

The Relationship Between Leptin Levels and CPAP Treatment: A Cluster Analysis

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Abstract

Background: Leptin is an appetite-suppressing hormone, released by adipose tissues, that plays an important role in severe obstructive sleep apnea syndrome (OSAS). However, it is unclear whether leptin levels are a useful OSAS biomarker. This study assessed the effect of continuous positive airway pressure (CPAP) treatment for OSAS according to leptin levels using a cluster classification based on OSAS clinical features.

Methods: Hierarchical cluster analysis was performed on 97 patients with OSAS who had been diagnosed via polysomnography. We also evaluated the adherence of CPAP data after 6 months of CPAP administration.

Results: Cluster 1 (49 subjects, 50.5%) had severe OSAS, were obese, and had normal leptin levels. Cluster 2 (6 subjects, 6.2%) had the most severe OSAS, were obese, normal leptin levels, and high adiponectin levels. Cluster 3 (11 subjects, 11.3%) had the most severe OSAS, severe obesity, and the highest leptin levels. Cluster 4 (31 subjects, 32%) had the most severe OSAS, severe obesity, and high leptin levels. After CPAP treatment, EDS improved in all clusters. In Clusters 3 and 4, leptin levels were significantly reduced after treatment.

Conclusions: To establish if leptin can be a biomarker for CPAP treatment, it is necessary to elucidate the mechanisms of lipid metabolism, leptin, and ventilatory responses in OSAS patients, for which further studies are necessary.

Background

Adult obstructive sleep apnea syndrome (OSAS) is classified as a sleep-related respiratory disorder, and can be fatal if complicated with cardiovascular disease [1]. The first-line of treatment for moderate or severe OSAS is continuous positive airway pressure (CPAP) prescribed according to the apnea-hypopnea index (AHI) [2]. Although polysomnography (PSG) is not conducted in many cases, people diagnosed with OSAS and who receive CPAP treatment is increasing due to the increase in out-of-center sleep testing [3].

One of the main goals of CPAP treatment is to control cardiovascular disease. However, most studies investigating the efficacy of CPAP treatment have been cohort trials, and randomized control trials [4] and a meta-analysis [5] have failed to find any significant CPAP-induced OSA improvement. Moreover, some reports have shown that moderate OSA confers a survival advantage in the elderly [6], and that OSA has a protective effect in cases of myocardial infarction [7]. Furthermore, although various factors can predict adherence to CPAP [8], it is difficult to comply with CPAP. There are reports that eHealth interventions and education can enhance adherence [9], but no strong solution has been found. Therefore, treatment decisions based on a single measure (e.g. AHI) are limited and that individual patient evaluation is necessary. Evaluation by phenotype, for example, is an appropriate tool for patient selection and CPAP treatment management [10].

The most distinctive OSAS phenotype is obesity hypoventilation syndrome (OHS). Burwell et al. described Pickwickian Syndrome, now known as OHS, more than 60 years ago, which was characterized by morbid obesity, somnolence, cyanosis, daytime hypoventilation, erythrocytosis, and right heart failure [11]. Currently, OHS is recognized as a type of OSAS that is more severe in people with obesity and aspects of sleep-related hypoventilation syndrome. The main OHS condition is that chronic hypoventilation causes hypercapnia. Specifically, the respiratory system is affected by (1) increased mechanical load, (2) sleep apnea syndrome, and (3) leptin resistance [12]. Leptin is an adipokine, an appetite-suppressing hormone, and is involved in oxidative stress, inflammation, thrombosis, and arteriosclerosis. Furthermore, leptin may predispose an individual to cardiovascular disease development [13, 14]. Leptin enhances ventilation response through the nervous system; thus, leptin resistance in obesity can diminish ventilatory response and may play an important role in OHS. [12, 15]. It is unclear whether leptin could be an OSAS biomarker [16, 17]. This study used cluster classification based on clinical features of OSAS patients to examine the effects of CPAP treatment using leptin as a biomarker. The aim was to promote a change in OSAS evaluation from evaluation based on AHI alone to evaluation based on the phenotype, in order to resolve the controversies surrounding evaluation of the treatment effectiveness.

Methods

The Clinical Research Ethics Committee of the Nihon University Hospital (protocol number RK-170509-07) approved our study. Written informed consent was waived by the ethics committee. All protocols and practices were conducted in accordance with the World Medical Association's Declaration of Helsinki.

Subjects

Between November 24, 2015 and January 9, 2018, 97 OSA patients visited the Nihon University Sleep Center, were diagnosed based on PSG results, and registered in a database. All patients at the Nihon University Itabashi Hospital Sleep Center during that period underwent overnight PSG and received CPAP treatment for at least 6 months. Only the patients whose clinical records and PSG data were available were enrolled in the present study. Exclusion criteria included patients who did not agree to participate in the study, were aged < 20 years, and had not undergone PSG and/or respiratory function tests.

Polysomnography

A full, attended PSG was performed in all cases. Measurement items were electroencephalogram, electrooculogram, electrocardiogram, electromyogram, nose and mouth airflow, chest and abdomen movement, and peripheral capillary oxygen saturation (SpO₂). Apnea was defined as airflow cessation in the nose and mouth that lasted at least 10 s. Hypopnea was defined as decreased airflow, thoracic excursion, or decreased oxygen desaturation below 3% of the previous baseline value, and a decreased abdominal excursion below 50%. AHI was calculated as the apnea and hypopnea counts per hour of sleep. The diagnostic OSA criterion was an AHI of ≥ 5 . The OSA severity was defined as mild (AHI = 5–15), moderate (AHI = 15–30), severe (AHI ≥ 30), and most severe (AHI ≥ 60). In Japan, it is standard to initiate CPAP if AHI ≥ 20 , and most patients in this study had AHI ≥ 20 . The mean and minimum SpO₂

values were also calculated from PSG data. Baseline clinical features (height, body weight, Epworth Sleepiness Scale (ESS)) were assessed. Height and weight were measured at the first medical examination, and at the same time, daytime sleepiness was evaluated using ESS in the questionnaire when the medical history was taken.

Blood tests

Blood counts and general biochemical testing data were obtained from general medical records. Serum leptin and adiponectin levels were measured in all patients. Leptin and adiponectin were measured using the Human Leptin Assay Kit (catalog number #27775; IBL, Inc., Gunma, Japan) and the Human Adiponectin ELISA Kit (catalog number #CY-8050; CircuLex, Inc., Nagano, Japan), respectively.

Spirometry

Conventional spirometry was performed using a Chestak auto-spirometer (Chest Co., Tokyo, Japan). Spirometric predictions were obtained from the literature [18], and for the arterial blood gas analysis were taken from the radial artery in a sitting position. The criterion for obstructive ventilatory dysfunction was defined as 70% or less per second, and the criterion for restrictive ventilatory dysfunction was 80% or less vital capacity.

ESS score

Daytime sleepiness was assessed using the ESS 19, a fully validated 8-item self-administered questionnaire. Each item is scored on a scale of 0–3, with a score of more than 10 out of a total possible score of 24 scores indicative of daytime sleepiness.

CPAP adherence

Before starting CPAP treatment, the CPAP therapist explained treatment precautions (e.g. how to put on and take off the mask, operate CPAP, and daily maintenance). Mask fitting was performed to select the appropriate mask. All patients were able to use CPAP more than 70% or more than 4 hours. The residual AHI, ESS score, leptin, adiponectin, high-sensitivity C-reactive protein (h-CRP), and body mass index (BMI) were measured six months after CPAP treatment. With regard to cardiovascular risk, although long-term observation is necessary, 6 months was considered sufficient for observing changes in the molecular pathology.

Statistical analysis

Ward's method was suitable for this study considering the relatively few samples were classified into four clusters. The cluster analysis (Ward's method) resulted in four subpopulations; the resulting data were tested for equality of variance between four clusters. For clusters with equal variance, a one-way ANOVA with Tukey's all column comparison test was used for intergroup comparisons. Otherwise, intergroup comparisons were performed using the nonparametric Games-Howell test. For F-values > 4, a p-value of < 0.05 was considered statistically significant. Analyses were performed using software Text Explorer module of JMP Pro 13.

The required number of subjects was determined from the independent variables while performing a multi-group comparison of each cluster, and the sample size was sufficient ($n > 64$) even when the power was set to 80%. Normality of the variables was checked, and the results are presented as mean \pm standard deviation (SD) values. We selected the four variables, AHI, BMI, leptin and adiponectin into four clusters (Table 1). AHI is considered to be the most important criterion for treatment indication [19]. High BMI is the most important factor in the severity of OSAS [20]. In obese patients, leptin and adiponectin are the most important biomarkers of lipid metabolism [21].

Results

Patient characteristics

The cluster analysis identified four clusters. Compared to Clusters 1 and 2, Cluster 3 and 4 were younger, had a higher male:female ratio, a higher BMI and history of smoking. The ESS scores were elevated in Cluster 3 compared to other clusters (Table 1).

Table 1
Summary of the main features in the identified clusters

	All Clusters (n = 97)	Cluster 1 (n = 49)	Cluster 2 (n = 6)	Cluster 3 (n = 11)	Cluster 4 (n = 31)	P-value
Age	56 \pm 13	57 \pm 11	65 \pm 13	52 \pm 15	51 \pm 12	< 0.0001
Sex (male) %	80	86	90	60	75	0.462
Body weight, kg	81 \pm 1.7	73 \pm 11	78 \pm 17	94 \pm 18	93 \pm 15	0.0001
Body mass index, kg/m ²	29 \pm 5.1	26 \pm 2.6	28 \pm 5.8	34 \pm 6.7	33 \pm 3.5	< 0.0001
Have never smoked %	16	38 \pm 28	20 \pm 31	40 \pm 41	56 \pm 16	0.562
ESS score	8.7 \pm 4.8	8.7 \pm 4.8	7.3 \pm 3.6	9.4 \pm 4.8	8.7 \pm 5.3	0.74
All data are expressed as mean + SD values.						
The P-values are derived by ANOVA with Turkey's post hoc tests.						
ESS: Epworth Sleepiness Scale						

The 97 OSA patients had a mean age of 56.0 years (22 to 82 years) and 80% were men, around 16% were current smokers, and the mean ESS score was 8.7. Of the identified clusters, Clusters 3 and 4 exhibited high leptin levels. The leptin/adiponectin ratio was high in Cluster 3, despite a similar BMI in Clusters 3 and 4. Cluster 2 exhibited high adiponectin levels.

Table 2 shows the PSG results. Of the enrolled OSA patients, 17 (18%) had moderate (AHI 20–30/h), 45 (46%) severe (AHI 30–60/h), and 35 (36%) most severe (AHI \geq 60) OSA, with a mean AHI of 57/h. The

data related to sleep-disordered breathing (obstructive apnea, apnea index, AHI, oxygen desaturation index 3%, mean SpO₂, and arousal index) were worse in Cluster 3, followed by Cluster 4.

Table 2
Sleep characteristics

	All Cluster (n = 97)	Cluster 1 (n = 49)	Cluster 2 (n = 6)	Cluster 3 (n = 11)	Cluster 4 (n = 31)	P Value
Wake time (%)	14 ± 10	13 ± 10	22 ± 14	14 ± 7.5	12 ± 8.3	0.952
NREM1 (%)	47 ± 19	40 ± 15	67 ± 22	56 ± 19	49 ± 20	0.968
NREM2 (%)	37 ± 15	42 ± 13	25 ± 18	28 ± 17	35 ± 14	0.67
NREM3 (%)	3.0 ± 4.5	2.9 ± 3.9	0.2 ± 0.7	3.5 ± 5.5	3.8 ± 5.5	0.503
REM (%)	13 ± 6.3	14 ± 5.7	11.7 ± 11.7	12 ± 4.8	12 ± 5.8	0.128
AHI (/hr)	57 ± 68	40 ± 15	60 ± 20	72 ± 25	84 ± 30	< 0.0001
CA (/hr)	0.7 ± 2.3	0.5 ± 1.2	2.7 ± 6.7	0.9 ± 2.6	0.3 ± 0.7	0.31
OA (/hr)	23 ± 20	17 ± 15	37 ± 31	37 ± 26	27 ± 20	0.01
MA (/hr)	3.7 ± 8.8	1.9 ± 3.7	13 ± 21	2.2 ± 5.1	5.4 ± 10	0.01
Apnea (/hr)	32 ± 43	20 ± 16	55 ± 26	42 ± 27	46 ± 75	0.113
Hypopnea (%)	23 ± 14	20 ± 9	11 ± 12	29 ± 21	29 ± 17	0.471
Mean SpO ₂ (%)	94 ± 2.6	95 ± 1.8	94 ± 3.0	92 ± 3.4	93 ± 2.9	0.321
Lowest SpO ₂ (%)	72 ± 13	76 ± 11	67 ± 14	62 ± 3	68 ± 12	0.458
SpO ₂ > 90% (%)	9.7 ± 14	5.0 ± 9.4	13 ± 16	18 ± 19	15 ± 17	< 0.0001
SpO ₂ > 85% (%)	4.9 ± 9.7	2.4 ± 6.4	6.7 ± 12	10 ± 14	7.5 ± 11	< 0.0001
Arousal (/hr)	54 ± 20	46 ± 14	74 ± 19	68 ± 29	60 ± 20	< 0.0001
All data are expressed as mean + SD values.						
The P-values are derived by ANOVA with Turkey's post hoc tests.						
NREM: Non Rapid Eye Movement; REM: Rapid Eye Movement; AHI: Apnea-Hypopnea Index; CA: Central apnea; OA: Obstructive apnea; MA: Mixed Apnea; SpO ₂ : peripheral capillary oxygen saturation						

Table 3 shows the lung function test results. Although no ventilatory dysfunction was observed, forced vital capacity and forced expiratory volume were lower in Clusters 2 and 3. There were no significant differences regarding peripheral airway obstruction.

Table 3
Lung function tests

	All Clusters (n = 97)	Cluster 1 (n = 49)	Cluster 2 (n = 6)	Cluster 3 (n = 11)	Cluster 4 (n = 31)	P-value
VC (%)	112.8 ± 15.7	100 ± 16	100 ± 16	1050 ± 15	113 ± 14	0.03
FVC (L)	3.8 ± 0.8	3.9 ± 0.8	3.2 ± 0.6	3.2 ± 0.9	3.9 ± 0.8	0.02
FVC (%)	110 ± 16	113 ± 16	98 ± 16	100 ± 17	110 ± 16	0.28
FEV _{1.0} (L)	2.8 ± 0.6	2.0 ± 0.7	2.5 ± 0.6	3.1 ± 0.8	2.8 ± 0.8	0.15
FEV _{1.0} (%)	102 ± 17	104 ± 15	101 ± 17	94 ± 24	103 ± 16	0.64
%FEV _{1.0}	75 ± 7.5	74 ± 7.7	78 ± 8.6	76 ± 7.1	79 ± 4.3	0.95
V50	65 ± 25	62 ± 22	69 ± 31	62 ± 21	67 ± 29	0.37
V25	65 ± 25	37 ± 19	51 ± 39	33 ± 14	45 ± 18	0.18
V50/25	5.0 ± 4.1	5.4 ± 4.2	5.1 ± 3.0	4.1 ± 1.0	5.0 ± 4.2	0.21
DL CO(%)	86 ± 21	92 ± 20	71 ± 16	78 ± 20	86 ± 21	0.01
FeNO (ppb)	25 ± 15	25 ± 15	16 ± 7.5	29 ± 29	27 ± 10	0.24
All data are expressed as mean + SD values.						
The P-values are derived by ANOVA with Turkey's post hoc tests.						
VC: Vital Capacity; FVC: Forced Vital Capacity; FEV: Forced Expiratory volume; MMF: maximal mid-expiratory flow						

According to the blood test results (see Table 4), the number of white blood cells and liver function parameters increased in Clusters 3 and 4. High-sensitivity test values that detect C-reactive protein (CRP) also increased, particularly in Cluster 3.

Table 4
Blood chemistry analysis

	All Clusters (n = 97)	Cluster 1 (n = 49)	Cluster 2 (n = 6)	Cluster 3 (n = 11)	Cluster 4 (n = 31)	P-value
WBC, mL	6.6 ± 1.7	6.5 ± 1.4	5.7 ± 1.6	7.4 ± 2.1	7.0 ± 2.1	0.315
T-bilirubin, mg/dl	0.57 ± 0.28	0.59 ± 0.28	0.62 ± 0.23	0.53 ± 0.36	0.55 ± 0.27	0.843
AST, U/L	27.7 ± 16.2	23 ± 13	23 ± 15	34 ± 51	35 ± 23	0.34
ALT, U/L	35.0 ± 32.0	28 ± 23	18 ± 13	27 ± 24	48 ± 55	<0.0001
HDL-C, mg/dl	50 ± 13	50 ± 12	54 ± 16	51 ± 20	47 ± 11	0.85
LDL-C, mg/dl	115 ± 35	120 ± 27	95 ± 36	119 ± 24	115 ± 35	0.21
TG, mg/dl	198 ± 195	189 ± 131	103 ± 42	200 ± 92	199 ± 95	0.35
eGFR ml/min/1.73 m ²	69 ± 22	71 ± 15	43 ± 34	66 ± 17	70 ± 28	0.234
UA, mg/dl	5.9 ± 1.6	6.3 ± 1.9	6.0 ± 1.3	6.4 ± 1.4	5.6 ± 1.5	0.605
h-CRP, ng/ml	0.19 ± 0.27	0.21 ± 0.27	0.21 ± 0.32	0.36 ± 0.14	0.26 ± 0.29	0.276
HbA1c, %	6.0 ± 4.6	6.0 ± 0.6	6.0 ± 0.6	6.2 ± 0.4	6.1 ± 0.5	0.62
NT-proBNP, pg/ml	434 ± 300	81.0 ± 166	355 ± 972	68.5 ± 148	238 ± 481	0.775
All data are expressed as mean + SD values.						
The P-values are derived by ANOVA with Turkey's post hoc tests.						
WBC: White blood cells; AST: aspartate aminotransferase; ALT: alanine aminotransferase; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglyceride; eGFR: estimated glomerular filtration rate; UA: uric acid; h-CRP: high-sensitivity C-reactive protein; HbA1c: hemoglobin A1c; NT por BNP: N-terminal pro-brain natriuretic peptide						

Cluster analysis

Cluster 1 (49 subjects, 50.5%) had severe OSAS, were obese, and normal leptin level. Cluster 1 primarily included middle-aged participants (mean age: 57 ± 12 years). The mean BMI of patients was 26 ± 2.6. Based on the BMI classification (normal weight, 18.5–24.9; overweight, 25.0–29.9; and obese, > 30), these patients were obese. The mean ESS score of this cluster was 8.7 ± 4.8, which indicates non-symptomatic excessive daytime sleepiness (EDS). The mean AHI, however, was 40 ± 15, which is below the overall average. Leptin and adiponectin levels were low compared to other clusters.

Cluster 2 (6 subjects, 6.2%) had the most severe OSAS, were obese, normal leptin levels, and high adiponectin levels. This cluster also mainly included older participants (mean age: 65 ± 13 years). Their

mean BMI was 28 ± 5.8 , indicating that they were mildly overweight. The mean ESS score of this cluster was 7.3 ± 3.6 , which is low, indicating that this cluster had the lowest likelihood of presenting with EDS symptoms among all clusters (Table 1). However, the AHI was 60 ± 20 , indicating severe apnea and hypopnea. Leptin levels were normal, and this cluster had the highest adiponectin levels of all clusters; however, the leptin/adiponectin ratio was the lowest among all clusters.

Cluster 3 (11 subjects, 11.3%) had the most severe OSAS, severe obesity, and the highest leptin levels. This cluster was centered on middle-aged patients (mean age: 52 ± 15 years). The mean BMI of patients in this cluster was 34 ± 6.7 , which indicates that they had severe obesity. The mean ESS score was 9.4 ± 4.8 (Table 1). The AHI was 72 ± 25 , which is far higher than the overall mean AHI. This cluster accordingly comprised the most severe OSAS patients who had severe obesity (Table 2). Leptin levels and the leptin/adiponectin ratio were the highest of all clusters.

Cluster 4 (31 subjects, 32%) had the most severe OSAS, severe obesity, and high leptin levels. It mainly included middle-aged patients (mean age: 51 ± 12 years). The mean BMI was 33 ± 3.5 , indicating severe obesity. The mean ESS score was 8.7 ± 5.3 (Table 1). The AHI was 84 ± 30 , which is much higher than the overall AHI mean. This cluster was accordingly composed of the most severe OSAS patients, and had mild-to-moderate symptoms (Table 2). Leptin levels were high and the leptin/adiponectin ratio was comparable to that of Cluster 3.

Table 5 shows AHI, ESS, leptin, adiponectin, h-CRP, BMI before and after 6 months using CPAP. After CPAP treatment, EDS improved in all clusters. In Cluster 3, which had high leptin levels before treatment, leptin levels were significantly reduced after treatment. These results were not accompanied by BMI changes. The adiponectin levels did not change. Clusters 3 and 4 h-CRP value improved but was not significant.

Table 5
Six months after CPAP treatment

		All Clusters (n = 97)	Cluster 1 (n = 49)	Cluster 2 (n = 6)	Cluster 3 (n = 11)	Cluster 4 (n = 31)	P-value
AHI/hr	Before	57 ± 68	40 ± 15	60 ± 20	72 ± 25	84 ± 30	< 0.0001
	CPAP	3.4 ± 3.7	3.3 ± 3.0	3.5 ± 2.8	2.6 ± 0.9	3.7 ± 5.2	0.67
ESS score	Before	8.7 ± 4.8	8.7 ± 4.8	7.3 ± 3.6	9.4 ± 4.8	8.7 ± 5.3	0.74
	CPAP	5.5 ± 3.7	5.2 ± 3.8	5.5 ± 2.3	7.6 ± 4.6	5.2 ± 3.4	0.65
Leptin (pg/ml)	Before	14 ± 11	7.0 ± 4.3	6.8 ± 3.8	39 ± 8.1	16 ± 7.0	< 0.0001
	CPAP	13 ± 12	8.3 ± 8.3	8.0 ± 2.1	27 ± 17	17 ± 9.0	< 0.0001
Adiponectin (ng/ml)	Before	15 ± 7.0	15 ± 7.0	64 ± 10	10 ± 4.1	16 ± 10	0.35
	CPAP	19 ± 22	16 ± 8.0	57 ± 9.1	10 ± 4.5	8.6 ± 4.9	0.23
h-CRP ng/ml	Before	0.19 ± 0.27	0.21 ± 0.27	0.21 ± 0.32	0.36 ± 0.14	0.26 ± 0.29	0.276
	CPAP	0.20 ± 0.58	0.22 ± 0.13	0.19 ± 0.33	0.28 ± 0.31	0.18 ± 0.10	0.276
Body mass index, kg/m ²	Before	29 ± 5.1	26 ± 2.6	28 ± 5.8	34 ± 6.7	33 ± 3.5	< 0.0001
	CPAP	28 ± 5.2	25 ± 2.7	27 ± 5.5	34 ± 6.2	32 ± 4.0	< 0.0001
All data are expressed as mean + SD values.							
The P-values are derived by ANOVA with Turkey's post hoc tests.							
AHI: apnea hypopnea index; ESS: Epworth Sleepiness Scale; h-CRP: high-sensitivity C-reactive protein; CPAP: continuous positive airway pressure							

Discussion

Summary of results

Four variables, AHI, BMI, leptin and adiponectin were selected. They are the most important factors to OSAS and Obesity [19–21]. The subjects were middle-aged patients around 50 years old, and had low levels of daytime sleepiness. The cluster analysis shows that the combination of high levels of AHI, BMI, and leptin can be considered an OHS phenotype, and are considered to be the most important phenotypes requiring CPAP treatment in this study. On the other hand, there are phenotypes where leptin is not elevated even in severe OSAS and obesity, making it necessary to examine CPAP effectiveness in these individuals. For example, just as obesity and metabolic syndrome [22] depend on the presence or absence of metabolic disorders, whether apnea is directly affecting metabolic dysfunction is important in increasing cardiovascular risk. Therefore, if it can be evaluated, it will help determine the effectiveness of CPAP. However, evaluating it accurately is difficult and requires further study.

Subgroup-specific links to adipokines

This study investigated whether adipokines, especially leptin, could be a biomarker for efficacy of CPAP. Leptin is a hormone produced by fat cells that suppresses appetite, and may play an important role in OHS since it enhances ventilation response through the nervous system. Obesity may cause leptin resistance in the central nervous system, leading to diminished ventilatory responses [15]. Previous studies did not identify leptin as a biomarker for efficacy of CPAP as obesity could not be ruled out as a confounding factor. Therefore, we attempted to eliminate any confounding effects by separating clusters according to BMI. Consequently, Clusters 1 and 2 had normal leptin levels while Clusters 3 and 4 had high leptin levels; Clusters 3 and 4 had different leptin levels but the same degree of obesity. Adiponectin and leptin-adiponectin ratios were calculated, but the differences between the clusters were unclear.

Prevalence of OSAS in women

Leptin levels differ between men and women; however, our data did not take these differences into account, as the main purpose was to determine changes before and after CPAP use in all patients, regardless of the sex. Previous studies have reported that OSAS prevalence in women is 9% (24% for men) [23]. Although there was no significant difference in the number of men and women, and the number of participants was small, the proportion of women in Cluster 3 was higher than other clusters. It has been reported that OSAS frequency suddenly increases in women after menopause and that postmenopausal women's hormone changes (particularly the decrease in progesterone) suppresses respiratory stimulation [24, 25]. Even then, men are more likely to suffer from OSAS, probably because of the shape of the throat and airways, respiratory stimulating hormone, and that upper body obesity is more common in men (women are more obese in the lower body) [26]. Conversely, OHS is reportedly more prevalent in women than OSAS [27]. Moreover, morbid obesity (BMI > 40) may be more common in women than men and associated with a higher OHS prevalence. Furthermore, OHS is more commonly associated with heart disease than OSAS, even at similar BMIs, and some reports have claimed that the untreated OHS mortality is 46% (over 50 months) [28]. In this study, Clusters 3 and 4 may have had more women because OHS was more common in these two clusters.

Therapeutic effects of CPAP

The therapeutic effect of CPAP on OSAS and current treatment practice are based on findings published many years ago [29]. However, a previous randomized control trial failed to find a therapeutic effect of CPAP on OSAS [4]. Therefore, selecting and using more cases than those selected by AHI-only criteria is necessary. Nonetheless, CPAP and non-invasive ventilation are effective treatments for OHS, the most serious form of OSAS, but blood gas analysis collection and invasive procedures are required to meet the diagnostic criteria [30]. Even if the criteria for OHS were not met, as was the case in this study, grouping by phenotype would make it possible to divide the population according to the CPAP treatment effect. Indeed, Clusters 3 showed markedly improved leptin levels after CPAP, and Clusters 3 and 4 showed a decline in CRP although this was not significant. Conversely, although OSAS severity according to the AHI is high, a group with moderate obesity and low leptin levels may have low risk of abnormal lipid metabolism.

In this group, CPAP may not decrease cardiovascular disease because of normal lipid metabolism. Several previous studies have reported that CPAP improves leptin level, while others have not [5]. Assessing efficacy based on a single factor is difficult; however, it is possible to find a population for which treatment is effective by cluster classification, which combines multiple factors. This study was limited by the small sample size, cluster sizes, and narrow capacity for generalization. Although the risk of cardiovascular disease was low, the patient number was small; further studies need to include a larger number of cases. In addition, we believe that the abnormal lipid metabolism in OSAS should be included as a factor in future cluster analyses. To generalize the present results, it is necessary to consider larger sample sizes, and non-hierarchical cluster classification should also be performed and re-evaluated.

Conclusions

To establish whether leptin could be a biomarker for CPAP treatment, it is necessary to elucidate the mechanisms of lipid metabolism, leptin, and ventilatory responses in patients with OSAS and accumulate more cases. A prospective study should examine whether the leptin level is a predictor of the CPAP effect on cardiovascular disease.

Abbreviations

OSAS
obstructive sleep apnea syndrome
CPAP
continuous positive airway pressure
AHI
apnea-hypopnea index
PSG
polysomnography
OHS
obesity hypoventilation syndrome

SpO2
peripheral capillary oxygen saturation
ESS
Epworth Sleepiness Scale
h-CRP
high-sensitivity C-reactive protein
BMI
body mass index
CRP
C-reactive protein
EDS
excessive daytime sleepiness

Declarations

Ethics approval and consent to participate: All procedures involving human participants were in accordance with the ethical standards of the Clinical Research Ethics Committee of the Nihon University Hospital (protocol number RK-170509-07) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The need for written informed consent was waived by the ethics committee.

Consent for publication: Not applicable.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: AH, HM, YK, SY, AF, HM analyzed and interpreted the patient data regarding the OSAS. SO, SS, HH, TA, SM analyzed and interpreted the patient data regarding the CPAP treatment. YG was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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