studies should be given importance considering the variability of polymorphism according to ethnic origin.

Limitation of The Study

There are several potential limitations in this study. First, Vitamin D serum levels should be measured and evaluated in OSAS patients. Second, the clinical features of control group should be obtained. Third, we need to investigate the detection of the VDR-Fok-I SNP in OSAS patient as well as Apa-I, Bsm-I and Taq-I polymorphisms.

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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