Research Article

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Evaluation of adropin indices and PCSK9 in non-diabetic men with severe obstructive sleep apnea

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Abstract

Objectives: We aimed to investigate the relationship among proprotein convertase subtilisin/kexin type 9 (PCSK9), adropin levels, inflammation, and sleep variables in non-diabetic males with severe obstructive sleep apnea (OSA).

Methods: This cross-sectional study included adults aged 18 to 65 who underwent polysomnography due to sleep problems between July 2019 and August 2020. Participants were grouped based on their apnea-hypopnea index (AHI). We included 32 males with simple snoring (AHI<5 events/h) as the controls and 48 males with severe OSA (AHI≥30 events/h). Furthermore, patients with severe OSA were divided into two groups based on body mass index (BMI), resulting in three groups in total. Adropin and PCSK9 were analyzed using the enzyme-linked immunosorbent assay method.

Results: In severe OSA with BMI≥30 kg/m^2, compared to the controls, blood pressure values, interleukin-6 (IL-6), white blood cell (WBC) count, systemic inflammation response index, neutrophil, monocyte counts were found to be higher, but adropin/BMI, adropin/waist circumference, adropin/neck circumference were significantly lower. Adropin/BMI had the highest correlation coefficient with IL-6. Although there was no significant difference in PCSK9 levels among the groups, PCSK9 was independently correlated with the WBC and its subsets.

Conclusions: Our study is of clinical importance as it is the first to show a relationship between PCSK9 and inflammation markers in severe OSA. Also, this study demonstrated the potential value of adropin, in combination with BMI, as a valuable indicator for assessing inflammation and OSA severity.

Keywords: proprotein convertase subtilisin/kexin type9; adropin; obstructive sleep apnea; obesity; inflammation

Introduction

Obstructive sleep apnea (OSA) is a complex disease characterized by episodes of upper respiratory tract obstruction, resulting in chronic intermittent hypoxia (CIH) [1]. CIH activates proinflammatory cytokines, adhesion molecules, and other inflammatory mediators, causing chronic inflammatory processes such as endothelial dysfunction and atherosclerosis [2, 3]. OSA could accelerate dyslipidemia-associated atherosclerosis by oxidizing low-density lipoprotein (LDL) [4]. Instead of focusing on a single component of dyslipidemia, searching for comprehensive biomarkers is recommended to evaluate lipid abnormalities in OSA [5].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease that belongs to the proprotein convertase family of enzymes. It affects plasma lipid and lipoprotein levels by reducing the clearance of lipoproteins by the liver and promoting lipid production in the liver [6]. Loss-of-function mutations in PCSK9 are related to decreased circulating low-density lipoprotein cholesterol (LDL-C) and cardiovascular disease (CVD) risk. Therefore, inhibition of PCSK9 by monoclonal antibodies has received significant attention as a promising target for lipid-lowering treatment.

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Beyond cholesterol metabolism, PCSK9 mediates many other physiological processes, such as immune response regulation, inflammation, and neurogenesis regulation during neuronal development [7]. It has been reported that PCSK9 expression is mediated by oxidized LDL in immune cells, such as dendritic cells and macrophages. PCSK9 influences the release of extracellular vesicles and microRNAs during inflammation, which are linked to various stages of atherosclerosis. Elevated PCSK9 levels contribute to vascular and systemic inflammation [8–12].

Adropin, a peptide hormone, has been identified as a crucial regulator of energy homeostasis and metabolic control. Adropin is recognized for regulating and maintaining fatty acid and glucose metabolism within the body's physiological processes. Decreased levels of adropin are associated with a higher body mass index (BMI) and indicate poorer metabolic health, including insulin resistance and increased adiposity. It was observed that adropin has an independent negative predictive value in OSA and could serve as a critical new indicator in risk stratification [1, 3].

The number of studies investigating PCSK9 levels in the OSA is very limited. This study aimed to investigate the effect of severe OSA on PCSK9, adropin indices and the patient's sleep laboratory variables. Also, we aimed to show the potential interaction between PCSK9 levels and factors such as obesity or inflammation markers [e.g. adropin, interleukin-6 (IL-6)], as well as hemogram parameters including white blood cell (WBC), neutrophil, lymphocyte, monocyte, platelet counts, and mean platelet volume (MPV) in this group of patients.

Materials and methods

This study was started after the University of Health Sciences Istanbul Training and Research Hospital Clinical Research Ethics Committee was approved on June 28, 2019, under registration number 1897. It was conducted in accordance with the Declaration of Helsinki, patient rights regulations, and ethical guidelines. Informed written consent was obtained from all participants involved in the research.

Study groups

This cross-sectional study encompassed adult male patients who were admitted to the University of Health Sciences Istanbul Training and Research Hospital's ear, nose, and throat, or neurology polyclinics from July 2019 to August 2020 and underwent polysomnography (PSG) due to sleep-related issues. Participants were divided into two primary groups: a control group of 32 individuals with simple snoring [apnea-hypopnea index (AHI)<5 events/h] and a severe OSA group consisting of 48 individuals (AHI≥30 events/h) [13]. Further, the severe OSA group was divided into two subgroups based on BMI thresholds of 30 kg/m^2. The study's inclusion criteria covered patients aged 18–65 years.

The exclusion criteria for this study were defined as follows: (1) cases with infectious and systemic inflammatory or autoimmune diseases, thyroid disorders, Diabetes Mellitus (DM), chronic kidney disease, severe liver failure; (2) Participants with a medical history of OSA treatment prior to study enrollment; (3) cases with known malignancy, hematological disorders, taking immunosuppressive treatment; (4) use of anti-lipidemic drugs (statin, fibrate, nicotinic acid, ezetimibe); (5) cases with BMI>40 kg/m^2.

Neck circumference, waist circumference, BMI, and blood pressure values were measured before sample collection. The BMI was calculated using the formula [weight (kg)/height (m)^2]. In addition, the patients were questioned about their history of acute or chronic diseases, medication use, as well as smoking and alcohol consumption.

Sleep study

Sleep patterns of the patients were evaluated in the sleep laboratory using the Embla S4500 system (Philips Respironics, UK). The PSG examination included assessments of brain activity through electroencephalogram recordings, bilateral eye movements via electrooculography, activity of the submental and bilateral tibialis anterior muscles through electromyography, airflow measured by a thermistor and nasal cannula, respiratory movements of the abdomen and chest captured with plethysmography, body position detected by position sensors, arterial oxygen saturation (SpO_2) monitored by a pulse oximeter, cardiac activity via electrocardiography, and the sleep environment observed through video recording with an infra-red-light camera. PSG results were obtained between 22.00 and 07.30 (full-night PSG). The study participants had a sleep record of at least 6 h. The collected data was evaluated by a neurologist based on the 2017 American Academy of Sleep Medicine criteria [14]. In PSG recordings, AHI, oxygen desaturation index (ODI), total sleep time (TST) and percentage of sleep stages [N1 (%TST), N2 (%TST), N3 (%TST)], non-rapid eye movement (NREM)-AHI, rapid eye movement (REM)-AHI, SpO_2 levels were evaluated. In polysomnography, a decrease in the peak signal of the oronasal thermal sensor by ≥90 % of the baseline value and persistence of this condition for ≥10 s is defined as apnea. Hypopnea is defined as a decrease of at least 30 % from the baseline value that persists for at least

10 s. The AHI represents the total number of apnea and hypopnea episodes per hour of sleep. The ODI quantifies the number of oxygen desaturations occurring per hour of sleep. TST is the sum of all REM and NREM sleep in a sleep episode. The percentages of NREM stages in the TST were indicated by N1, N2, and N3. REM (%TST) indicates the percentage of REM stage in the total sleep time [14]. The term "Mean SpO_2" refers to the average oxygen saturation level over a specified period, indicating oxygen level stability within that timeframe. "Minimum SpO_2" refers to the lowest level of oxygen saturation measured in each period. This value is often used during sleep, especially in individuals with respiratory problems like sleep apnea. "SpO_2<90 %" indicates the percentage of an individual's TST during which the oxygen saturation falls below 90 % [15].

Laboratory analysis

Venous blood samples were collected from the patients between 8:00 and 10:00 AM, after a minimum fasting period of 8 h. Both routine and enzyme-linked immunosorbent assay (ELISA)-specific samples were drawn simultaneously, ensuring collection occurred no more than two weeks following the sleep study. Hemogram samples were collected into K_2EDTA tubes (EDTA tubes, BD Vacutainer, 13 × 75 mm, 3.0 mL) from Becton Dickinson (Franklin Lakes, NJ, USA). The other samples were taken using serum separator tubes (SST, BD Vacutainer, SST™ II Advance, 13 × 100 mm, 5.0 mL) from Becton Dickinson (Franklin Lakes, NJ, USA). They were allowed to clot for at least 30 min before being centrifuged at 2000×*g* for 10 min at room temperature using a Beckman Coulter Allegra X-15R Centrifuge. Subsequently, the serum was aliquoted into Eppendorf tubes and stored at −80 °C until analysis.

Serum adropin (Catalog number: E-EL-H5307; Elabscience Biotechnology Co., Ltd, Houston, TX, USA) and serum PCSK9 (Catalog number: RD191473200R; Biovendor R&D Products, Brno, Czech Republic) concentrations were evaluated via ELISA (Synergy HT, Biotek Instruments, Inc., USA) device. The absorbance for adropin was photometrically measured at a wavelength of 450 nm. For PCSK9, absorbance was determined photometrically at two wavelengths: 450 and 630 nm. The analytical sensitivity for adropin is 7.5 pg/mL, with a linear measurement range of 12.5–800 pg/mL. For PCSK9, the minimum detectable value is 9.0 pg/mL, with an analytical measurement range of 125–4,000 pg/mL. Based on the manufacturer's recommendation for PCSK9, results obtained using a dilution coefficient of 200 are expressed in ng/mL rather than pg/mL. The final sample concentrations, derived from the standard curve, were adjusted by the specified dilution factor of 200. The coefficient of variation for within-run and between-day precision for both assays is reported to be less than 10 %.

Serum IL-6 levels were determined by electrochemiluminescence immunoassay (Cobas 8,000 modular analyzer, Roche Diagnostics, Mannheim, Germany), in which measurement ranges were 1.5–5,000 pg/mL. Other laboratory data were received via hospital/laboratory information systems: total cholesterol, triglycerides (TG), LDL-C, and high-density lipoprotein cholesterol (HDL-C) tests via AU5800 (Beckman Coulter Inc., Brea, CA, US.) biochemistry autoanalyzer. Non-HDL-C was determined by subtracting HDL-C from total cholesterol levels. In addition, hemogram parameters, such as WBC, neutrophil, lymphocyte, monocyte, platelet counts, MPV were measured in XN-9000 (Sysmex Corporation, Kobe, Japan) autoanalyzer. Adropin values were divided by BMI, waist circumference, and neck circumference values, and the adropin/BMI, adropin/waist circumference, and adropin/neck circumference indices were calculated. Systemic inflammation response index (SIRI): (monocyte count * neutrophil count)/lymphocyte count [16]. The monocyte count divided by HDL-C values was used to calculate the monocyte count to high-density lipoprotein cholesterol ratio (MHR).

Statistical evaluation

All statistical evaluations were conducted using SPSS version 26.0 (IBM Corp, Armonk, NY, US) and GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, US). The conformity of the data to normal distribution was assessed through the application of the Shapiro–Wilk test. Normally distributed data were expressed as mean±standard deviation, whereas non-normally distributed data were expressed as median (1st and 3rd quartile values). Categorical variables were expressed as numbers and percentages, and comparisons between groups were analyzed with the Chi-Square test. The Student-t or Mann–Whitney U test was applied to compare two independent groups. The one-way ANOVA or Kruskal–Wallis test was utilized to compare three independent groups. Pairwise comparisons for both one-way ANOVA and Kruskal–Wallis tests were conducted using Bonferroni correction, accepting a significance level of 0.017 for the adjusted p-value. Correlations between variables were examined using Pearson or Spearman correlation analyses, depending on data distribution. Multivariate linear regression analyses were applied to evaluate the association between log-transformed PCSK9 levels and inflammatory parameters. $p<0.05$ was considered significant.

Results

There were no statistically significant differences in terms of age, smoking and alcohol consumption, rates of coronary artery disease (CAD) and chronic obstructive pulmonary disease (COPD) between the controls and the severe OSA group. Nevertheless, patients with severe OSA demonstrated significantly higher neck circumference, BMI, waist circumference, hypertension frequency, and blood pressure levels (Table 1).

There was no statistically significant difference in PCSK9 levels between the severe OSA and the simple snoring groups. However, adropin, adropin/BMI, adropin/waist circumference and adropin/neck circumference were lower in the severe OSA group. There were no significant differences in platelet counts, lymphocyte counts, total cholesterol, TG, LDL-C, non-HDL-C, and HDL-C between the groups. On the other hand, IL-6, SIRI, MPV, MHR, neutrophil counts, WBC, and monocyte counts were significantly higher in the severe OSA group (Table 2). In univariate linear regression analysis, PCSK9 was positively related to WBC (β=0.451, p=0.001), neutrophil (β=0.431, p=0.002), monocyte (β=0.318, p=0.028) in severe OSA group. Furthermore, after adjusting for covariates (age, BMI, neck circumference, smoking, alcohol consumption, hypertension, COPD, CAD history), PCSK9 was independently correlated with WBC and its subsets (WBC β=0.582, p=0.002; neutrophil β=0.570, p=0.003; monocyte β=0.355, p=0.049) in a multivariable linear regression model (Supplement Table 1).

Table 1: Comparison of demographic findings, comorbidities between simple snoring group and severe obstructive sleep apnea.

Parameter	Simple snoring (n=32)	Severe OSA (n=48)	p-Value
Age, years	41.8 ± 10.1	44.7 ± 10.2	0.210[a]
Neck circumference, cm	38.5 (37.0–40.0)	42.0 (40.5–45.0)	**<0.001**[b]
Waist circumference, cm	92.9 ± 9.85	112 ± 13.8	**<0.001**[a]
BMI, kg/m^2	26.2 ± 3.28	32.6 ± 4.63	**<0.001**[a]
Systolic BP, mmHg	119 ± 9.00	131 ± 15.4	**<0.001**[a]
Diastolic BP, mmHg	78.6 ± 7.60	86.7 ± 9.02	**<0.001**[a]
Hypertension, n (%)	3 (9)	14 (29)	**0.034**[c]
Smoking, n (%)	12 (37)	16 (33)	0.886[c]
Alcohol consumption, n (%)	9 (28)	7 (15)	0.138[c]
COPD, n (%)	0 (0)	2 (4.2)	0.514[c]
CAD, n (%)	2 (6.2)	6 (12.5)	0.466[c]

p-Value<0.05 was considered significant. Numerical data are expressed as mean±standard deviation or median (1st and 3rd quartile values). Categorical variables were expressed as numbers and percentages. [a]Student t-test, [b]Mann–Whitney U test, [c]Chi-Square test for categorical variables. OSA, obstructive sleep apnea; BMI, body mass index; BP, blood pressure; COPD, chronic obstructive pulmonary disease; CAD, coronary artery disease.

Table 2: Comparison of laboratory findings between simple snoring and severe obstructive sleep apnea.

Parameter	Simple snoring (n=32)	Severe OSA (n=48)	p-Value
PCSK9, ng/mL	369 (296–419)	353 (304–439)	0.860[b]
Adropin, pg/mL	69.8 (35.7–143)	43.9 (16.0–89.2)	**0.018**[b]
Adropin/BMI	2.76 (1.31–5.36)	1.38 (0.46–2.90)	**0.002**[b]
Adropin/WC	0.75 (0.38–1.50)	0.40 (0.14–0.79)	**0.004**[b]
Adropin/NC	1.89 (0.91–3.70)	1.04 (0.37–2.13)	**0.008**[b]
Interleukin-6, pg/mL	1.50 (1.50–3.01)	2.30 (1.51–3.29)	**0.006**[b]
WBC, 10^9/L	6.12 (5.63–7.32)	7.17 (6.41–8.86)	**0.005**[b]
Neutrophil count, 10^9/L	3.42 (2.89–3.98)	4.10 (3.31–5.14)	**0.014**[b]
Lymphocyte count, 10^9/L	2.29 ± 0.61	2.51 ± 0.72	0.170[a]
Monocyte count, 10^9/L	0.51 (0.43–0.61)	0.65 (0.52–0.72)	**0.003**[b]
Platelet count, 10^9/L	235 ± 29.8	255 ± 66.8	0.071[a]
MPV, fL	10.2 ± 0.67	10.5 ± 0.74	**0.025**[a]
SIRI	0.84 (0.61–1.09)	0.98 (0.75–1.42)	**0.023** [b]
Total cholesterol, mg/dL	207 (174–234)	210 (180–239)	0.817[b]
Trygliceride, mg/dL	133 (93.0–193)	143 (114–178)	0.432[b]
LDL-C, mg/dL	144 (117–160)	142 (122–170)	0.910[b]
HDL-C, mg/dL	43.5 (36.0–49.5)	42.0 (37.5–47.0)	0.513[b]
Non-HDL-C, mg/dL	167 (130–182)	167 (141–194)	0.648[b]
MHR	12.4 ± 4.60	15.5 ± 5.53	**0.012**[a]

p-Value<0.05 was considered significant. Numerical data are expressed as mean±standard deviation or median (1st and 3rd quartile values). [a]Student t-test, [b]Mann–Whitney U test. OSA, obstructive sleep apnea; PCSK9, proprotein convertase subtilisin/kexin type 9; BMI, body mass index; WC, waist circumference; NC, neck circumference; WBC, white blood cell count; MPV, mean platelet volume; SIRI, systemic inflammation response index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Non-HDL-C, non-high density lipoprotein cholesterol; MHR, monocyte count to high-density lipoprotein cholesterol ratio.

The severe OSA group was divided into two subgroups based on BMI thresholds of 30 kg/m^2. Across these subgroups, including the control group, there were no statistically significant differences observed in smoking habits, alcohol consumption, or the prevalence of co-morbidities such as hypertension, COPD, and CAD. Similarly, levels of PCSK9, adropin, MPV, platelet counts, and lymphocyte counts did not differ significantly. In the severe OSA with obesity group, blood pressure values, IL-6, WBC, SIRI, neutrophil, monocyte, and MHR levels were higher compared to the control group. However, adropin/BMI, adropin/waist circumference, and adropin/neck circumference were significantly lower compared to the control group (Table 3 and Figure 1A–D).

A significant positive relationship was found between PCSK9 and monocyte (r=0.249, p=0.026), total cholesterol (r=0.252, p=0.024), non-HDL-C (r=0.251, p=0.025), LDL-C (r=0.242, p=0.031), and MPV (r=0.276, p=0.013) in all groups. PCSK9 correlated negatively with the N1 (%TST) (r=−0.281, p=0.012), and correlated positively with N2 (%TST) (r=0.220, p=0.049) (Table 4). The adropin/BMI ratio had the highest

Table 3: Comparison of clinical and laboratory findings among individuals with the simple snoring, non-obese severe OSA and obese severe OSA.

Parameter	Simple snoring (n=32)	Non-obese severe OSA (n=13)	Obese severe OSA (n=35)	p-Value
Age, years	41.8 ± 10.1	50.6 ± 9.47	42.5 ± 9.68	**0.020**[f]
BMI, kg/m^2	25.8 (24.3–28.1)	28.1 (26.4–28.7)	34.7 (32.1–38.0)[b,c,d]	**<0.001**[g]
Systolic BP, mmHg	119 ± 9.01	125 ± 13.6	133 ± 15.7[b,c]	**<0.001**[f]
Diastolic BP, mmHg	78.6 ± 7.60	86.7 ± 6.98[a,c]	86.7 ± 9.76[a,c]	**<0.001**[f]
Hypertension, n (%)	3 (9)	5 (39)	9 (26)	0.080[e]
Smoking, n (%)	12 (38)	6 (46)	10 (29)	0.492[e]
Alcohol consumption, n (%)	9 (28)	1 (8)	6 (17)	0.290[e]
COPD, n (%)	0 (0)	1 (8)	1 (3)	0.300[e]
CAD, n (%)	2 (6)	3 (23)	3 (9)	0.271[e]
PCSK9, ng/mL	369 (296–419)	376 (344–410)	335 (292–442)	0.716[g]
Adropin, pg/mL	69.8 (35.7–143)	58.1 (14.9–93.9)	41.7 (16.7–86.3)	0.060[g]
Adropin/BMI	2.76 (1.31–5.36)	2.02 (0.51–3.28)	1.18 (0.44–2.54)[a,c]	**0.005**[g]
Adropin/WC	0.75 (0.38–1.50)	0.58 (0.15–0.93)	0.36 (0.12–0.73)[a,c]	**0.013**[g]
Adropin/NC	1.89 (0.91–3.70)	1.42 (0.35–2.41)	0.94 (0.37–1.94)[a,c]	**0.025**[g]
Interleukin-6, pg/mL	1.50 (1.50–3.01)	2.33 (1.63–3.85)	2.27 (1.51–3.25)[a,c]	**0.024**[g]
WBC, 10^9/L	6.12 (5.63–7.32)	6.75 (5.99–7.73)	7.46 (6.50–9.10)[a,c]	**0.006**[g]
Neutrophil count, 10^9/L	3.42 (2.89–3.98)	3.60 (3.02–4.02)	4.47 (3.59–5.31)[a,c]	**0.007**[g]
Lymphocyte count, 10^9/L	2.25 (1.80–2.70)	1.93 (1.76–3.11)	2.53 (2.06–2.91)	0.218[g]
Monocyte count, 10^9/L	0.51 (0.43–0.61)	0.62 (0.41–0.64)	0.66 (0.52–0.75)[a,c]	**0.004**[g]
Platelet count, 10^9/L	235 ± 29.8	238 ± 59.8	262± 68.9	0.125[f]
MPV, fL	10.2 ± 0.67	10.4 ± 0.78	10.6 ± 0.73	0.062[f]
SIRI	0.84 (0.61–1.09)	0.87 (0.68–0.96)	1.31 (0.76–1.46)[a,c]	**0.014**[g]
MHR	11.5 (8.55–15.6)	14.4 (9.70–16.4)	16.3 (11.7–19.6)[a,c]	**0.028**[g]

Pairwise comparisons for both one-way ANOVA and Kruskal-Wallis tests were conducted using Bonferroni correction, accepting a significance level of 0.017 for the adjusted p-value. [a]p<0.017, [b]p<0.001. Numerical data were expressed as mean±standard deviation or median (1st and 3rd quartile values). Categorical variables are expressed as numbers and percentages. [c]Comparison with simple snoring group, [d]comparison with non-obese severe OSA group, [e]Chi-Square test for categorical variables, [f]One-way ANOVA test, [g]Kruskal–Wallis test. OSA, obstructive sleep apnea; BMI, body mass index; BP, blood pressure; COPD, chronic obstructive pulmonary disease; CAD, coronary artery disease; PCSK9, proprotein convertase subtilisin/kexin type 9; WC, waist circumference; NC, neck circumference; WBC, white blood cell count; MPV, mean platelet volume; SIRI, systemic inflammation response index; MHR, monocyte count to high-density lipoprotein cholesterol ratio.

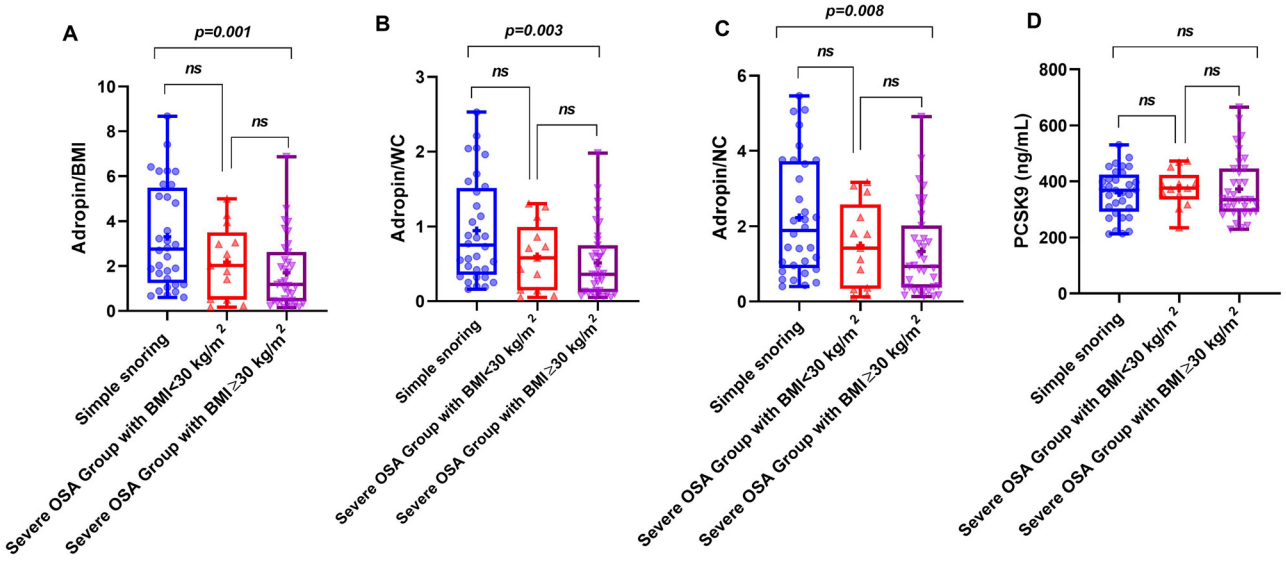

Figure 1: Comparing adropin indices and PCSK9 levels among the groups using box-plot graphics. (A) Adropin/BMI. (B) Adropin/WC. (C) Adropin/NC. (D) PCSK9 levels. BMI, body mass index; WC, waist circumference; NC, neck circumference; PCSK9, proprotein convertase subtilisin/kexin type 9; OSA, obstructive sleep apnea; ns, non-significant. Pairwise comparisons were conducted using Bonferroni correction for the Kruskal–Wallis test, accepting a significance level of 0.017 for the adjusted p-value.

correlation coefficient with IL-6. Furthermore, the adropin/BMI ratio was linked to AHI, ODI, and SpO_2 levels, which indicate the severity of OSA and other inflammatory markers, including IL-6, SIRI, WBC, and MHR. Additionally, the adropin/BMI ratio was inversely correlated with systolic and diastolic blood pressure values (Table 4).

In the severe OSA group, AHI, ODI, NREM AHI, and REM AHI parameters were significantly higher. In the severe OSA group, mean and minimum SpO_2 values were significantly lower, and the frequency of SpO_2<90 % was significantly higher, compared to the simple snoring group. Moreover, N2 (%TST) was found to be significantly higher, and N3 (%TST) was significantly lower (Supplement Table 2).

Table 4: Significant correlations between adropin/BMI, PCSK9 and other parameters in all groups.

Parameter	r	p-Value
Adropin/BMI		
AHI (events/h)	−0.327	0.003
ODI (events/h)	−0.321	0.004
NREM AHI (events/h)	−0.305	0.006
REM AHI (events/h)	−0.365	0.001
Mean SpO_2%	0.382	<0.001
Minimum SpO_2%	0.374	0.001
SpO_2<90 %	−0.367	0.001
Systolic BP, mmHg	−0.274	0.014
Diastolic BP, mmHg	−0.349	0.001
Interleukin-6, pg/mL	−0.512	<0.001
WBC, 10^9/L	−0.421	<0.001
SIRI	−0.396	<0.001
Neutrophil count, 10^9/L	−0.423	<0.001
Monocyte count, 10^9/L	−0.401	<0.001
MHR	−0.430	<0.001
PCSK9 (ng/mL)		
N1 (%TST)	−0.281	0.012
N2 (%TST)	0.220	0.049
Monocyte count, 10^9/L	0.249	0.026
Total cholesterol, mg/dL	0.252	0.024
Non-HDL-C, mg/dL	0.251	0.025
LDL-C, mg/dL	0.242	0.031
MPV, fL	0.276	0.013

p-Value<0.05 was considered significant. The associations between variables were assessed using Pearson or Spearman correlation analyses. BMI, body mass index; AHI, apnea-hypopnea index; ODI, oxygen desaturation index; NREM, non-rapid eye movement phase; REM, rapid eye movement; SpO_2, arterial oxygen saturation; BP, blood pressure; WBC, white blood cell count; SIRI, systemic inflammation response index; MHR, monocyte count to high-density lipoprotein cholesterol ratio; PCSK9, proprotein convertase subtilisin/kexin type 9; TST, total sleep time; N1 (%TST), per cent of non-rapid eye movement phase 1 in TST; N2 (%TST), per cent of NREM phase 2 in TST; Non-HDL-C, non-high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MPV, mean platelet volume.

Discussion

OSA is associated with various metabolic and inflammatory changes that can be influenced by factors such as obesity. To see the effect of BMI on serum PCSK9 levels, we grouped patients with severe OSA according to a BMI threshold of 30 kg/m^2. Compared to simple snoring, both obese and non-obese patients with severe OSA had no significant change in serum PCSK9 levels. PCSK9 is involved in lipid metabolism and plays a crucial role in inflammation, particularly in the context of atherosclerosis and other inflammatory conditions. While there is extensive research on the association between PCSK9 and inflammation in various health conditions, including CVD and metabolic disorders [17], there is a gap in understanding how PCSK9 levels correlate with inflammation markers, specifically in OSA patients. In our study, PCSK9 levels independently correlated with WBC subsets, such as neutrophils and monocytes, suggesting a potential association with systemic inflammation. Indices of adropin/BMI, adropin/waist circumference, and adropin/neck circumference were significantly lower in severe OSA with obesity compared to those with simple snoring. The adropin/BMI index exhibited a significant correlation with IL-6 and other inflammation markers, and it is linked to parameters indicative of OSA severity. These relations showed us the potential of adropin/BMI as an index for assessing inflammation and disease severity in OSA patients.

In a study [18], no significant difference was observed in PCSK9 levels between the control group and the OSA group that did not use statins. However, in the same study, PCSK9 levels were higher in obese OSA patients who used statins compared to both the control group and OSA patients who did not use statins. Such findings indicate that OSA, obesity, and statin use may each contribute to elevated PCSK9 levels [18]. The mechanism behind this increase is attributed to statin treatment leading to the depletion of cellular cholesterol levels, which in turn activates PCSK9 production through the sterol regulatory element-binding protein-2 pathway [18, 19]. Given that our study excluded patients undergoing statin therapy, we similarly observed no significant difference in PCSK9 levels between the control group and the severe OSA group.

Data on the relationship between PCSK9 and sleep architecture is very limited. NREM AHI had a stronger relationship with lipid metabolism and might have had a better predictive value than REM AHI. In a study, patients with OSA exhibited a significant increase in free fatty acids compared to control participants, and this increase persisted throughout the sleep period, particularly during NREM

sleep. The rise in free fatty acids might have reduced growth hormone production, mainly secreted during deep sleep (N3 stage). Reduced growth hormone levels seemed to independently contribute to dyslipidemia [20]. There were positive correlations between NREM AHI and TG, apoB, and a negative correlation between HDL-C and apoA1 levels [21]. We found a negative correlation between PCSK9 levels and the N1 (%TST) phase duration. Further investigation into the molecular and physiological mechanisms underlying NREM sleep may uncover potential connections to PCSK9 metabolism and its implications for health and disease.

The relationship between PCSK9 and inflammation markers has been demonstrated, especially in CVD. PCSK9 has been shown to promote plaque inflammation regardless of changes in plasma lipid levels, suggesting a potential connection between PCSK9 levels and inflammation in atherosclerotic plaque development [22, 23]. PCSK9 significantly correlated with high sensitivity C-reactive protein (hs-CRP) and platelet activation in ST-elevation myocardial infarction patients with DM [24] and with hs-CRP and fibrinogen levels in stable CAD patients [25]. It was reported that PCSK9 positively correlated with WBC, especially neutrophils, in stable CAD [26]. A similar relationship was shown in our cases, where the risk of CVD is high. A significant relationship was also detected between PCSK9 levels and MPV, accompanied by an elevation in MPV levels. Given the known associations of PCSK9 with inflammation and CVD, further exploration of the interplay between PCSK9 levels and MPV in conditions like severe OSA could offer valuable insights into their relationship and clinical significance.

Adropin has been identified as a critical regulator of energy and metabolic homeostasis, exhibiting a protective function in vascular health. It was observed that adropin could be a critical new indicator for the severity of OSA [1, 3, 27–30], suggesting that adropin plays a vital role in the pathophysiology of OSA. In studies involving OSA patients with endothelial dysfunction, low adropin levels were also observed [27, 28]. A significant increase in adropin levels was shown after tonsillectomy in pediatric OSA patients with endothelial dysfunction [29].

Sleep architecture in OSA patients is characterized by an increased light sleep (N1) of NREM sleep, a decrease in deep sleep (N3) of NREM sleep, and alterations in REM sleep, which may contribute to daytime cognitive and mood disturbances [31, 32]. In the study, despite the small sample sizes of the subgroups, the N3 (%TST) sleep phase in patients with severe OSA was significantly reduced compared to those in the moderate and control groups [33]. When our PSG findings are examined in detail, N2 (%TST) was significantly higher, and N3 (%TST) was significantly lower in the severe OSA group. SpO_2 values could be associated with healthcare complications, such as cognitive dysfunction, arrhythmia, CVD and ischemic stroke [34–36]. Monitoring SpO_2 or SpO_2<90 % levels allow clinicians to guide treatment strategies and alleviate potential complications associated with oxygen desaturation during sleep. In our study, reflecting the severity of hypoxia, SpO_2<90 % levels were significantly higher, while the mean and minimum SpO_2 values were significantly lower in the severe OSA group.

Since age, gender, menopause, and statin therapy affect PCSK9 levels [19], we included age-matched males not undergoing statin therapy. As an advantage, the sampling was conducted prior to the Covid-19 period. Additionally, we excluded patients with conditions that might induce inflammation.

The limitations of our study include a relatively small sample size and the absence of patients with mild and moderate OSA. Furthermore, the participants lacked radiological evidence of atherosclerotic lesions, precluding the monitoring of CVD and ischemic vascular events. Serum PCSK9 levels were only measured via ELISA, without the opportunity to assess PCSK9 gene expression levels to investigate functional status. Furthermore, due to the method used to measure serum IL-6 levels, with a quantitation limit of 1.50 pg/mL, the analysis, particularly in control subjects, often yielded results near this threshold.

Conclusions

Although we did not observe significant increases in PCSK9 levels, our study is of clinical importance because it is the first study showing a relationship between PCSK9 and inflammation markers in patients with severe OSA. In addition, our findings reveal the potential value of adropin, in combination with BMI, as a valuable indicator of assessing inflammation and the severity of OSA. Further investigations are necessary to uncover the PCSK9's expression or role in the inflammatory processes occurring within atherosclerotic plaques or impaired adipose tissue in individuals with sleep disorders.

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