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Original Research

Different *VDR*, *VDBP* genotypes and vitamin D levels may effect obstructive sleep apnea syndrome

Deniz Kirac[1*], Ozge Yagcioglu Yassa[2], Hazal Gezmis[1], Saime Fusun Mayda Domac[2], Elif Cigdem Altunok[3], Ece Genc[4]

[1] Department of Medical Biology, Faculty of Medicine, Yeditepe University, Istanbul, Turkey
[2] Department of Neurology, Erenkoy Mental and Nervous Diseases Training and Reserach Hospital, Istanbul, Turkey
[3] Department of Biostatistics, Faculty of Medicine, Yeditepe University, Istanbul, Turkey
[4] Department of Medical Pharmacology, Faculty of Medicine, Yeditepe University, Istanbul, Turkey

Correspondence to: denizyat@hotmail.com

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Abstract: Obstructive sleep apnea syndrome (OSAS) is a highly prevalent disorder which results in markedly reduced (hypopnea) or absent (apnea) airflow at the nose/mouth. Since vitamin D deficiency has found in an association with some disorders it is thought to be related with OSAS progression. The aim of this study is to investigate the association between *VDR*, *VDBP* mutations, vitamin D level and some environmental risk factors with OSAS. Fifty individuals who were diagnosed as OSAS were selected as patients, 50 healthy volunteers without any disease were selected as controls. *FokI* (rs2228570) and *BsmI* (rs1544410) mutations in *VDR*; rs4588 and rs7041 mutations in *VDBP* were investigated with quantitative real-time polymerase chain reaction (qPCR). Other risk factors were also investigated. Results were evaluated statistically. Statistically significant differences were observed according to the baseline characteristics between the groups, When groups were compared with each other, CA genotype in rs4588, CC genotype in rs2228570 and AA genotype in rs1544410 mutations were found statistically significant in patients whereas TC genotype in rs2228570 and GA genotype in rs1544410 mutations were found statistically significant in controls. When the relation between risk factors and genotypes were investigated, statistically significant associations were detected for body mass index (BMI), waist circumference, Apnea–Hypopnea Index (AHI), excessive daytime sleepiness (EDS), vitamin D and triglyceride levels. *VDR* and *VDBP* mutations were found highly related with OSAS. Possible tracking of these mutations and risk factors may help to understand the metabolism as well as the progression of the disease.

Key words: OSAS; *VDR*; *VDBP*; Vitamin D; qPCR.

Introduction

Obstructive sleep apnea syndrome (OSAS) is a common disorder characterized by repeated episodes of upper airway obstruction and airflow cessation (apneas) that causes to arterial hypoxemia and sleep disruption during individual's sleep (1). Patient with OSAS shows some clinical features such as snoring, witnessed apnea, excessive daytime sleepiness as well as neuropsychiatric symptoms such as headache, memory impairments, difficulty in adaptation to environment, and depression. Growing evidences suggest that obesity, type II diabetes, metabolic syndromes and cardiovascular diseases are closely related to OSAS (1,2). Although the current knowledge about the history of the disease is still limited, the long-term results obtained are considered significant. Untreated OSAS increases the risk of car accidents, worsening quality of life and mood (3). Although the occurrence of OSAS is linked to anatomical and mechanical factors (such as recurrent episodes of pharyngeal collapse etc.), it is not yet clear which pathophysiological mechanisms are associated with OSAS. The obtained data suggest that OSAS is a complex disorder with multiple features, especially a hereditary component (4).

Vitamin D is a hormone that interacts with intranuclear receptors and performs its biological function by affecting transcriptional changes in many cell types including those in gut, bone, breast, prostate, brain, skeletal muscle, and the immune system (5,6). Studies showed that vitamin D has immunmodulatory properties which is linked to pulmonary disease, musculoskeletal pain, metabolic syndrome, hypertension, poor stress resilience, altered emotional functioning, and cognitive decline when there is not enough in the body (7). Especially after discovery of vitamin D receptors (VDR) which is main gene and its product is responsible from the transport of the active form of 1,25OH]D, the studies in this area have been increased.

25-Hydroxyvitamin D (25[OH]D) is the primary form of vitamin D and the precursor of the active form 1,25[OH]D. This active form binds to vitamin D-binding protein (VDBP) in order to transport vitamin D metabolites to targets where it shows its biological effects by binding to VDR (8). *VDR* is located in the 12q13 chromosome region and is known to be present in almost all cells of the body (9). With the identification of many polymorphisms on the *VDR* gene; the rs2228570 polymorphism observed in the second exon region of the gene is identified during translation of the ATG promoter region in the *VDR* complementary DNA. T>C polymorphism (ATG<ACG) was observed in the first

translation initiation codon resulting with 3 amino acid protein shortening. This initial codon polymorphism is also referred to as *Fok1* polymorphism since it is defined using the Fok1 restriction enzyme (10). Another important polymorphism identified in the *VDR* gene is rs1544410 which is detected using the Bsm1 restriction enzyme. *Bsm1* polymorphism has been localized in the 8th intron of the related gene. As a result of G> A polymorphic change, mRNA stability is impaired and a decrease in the amount of VDR protein is observed (11).

VDBP encoded by the GC gene binds 85-90% of circulating 25[OH]D, thus regulating the bioavailability of vitamin D (12). Three common alleles in the *VDBP* gene have been identified: Gc1s (slow), Gc1f (rapid), and Gc2. These alleles are separated from each other by the combination of two single nucleotide polymorphisms (SNPs) known as rs4588 and rs7041. Two polymorphisms in the *VDBP* gene reveal different phenotypic alleles by glycosylation patterns and amino acid substitutions in the *VDBP* gene. In the Gc1s and Gc1f alleles, galactose and sialic acid exchange were observed in this way, whereas only differences in galactose amino acid were observed in Gc2. rs7041 (G>T) polymorphism is glutamic acid aspartic acid conversion, while rs4588 (C>A) is the lysine conversion of threonine amino acid (13).

In human body, vitamin D level is referred as normal (>30 ng/mL), insufficient (20–30 ng/mL), and deficient (<20 ng/mL) (14). McCarty and his colleagues observed that more than half of the their patients who complained about sleep disruption and nonspecific somatic pain also showed vitamin D deficiency (7). Similar to OSAS, vitamin D deficiency has found in a association with adiposity, dark skin pigmentation, winter season, and physical inactivity (6). Therefore there might be possible relation between vitamin D and sleep apnea disorder. For the identification of genetic alterations which is very valuable at this matter, the aim of the present study was to investigate the effect of *VDBP* and *VDR* gene polymorphisms and other risk factors which may cause OSAS.

Materials and Methods

Study population

Totally,100 individuals were included from Erenkoy Mental and Neurological Diseases Training and Research Hospital, Sleep Center Clinic in this study. Fifty individuals who have Apnea–Hypopnea Index (AHI)≥5 and were diagnosed as OSAS according to the standard overnight whole-night polysomnography (PSG) results were selected as patients and 50 healthy volunteers without any disease and have AHI<5 were selected as controls. Electroencephalography (EEG), chin surface electromyography (EMG) and electrocardiography (ECG), oral and nasal air flow, thoracic and abdominal movements, arterial oxygen saturation and snoring count were recorded on the computer, additionally filled with epworth sleepiness test, consisted of both the aforementioned test and polysomnographic examiner and confirmed OSAS diagnosis. Total cholesterol, low density lipoprotein (LDL), high density lipoporotein (HDL) and triglyceride levels were also measured for all individuals.

Individuals who are thought to be needed an operation after otorhinolaryngologic examination were not included into the study. Also, to exclude the obesity factor, subjects whose body mass index (BMI) value is greater than 30 were left out the study.

The present study protocol conforms to the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Yeditepe University, Istanbul, Turkey. Informed consent was obtained from all individual participants included in the study.

Blood sampling and genotyping

Whole blood samples were taken from all patients and volunteers. DNA was extracted from 200 µL of peripheral blood using commercially available kits (Qiagen, Foster City, USA) according to manufacturer's instructions. DNA purity and concentrations were determined by NanoDrop spectrophotometer (Thermo Scientific, Wilmington, USA). Quantitative real-time polymerase chain reaction (qPCR) reactions for rs2228570, rs1544410 in *VDR*, rs4588 and rs7041 in *VDBP* were carried out on 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, USA) (15,16,17). The reaction was performed according to the manufacturer's instructions.

Statistical analysis

Statistical analyses was performed using IBM SPSS (Statistical Package of Social Sciences, Version 23.0. Armonk, NY: IBM Corp. Descriptive analysis was presented using means ± standard deviations (SD) for contionus data and frequecies and percentages for categorical data. The variables were inverstigated using Kolmogorov Smirnov test to determine whether they are normally distributed. If the variables are not normally distributed, Mann-Whitney U test was used to compare the two independent groups. Since the variables are not normally distributed, Kruskal-Wallis test were conducted to compare risk factors among genotypes. Mann-whitney U test was performed to test the significance of pairwise differences using Bonferroni correction adjust for multiple comparasions The Chi-Square and Fisher's exact test, where appopriate, was used to compare the proportions of the groups. Relationships between the variables were determined using Contengency Coefficients. A 5% type-I error level was used to infer a statistical significance.

Results

Study population

Table 1 shows the baseline characteristics of the study population. Significant differences were observed with regard to all baseline characteristics between the two groups, except age, neck circumference, hypertension and coronary artery disease (CAD) ($p<0.001$).

VDR and *VDBP* genotyping

Table 2 shows genetic analyses of cases and controls. When groups were compared with each other, CA genotype in rs4588, CC genotype in rs2228570 and AA genotype in rs1544410 mutations were found statistically significant in patients ($p<0.05$) whereas TC genotype in rs2228570 and GA genotype in rs1544410 mutations

Table 1. Baseline characteristics of the study population.

Baseline Characteristics		Groups (number of participants)		p values
		Controls (n=50)	Patients (n=50)	
Age (years)		45.86 ± 8.51	48.82 ± 11.03	0.14
Gender	Male	14 (28%)	32 (64%)	**<0.001***
	Female	36 (%72)	18 (%36)	
BMI (kg/m^2)		21.91 ± 1.95	27.45 ± 2.52	**<0.001***
Total cholesterol (mg/dL)		180.72 ± 38.62	217.40 ± 41.85	**<0.001***
LDL (mg/dL)		101.63 ± 30.80	130.36 ± 33.69	**<0.001***
HDL (mg/dL)		64.84 ± 20.74	44.78 ± 12.01	**<0.001***
Triglyceride (mg/dL)		71.35 ± 31.69	203.22 ± 98.95	**<0.001***
Hypertension (%)		1 (2%)	7 (14%)	0.059
Current smoker (%)		1 (2%)	18 (36%)	**<0.001***
ESS		2.2 ± 0.76	4.68 ± 4.43	**<0.001***
AHI (events/h)		2.68 ± 0.82	33.37 ± 17.36	**<0.001***
CAD (%)		0 (0%)	3 (6%)	0.242
EDS		0 (0%)	22 (44%)	**<0.001***
Neck circumference (cm)		38.54 ± 2.21	39.42 ± 2.94	0.094
Waist circumference (cm)		72.38 ± 7.97	102.54 ± 7.42	**<0.001***
Vitamin D levels (ng/mL)		21.81 ± 7.24	11.65 ± 3.75	**<0.001***
Alcohol intake (g/d)		0 (0%)	0 (0%)	n. e.
Story of stroke		0 (0%)	0 (0%)	n. e.

BMI: Body mass index, LDL: Low density lipoprotein, HDL: High density lipoprotein, AHI: Apnea–hypopnea index, EDS: Excessive daytime sleepiness, ESS: Epworth sleepiness scale, CAD: Coronary artery disease *p<0,001, n.e.: not evaluated.

Table 2. Genetic analysis in cases and controls.

Gene, genotype and nucleotide variation	Groups		p values
	Controls (n=50)	Patients (n=50)	
VDBP			
rs4588			
CC	37 (74%)	23 (46%)	
CA	8 (16%)	21 (42%)*	**0.01***
AA	5 (10%)	6 (12%)	
rs7041			
GG	8 (16%)	15 (30%)	
GT	19 (38%)	23 (46%)	0.051
TT	23 (46%)	12 (24%)	
VDR			
rs2228570			
TT	6 (12%)	4 (8%)	
TC	25 (50%)*	12 (24%)	**0.01***
CC	19 (38%)	34 (68%)*	
rs1544410			
GG	13 (26%)	24 (48%)	
GA	34 (68%)*	18 (36%)	**0.005***
AA	3 (6%)	8 (16%)*	

VDBP: Vitamin D binding protein gene, *VDR*: Vitamin D receptor gene, *p<0.05; ´p<0.001.

were found statistically significant in controls (p<0.05).

Relation between risk factors, *VDR* and *VDBP* mutations

Table 3 shows statistically significant relations between risk factors and mutations. Statistically significant relations were detected between GG and TT genotypes of rs7041 according to the triglyceride and AHI values. Also statistically significant relations were detected between CC and CA genotypes of rs4588 according to the waist circumference and vitamin D levels. Additionally statistically significant relations were detected between GG and GA genotypes of rs1544410 according to the BMI, triglyceride, AHI, waist circumference levels and AHI as well as between GA and AA genotypes of rs1544410 according to the AHI. Also it was found that homozygous mutant for rs1544410 and rs2228570 were found statsitically significant for EDS.

Table 3. Relation between disease associated risk factors and *VDR*, *VDBP* mutations.

Risk factors and mutations	Mean values for genotypes			p values
rs7041	**GG (n=23)**	**GT(n=42)**	**TT(n=35)**	
Triglyceride (mg/dl)	167.48 ± 106.32*	148.96 ± 103.51	103.43 ± 78.38*	**0.026***
AHI	27.36 ± 25.42*	17.07 ±16.13	13.04 ± 11.61*	**0.008***
rs4588	**CC (n=60)**	**CA (n=29)**	**AA (n=11)**	
Waist circumference (cm)	84.12 ± 16.8*	94.07 ± 15.77*	88.27 ± 17.28	**0.043***
Vitamin D levels (ng/mL)	18.78 ± 8.44*	13.08 ± 4.00*	15.17 ± 7.20	**0.002***
rs1544410	**GG (n=37)**	**GA (n=52)**	**AA (n=11)**	
BMI (kg/m^2)	25.71 ± 3.81*	23.68 ± 3.57*	26.01 ± 2.52	**0.036***
Triglyceride (mg/dl)	156.10 ± 96.74*	113.73 ± 88.16*	185.36 ± 127.70	**0.042***
AHI	21.71 ± 18.11*	13.40 ± 11.45*′	27.48 ± 25.64′	**0.047*/0.002′**
Waist circumference (cm)	94.86 ± 17.48*	82.46 ± 16.42*	92.91 ± 10.47	**0.008***
EDS	12	6	4*	**0.03***
rs2228570	TT (n=10)	TC (n=37)	CC (n=53)	
EDS	0	5	17*	**0.023***

BMI: Body mass index, AHI: Apnea–hypopnea index, EDS: Excessive daytime sleepiness, *p<0,05, ′p<0,05.

Correlation results

When the relations between mutations were investigated, a significant correlation was detected between rs4588 and rs7041 ($p<0.05$). It means that mutations/polymorphisms are tend to be related with each other and they are mostly found together in the same individual ($p<0.05$).

Discussion

OSAS is a common sleeping disorder characterized by repeated recurrent paralysis of the airway during sleep, which causes a significant decrease in airflow (hypopnea) or complete disruption (apnea) despite ongoing breathing. It is known that the majority of such sleep disorders are the result of a complex interaction between environmental factors and individual genetic predisposition (18). In this study, we investigated the possible relations between genetic or environmental risk factors with OSAS.

To our knowledge any other study, which investigate the relation between rs4588, rs7041, rs1544410 and rs2228570 mutations and OSAS, were not found in the literature. In this study heterozygous mutation for rs4588, homozygote mutations for rs2228570 and rs1544410 were found statistically high in patients. Also heterozygous mutations for rs2228570 and rs1544410 were found statistically high in controls ($p<0.05$).

In some studies, it was found that males have higher risk than females to get caught OSAS. This result overlaps with other studies (6, 19,20), whereas some studies could not found any association (21). In our study it was found that mostly males have got OSAS. Therefore it was considered that gender is an important factor which effect OSAS.

Approximately 60% of adults in many developed countries are overweight, defined as BMI> 25 kg/m^2, and 1/3 of adults are obese, defined as a BMI> 30 kg/m^2. BMI is one of the predictor of obesity and obesity is the largest risk factor for OSAS. The overall prevalence of OSAS in the general population is 2% to 4% and in obese individuals the prevalence of OSAS is 30% (22-24). In the present study, we found that patient with OSAS have significantly higher BMI score (27.45 ± 2.52) than control group (21.91 ± 1.95). When we investigated the relation between risk factors and vitamin D related polymorphisms, we obtained statistically significant association for BMI score between GG and GA genotypes of rs1544410 polymorphism. Also lubrication around waist is another predictor for obesity therefore the result for higher score of waist circumference was an expected result. Similar relation between risk factors and polymorhisms were found for waist circumference in rs4588 and rs1544410 between wild type and heterozygous mutant alleles.

The main cause for OSAS explained in the literature is the reduction of the expansion forces of the pharyngeal dilator muscles and the reduction of the discordance between the inspiratory activity of the muscles and the breathing effort (25). Recent studies clarified that vitamin D plays an important role in pleiotropic effects on many organ functions (1). Vitamin D deficiency causes muscle weakness and skeletal muscles have a vitamin D receptor, also may require vitamin D for maximum function (26). Therefore it was considered that there is an association between OSAS and vitamin D deficiency. Similar to these findings, in our study it was found that vitamin D level is statistically lower in patients than controls, and individuals who carry heterozygous allele for rs4588 genotype have lower vitamin D level than wild type allele.

Adults with sleep-disordered breathing (SDB) have many features in common with the metabolic syndrome, including systemic hypertension, central obesity, and insulin resistance (27,28). Some evidences have consistently supported the association of OSAS with an increased prevalence of hypertension but we could not obtain statistically significant relation between them.

In some studies, obesity was found to correlate with respiratory distress index (RDI), total serum cholesterol levels, LDL, HDL and triglyceride levels (29,30). In our study, it was found that total cholesterol, LDL and triglyceride levels are statistically high and HDL level is statistically low in patient group. Additionally it was found that individuals who carry homozygous mutant allele for rs7041 and heterozgous allele for rs1544410 have statistically low triglyceride levels.

Smoking is a predisposing factor for pulmonary and

cardiovascular diseases and is considered a risk factor for OSAS development. Current studies indicate that there is a synergistic effect between OSAS and cigarette consumption, which increases both cardiovascular disease risk through oxidative stress and endothelial dysfunction and abnormal inflammatory response. It can also be assumed that OSAS is responsible for nicotine addiction (31,32). But Schafer *et al.* could not found any association between smoking and OSAS (30) . In our study it was found that smoking is an important risk factor for OSAS.

Sleepiness is the most important daytime symptom of OSAS, and is due to the fragmentation of sleep caused by recurrent electroencephalographic awakening that usually terminate the apneas and hypopneas (3). The ESS is a simple, self-designed questionnaire that shows when a person is taking a measure of the general level of daytime sleepiness. The total ESS score performs significantly distinguishment of normal individual from patients in various diagnostic groups such as OSAS, narcolepsy and idiopathic hypersomnia (33). Also studies suggested that observed apneas are more predictive of a high AHI than either snoring or excessive daytime sleepiness (25). In our study, ESS, AHI and EDS were found statistically high in patient group. Also different relations were detected for rs7041 and rs1544410 mutations according to the AHI. Additionally it was found that rs2228570 and rs1544410 mutations cause different effects to EDS ($p<0.05$).

Additionally, in our study, statistically significant correlations was detected between rs4588 and rs7041 ($p<0.05$). It means that mutations/polymorphisms are tend to be related with each other and they are mostly found together in the same patient and may increase the risk of OSAS.

In conclusion, abnormally low levels of vitamin D are common in populations seeking care for sleep-medicine complaints, and may be causes or contributors to common sleep-disorder symptoms. This study shows that lower plasma vitamin D levels were associated with a higher prevalence of OSAS. In addition, elevated LDL and trigliceride levels as well as lower HDL level were associated with an increased prevalence of OSAS. *VDR* and *VDBP* gene polymorphisms were found highly related to the OSAS which have not been investigated. Possible tracking of these mutations and risk factors may help to understand the metabolism as well as the progression of the disease.

Conflict of interest

All of the authors have no conflict of interest to declare.

References

1. Barcelo A, Esquinas C, Pierola J, De la Pena M, Sanchez-de-la-Torre M, Montserrat JM et al. Vitamin D status and parathyroid hormone levels in patients with obstructive sleep apnea. Respiration 2013; 86(4):295-30. doi: 10.1159/000342748

2. McNicholas WT, Bonsigore MR. Management Committee of ECAB. Sleep apnoea as an independent risk factor for cardiovascular disease: current evidence, basic mechanisms and research priorities. Eur Respir J 2007; 29(1):156-78. doi: 10.1183/09031936.00027406

3. McNicholas WT. Diagnosis of obstructive sleep apnea in adults. Proc Am Thorac Soc 2008; 5(2):154-60. doi: 10.1513/pats.200708-118MG

4. Xu H, Guan J, Yi H, Zou J, Meng L, Tang X et al. Elevated low-density lipoprotein cholesterol is independently associated with obstructive sleep apnea: evidence from a large-scale cross-sectional study. Sleep Breath 2016; 20(2):627-34. doi: 10.1007/s11325-015-1262-3

5. McCarty DE, Chesson AL, Jr., Jain SK, Marino AA. The link between vitamin D metabolism and sleep medicine. Sleep Med Rev 2014; 18(4):311-9. doi: 10.1016/j.smrv.2013.07.001

6. Kerley CP, Hutchinson K, Bolger K, McGowan A, Faul J, Cormican L. Serum Vitamin D Is Significantly Inversely Associated with Disease Severity in Caucasian Adults with Obstructive Sleep Apnea Syndrome. Sleep 2016; 39(2):293-300. doi: 10.5665/sleep.5430

7. McCarty DE, Reddy A, Keigley Q, Kim PY, Marino AA. Vitamin D, race, and excessive daytime sleepiness. J Clin Sleep Med 2012; 8(6):693-7. doi: 10.5664/jcsm.2266

8. Young KA, Snell-Bergeon JK, Naik RG, Hokanson JE, Tarullo D, Gottlieb PA et al. Vitamin D deficiency and coronary artery calcification in subjects with type 1 diabetes. Diabetes Care 2011; 34(2):454-8. doi: 10.2337/dc10-0757

9. Abu El Maaty MA, Hassanein SI, Gad MZ. Genetic variation in vitamin D receptor gene (Fok1:rs2228570) is associated with risk of coronary artery disease. Biomarkers 2016; 21(1):68-72. doi: 10.3109/1354750X.2015.1118535

10. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene 2004; 338(2):143-56. doi: 10.1016/j.gene.2004.05.014

11. Laczmanski L, Milewicz A, Lwow F, Puzianowska-Kuznicka M, Pawlak M, Kolackov K et al. Vitamin D receptor gene polymorphism and cardiovascular risk variables in elderly Polish subjects. Gynecol Endocrinol 2013; 29(3):268-72. doi: 10.3109/09513590.2012.736562

12. Yao P, Sun L, Lu L, Ding H, Chen X, Tang L et al. Effects of Genetic and Nongenetic Factors on Total and Bioavailable 25[OH]D Responses to Vitamin D Supplementation. J Clin Endocrinol Metab 2017; 102(1):100-10. doi: 10.1210/jc.2016-2930

13. Verschuren JJ, Trompet S, Sampietro ML, Heijmans BT, Koch W, Kastrati A et al. Pathway analysis using genome-wide association study data for coronary restenosis--a potential role for the PARVB gene. PLoS One 2013; 8(8):e70676. doi: 10.1371/journal.pone.0070676

14. Pilz S, Tomaschitz A, Ritz E, Pieber TR. Vitamin D status and arterial hypertension: a systematic review. Nat Rev Cardiol 2009; 6(10):621-30. doi: 10.1038/nrcardio.2009.135

15. Kirac D, Dinçer Yazan C, Gezmis H, Yaman A, Haklar G, Sirikci O, Altunok EC, Deyneli O. VDBP, VDR mutations and other factors related with vitamin D metabolism may be associated with type 1 diabetes mellitus. Cell Mol Biol (Noisy-le-grand) 2018; 64(3):11-16. doi: http://dx.doi.org/10.14715/cmb/2018.64.3.3

16. Kirac D, Erdem A, Gezmis H, Yesilcimen K, Altunok EC, Isbir T. Effects of VDBP and VDR Mutations and Other Factors to the Development of Stent Thrombosis in Coronary Artery Disease Patients. Clin Exp Health Sci 2018; 8:1-8. doi:10.5152/clinexphealthsci.2017.540

17. Eken BF, Sercan C, Gezmis H, Moufti Chousen O, Kirac D, Akyuz S, Ulucan K. Effect of Vitamin D Receptor rs1544410 Polymorphism on Tooth Decays. European Journal of Research in Dentistry 2018; 2-1:1-5. doi:10.12990/MDJ.2018.13

18. Pellegrino R, Mazzotti DR, Guindalini C, Santos-Silva R, Bittencourt LR, Tufik S. Apolipoprotein E polymorphisms and sleep quality in obstructive sleep apnea syndrome. Clin Chim Acta 2011; 412(23-24):2223-7. doi: 10.1016/j.cca.2011.08.007

19. Basoglu OK, Tasbakan MS. Gender differences in clinical and polysomnographic features of obstructive sleep apnea: a clinical

study of 2827 patients. Sleep Breath 2017; doi: 10.1007/s11325-017-1482-9
20. Valipour A. Gender-related differences in the obstructive sleep apnea syndrome. Pneumologie 2012; 66(10):584-8. doi: 10.1055/s-0032-1325664
21. Kheirandish-Gozal L, Peris E, Gozal D. Vitamin D levels and obstructive sleep apnoea in children. Sleep Med 2014; 15(4):459-63. doi: 10.1016/j.sleep.2013.12.009
22. Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. JAMA 2002; 288(14):1723-7.
23. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. N Engl J Med 1993; 328(17):1230-5. doi: 10.1056/NEJM199304293281704
24. Kyzer S, Charuzi I. Obstructive sleep apnea in the obese. World J Surg 1998; 22(9):998-1001.
25. Azagra-Calero E, Espinar-Escalona E, Barrera-Mora JM, Llamas-Carreras JM, Solano-Reina E. Obstructive sleep apnea syndrome (OSAS). Review of the literature. Med Oral Patol Oral Cir Bucal 2012; 17(6):e925-9.
26. Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357(3):266-81. doi: 10.1056/NEJMra070553
27. Tauman R, O'Brien LM, Ivanenko A, Gozal D. Obesity rather than severity of sleep-disordered breathing as the major determinant of insulin resistance and altered lipidemia in snoring children. Pediatrics 2005; 116(1):e66-73. doi: 10.1542/peds.2004-2527
28. Meslier N, Gagnadoux F, Giraud P, Person C, Ouksel H, Urban T et al. Impaired glucose-insulin metabolism in males with obstructive sleep apnoea syndrome. Eur Respir J 2003; 22(1):156-60.
29. Borgel J, Sanner BM, Bittlinsky A, Keskin F, Bartels NK, Buechner N et al.Obstructive sleep apnoea and its therapy influence high-density lipoprotein cholesterol serum levels. Eur Respir J 2006; 27(1):121-7. doi: 10.1183/09031936.06.00131304
30. Schafer H, Pauleit D, Sudhop T, Gouni-Berthold I, Ewig S, Berthold HK. Body fat distribution, serum leptin, and cardiovascular risk factors in men with obstructive sleep apnea. Chest 2002; 122(3):829-39.
31. Pampati S, Manchikanti L. What Is the Prevalence of Symptomatic Obstructive Sleep Apnea Syndrome in Chronic Spinal Pain Patients? An Assessment of the Correlation of OSAS with Chronic Opioid Therapy, Obesity, and Smoking. Pain Physician 2016; 19(4):E569-79.
32. Trenchea M, Deleanu O, Suta M, Arghir OC.) Smoking, snoring and obstructive sleep apnea. Pneumologia 2013; 62(1):52-5.
33. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991; 14(6):540-5.