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Original Article

The Relationship between Leptin Levels and Continuous Positive Airway Pressure Treatment: A Cluster Analysis

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Sleep Sci

Abstract

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

Materials and Methods We performed a hierarchical cluster analysis of data from 97 OSAS patients diagnosed via polysomnography. We also evaluated the effect after 6 months of CPAP administration.

Results Clusters 1 (49 patients; 50.5%) and 2 (6 patients; 6.2%) presented normal leptin levels, and clusters 3 (11 patients; 11.3%) and 4 (31 patients; 32%) presented high leptin levels. Clusters 3 and 4 presented different leptin levels, but the same degree of obesity. After treatment, the levels of excessive daytime sleepiness improved in all clusters. In Cluster 3, leptin levels were significantly reduced after treatment.

Conclusion Using the conventional diagnostic method of the apnea-hypopnea index, it was not clear whether leptin is a useful biomarker for the CPAP treatment. However, it may be helpful for particular clusters, including obese women, and where particular populations require CPAP treatment.

Keywords
- obstructive sleep apnea
- phenotype
- cluster analysis
- leptin
- continuous positive airway pressure

Introduction

Adult obstructive sleep apnea syndrome (OSAS) is classified as a sleep-related respiratory disorder, and it can be fatal if complicated by cardiovascular disease.[1] The first line of treatment for moderate or severe OSAS is continuous positive airway pressure (CPAP), which is prescribed according to the apnea-hypopnea index (AHI).[2] Polysomnography (PSG) is not used in many cases, but the number of people diagnosed with this syndrome and receiving this treatment is increasing because of the rise in out-of-center sleep testing.[3]

One of the main goals of the CPAP treatment is to control cardiovascular disease. However, most studies on the efficacy of this treatment have been cohort trials. Randomized

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control trials[4] and meta-analyses[5] have failed to find any significant treatment-induced improvement in apnea. Some reports have shown that moderate obstructive sleep apnea (OSA) confers a survival advantage in older people[6] and has a protective effect in cases of myocardial infarction.[7] It is also difficult to comply with the CPAP treatment, although various factors can predict adherence.[8] There are reports that eHealth interventions, and education can enhance adherence,[9] but no good solution has been found. Therefore, treatment decisions based on a single measure such as the AHI are limited, and individual patient evaluation is necessary. Evaluation by phenotype, for example, is an appropriate tool for patient selection and treatment management.[10]

The most distinctive OSAS phenotype is obesity hypoventilation syndrome (OHS), formerly known as Pickwickian syndrome, which was described by Bickelmann et al.[11] Characterized by morbid obesity, somnolence, cyanosis, daytime hypoventilation, erythrocytosis, and right heart failure,[11] OHS is now recognized as a type of OSA that is more severe in people with obesity and aspects of sleep-related hypoventilation syndrome. The main element of OHS is that chronic hypoventilation causes hypercapnia. The respiratory system is affected by increased mechanical load, sleep apnea syndrome, and leptin resistance.[12] Leptin is an adipokine, an appetite-suppressing hormone, involved in oxidative stress, inflammation, thrombosis, and arteriosclerosis.[13] It may also predispose an individual to develop cardiovascular disease.[14] Leptin enhances ventilation response through the nervous system, and leptin resistance in obesity can therefore diminish the ventilatory response and may play an important role in OHS.[12,15] However, it is unclear whether leptin could be a biomarker for OSAS.[5,16] The present study used cluster classification based on clinical features of OSAS patients to examine the effects of the CPAP treatment using leptin as a biomarker. The aim was to promote a change in the evaluation of sleep apnea from the use of the AHI alone to an evaluation based on the phenotype, to resolve the controversies surrounding the assessment of treatment effectiveness.

Materials and Methods

The Clinical Research Ethics Committee of the Nihon University Hospital (protocol number RK-170509-07) approved the present study and waived written informed consent. All protocols and practices followed the World Medical Association's Declaration of Helsinki.

Participants

Between November 24, 2015, and January 9, 2018, in the Nihon University Itabashi Hospital Sleep Center, 97 patients were diagnosed with OSA through PSG and registered in a database. During this period, all patients underwent overnight PSG and received the CPAP treatment for at least six months. Only patients whose clinical records and PSG data were available were enrolled in the present study. The exclusion criteria were patients who did not agree to participate in the study, were younger than 20 years of age, and had not undergone PSG and/or respiratory function tests.

Polysomnography

A full, attended PSG examination was performed in all cases. The measurement items were electroencephalogram, electrooculogram, electrocardiogram, electromyogram, nose and mouth airflow, chest and abdomen movement, and peripheral capillary oxygen saturation (SpO_2). Apnea was defined as airflow cessation in the nose and mouth that lasted at least 10 seconds. Hypopnea was defined as decreased airflow, thoracic excursion, or oxygen desaturation $< 3\%$ of the previous baseline value and a abdominal excursion $< 50\%$. The AHI was calculated as the apnea and hypopnea events per hour of sleep. The diagnostic criterion for OSA was an AHI ≥ 5. The degree of apnea 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 the CPAP treatment if the AHI is ≥ 20, which applied to most patients in the present study. The mean and minimum SpO_2 values were also calculated based on PSG data. Baseline clinical features (height, body weight, and Epworth Sleepiness Scale, ESS) were assessed. At the first medical examination, height and weight were measured, and daytime sleepiness was evaluated using the ESS as part of the questionnaire about medical history.

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 was measured using the Human Leptin Assay Kit (catalog number #27775; Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) and adiponectin using the Circulex Human Adiponectin ELISA Kit (catalog number #CY-8050; Medical & Biological Laboratories Co. Ltd., Tokyo, Japan).

Spirometry

Conventional spirometry used a Chestak autospirometer (Chest Co., Tokyo, Japan). Spirometric predictions were obtained from the literature,[17] and patient samples 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 $\leq 70\%$ per second, and the criterion for restrictive ventilatory dysfunction was a vital capacity of 80% or less.

Epstein Sleepiness Scale Score

Daytime sleepiness was assessed through the ESS, a fully validated 8-item self-administered questionnaire. Each item is scored on a scale of 0–3, and scores > 10 out of a total possible score of 24 indicate daytime sleepiness.

Effect of Treatment after Six Months

Before starting the CPAP treatment, the therapist explained treatment precautions (such as how to put on and take off the mask, how to operate the machine, and daily maintenance). Mask fitting was used to select the appropriate mask.

All patients were able to undergo the treatment more than 70% of the time or for more than 4 hours. The residual AHI, the ESS score, the levels of leptin, adiponectin, and high-sensitivity C-reactive protein (h-CRP), as well as the body mass index (BMI) were measured six months after the beginning of the treatment.

Statistical Analysis

The Ward method was considered suitable for the present study because the relatively few samples were classified into four clusters. The cluster analysis (Ward method) resulted in four subpopulations: clusters 1 and 2 presented normal leptin levels, and clusters 3 and 4 presented high leptin levels. Clusters 3 and 4 presented different leptin levels, but the same degree of obesity. The resulting data were tested for equality of variance among four clusters. For clusters with equal variance, a one-way analysis of variance (ANOVA) with the Tukey all-column comparison test was used for intergroup comparisons. Otherwise, intergroup comparisons used the nonparametric Games–Howell test. For F-values > 4, values of $p < 0.05$ were considered statistically significant. The analyses were made using the Text Explorer module of the JMP Pro 13 (SAS Institute Inc., Cary, NC, United States) software.

The required number of participants was determined from the independent variables while performing a multigroup comparison of each cluster. The sample size was sufficient ($n > 64$) even when the power was set to 80%. The normality of the variables was checked, and the results are shown as mean $\pm$ standard deviation (SD) values. We used the four variables—AHI, BMI, level of leptin, and level of adiponectin—to identify four clusters (►**Table 1**). The AHI was considered the most important criterion for treatment indication.[18] High BMI was the most important factor in the severity of OSAS.[19] In obese patients, leptin and adiponectin were the most important biomarkers of lipid metabolism.[20] The reason for the categorization into four clusters was that when we investigated the relationship between OSA and lipid metabolism, selecting two factors for each was found to provide a good balance between data characteristics and computational efficiency and accuracy. Another reason to choose four clusters could be interpretability. Making intuitive sense of more than four clusters in the context of the problem that we were investigating was difficult. However, four clusters provided a clear and meaningful way to categorize the data.

Results

Patient Characteristics

The analysis identified four clusters. Compared with the patients in clusters 1 and 2, those in clusters 3 and 4 were younger, had a higher male-to-female ratio, a higher BMI, and history of smoking. The ESS scores were higher in cluster 3 than in the other clusters (►**Table 1**). The 97 patients had a mean age of 56.0 (range: 22 to 82) years, and 80% were men. Approximately 16% were current smokers, and the mean ESS score was of 8.7. The patients in cluster 3 presented significantly higher leptin levels, and those in cluster 2 showed high adiponectin levels, but the difference was not significant.

►**Table 2** shows the PSG results. Overall, OSA was moderate in 17 (18%) of the enrolled patients (AHI: 20 to 30 events/h), severe in 45 (46%) (AHI: 30 to 60 events/h), and most severe in 35 (36%; AHI: $\geq$ 60 events/h), with a mean AHI of 57 events/h.

The data related to sleep-disordered breathing (obstructive apnea, apnea index, AHI, oxygen desaturation index $< 3\%$, mean SpO_2, and arousal index) were worse in cluster 3 than in cluster 4. Cluster 1 showed significantly milder symptoms than clusters 3 and 4. Leg movement and arousal are essential items for the evaluation of quality of sleep. However, we found no significant differences in leg movement and arousal. These data should affect CPAP adherence, but we observed good adherence on all four clusters. We therefore focused only on critical respiratory parameters and omitted leg movement and arousal data.

►**Table 3** shows the results of the lung function test. No ventilatory dysfunction was observed, but forced vital

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 (in years)	56 ± 13	57 ± 11	$65 \pm 13^*$	52 ± 15	51 ± 12	< 0.0001
Male sex (%)	80	86	90	60	75	0.462
Body weight (kg)	81 ± 1.7	$73 \pm 11^*$	78 ± 17	94 ± 18	93 ± 15	< 0.0001
Body mass index (kg/m^2)	29 ± 5.1	$26 \pm 2.6^*$	28 ± 5.8	34 ± 6.7	33 ± 3.5	< 0.0001
Subjects who had never smoked (%)	16	38 ± 28	20 ± 31	40 ± 41	56 ± 16	0.562
Score on the Epworth Sleepiness Scale	8.7 ± 4.8	8.7 ± 4.8	7.3 ± 3.6	9.4 ± 4.8	8.7 ± 5.3	0.74
Leptin (pg/mL)	14 ± 11	7.0 ± 4.3	6.8 ± 3.8	$39 \pm 8.1^*$	16 ± 7.0	< 0.0001
Adiponectin (ng/mL)	15 ± 7.0	15 ± 7.0	64 ± 10	10 ± 4.1	16 ± 10	0.35

Notes: Data are shown as mean $\pm$ standard deviation values, except for the male sex variable. The *p*-values were derived by analysis of variance (ANOVA) with Tukey post hoc tests. *Significant change from other clusters.

Table 2 Sleep characteristics by cluster.

	All clusters ($n = 97$)	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 (events/h)	57 ± 68	40 ± 15*	60 ± 20	72 ± 25	84 ± 30	< 0.0001
CA (events/h)	0.7 ± 2.3	0.5 ± 1.2	2.7 ± 6.7	0.9 ± 2.6	0.3 ± 0.7	0.31
OA (events/hr)	23 ± 20	17 ± 15	37 ± 31	37 ± 26	27 ± 20	0.01
MA (events/h)	3.7 ± 8.8	1.9 ± 3.7	13 ± 21	2.2 ± 5.1	5.4 ± 10	0.01
Apnea (events/h)	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 (events/h)	54 ± 20	46 ± 14*	74 ± 19	68 ± 29	60 ± 20	< 0.0001

Abbreviations: AHI, apnea-hypopnea index; CA, central apnea; MA, mixed apnea; NREM, nonrapid eye movement; OA, obstructive apnea; REM, rapid eye movement; SpO_2, peripheral capillary oxygen saturation.
Notes: All data are shown as mean ± standard deviation values. The *p*-values were derived by analysis of variance (ANOVA) with Tukey post hoc tests.
*Significant change from other clusters.

Table 3 Results of lung function tests by cluster.

	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 (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 (%)	102 ± 17	104 ± 15	101 ± 17	94 ± 24	103 ± 16	0.64
FEV_1 (%)	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
DLCO (%)	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

Abbreviations: DLCO, diffusing lung capacity for carbon monoxide; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; ppb, parts per billion; V25, flow at 25% of remaining vital capacity; V50/V25, ratio of V50 to V25; V50, flow at 50% of remaining vital capacity; VC, vital capacity.
Notes: All data are shown as mean ± standard deviation values. The *p*-values are derived by analysis of variance (ANOVA) with Tukey post hoc tests.

capacity and forced expiratory volume were lower in clusters 2 and 3. There were no significant differences in peripheral airway obstruction. The results of the blood test (see ►**Table 4**) showed that the number of white blood cells and liver function parameters increased in cluster 4. The values for the h-CRP test also increased, particularly in cluster 3, but this was not significant.

Cluster Analysis

The patients in cluster 1 (49; 50.5%) presented severe OSAS and normal leptin levels. This cluster primarily included middle-aged participants (mean age: 57 ± 12 years) with a mean BMI of 26 ± 2.6 kg/m^2. Based on the BMI classification (normal weight: 18.5 to 24.9 kg/m^2; overweight: 25.0 to 29.9 kg/m^2; and obese: > 30 kg/m^2), these patients were

Table 4 Results of blood chemistry analysis by cluster.

	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/1.73m^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

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; h-CRP, high-sensitivity C-reactive protein; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NT-proBNP, N-terminal pro-brain natriuretic peptide; TG, triglyceride; UA, uric acid; WBC, white blood cells.
Notes: All data are shown as mean ± standard deviation values. The *p*-values are derived by analysis of variance (ANOVA) with Tukey post hoc tests.

overweight. The mean ESS score of this cluster was of 8.7 ± 4.8, which indicates nonsymptomatic excessive daytime sleepiness. The mean AHI, however, was of 40 ± 15, which is below the overall average. The leptin and adiponectin levels were low compared with those of the other clusters.

The patients in cluster 2 (6; 6.2%) presented most severe OSAS, normal leptin levels, and high adiponectin levels. This cluster also mainly included older participants (mean age: 65 ± 13 years). Their mean BMI was of 28 ± 5.8 kg/m^2, indicating that they were overweight, and more so than those in cluster 1. Their mean ESS score was of 7.3 ± 3.6, which is low, indicating that this cluster had the lowest likelihood of having excessive daytime sleepiness (►**Table 1**). However, the AHI was of 60 ± 20, indicating severe apnea and hypopnea. The leptin levels were normal, and this cluster had the highest adiponectin levels of all clusters. The leptin-to-adiponectin ratio was the lowest in this cluster.

The patients in cluster 3 (11; 11.3%) presented most severe OSAS and the highest leptin levels. This cluster was largely composed of middle-aged patients (mean age: 52 ± 15 years). Their mean BMI was of 34 ± 6.7 kg/m^2, which means that they were severely obese. The mean ESS score was of 09.4 ± 4.8 (►**Table 1**). The AHI was of 72 ± 25, far higher than the overall mean. This cluster, therefore, contained the patients with severe apnea, who were also severely obese (►**Table 2**). The leptin levels and the leptin-to-adiponectin ratio were highest in this cluster.

The patients in cluster 4 (31; 32%) presented most severe OSAS and high leptin levels. It mainly included middle-aged patients (mean age: 51 ± 12 years). The mean BMI was of 33 ± 3.5 kg/m^2, indicating severe obesity. The mean ESS score was of 8.7 ± 5.3 (►**Table 1**). The AHI was of 84 ± 30, much higher than the overall mean. This cluster, therefore, contained patients with the most severe apnea, and mild-to-moderate symptoms (►**Table 2**). The leptin levels were high, and the leptin-to-adiponectin ratio was similar to that of cluster 3.

►**Table 5** shows the AHI, ESS score, the levels of leptin, adiponectin, and h-CRP, and BMI before and after six months of the CPAP treatment. After treatment, excessive daytime sleepiness improved in all clusters. Cluster 3, presented high leptin levels before the treatment, which were significantly reduced after treatment. These results were not accompanied by BMI changes. Neither did the adiponectin levels change. The h-CRP values improved in clusters 3 and 4, but this was not significant.

Discussion

Summary of Results

We selected four variables to use in the present study: the AHI, the BMI, the level of leptin, and the level of adiponectin, which are the most important factors to evaluate OSAS and obesity.[16,18–20] The study participants were aged ~ 50 years, with low levels of daytime sleepiness. The cluster analysis showed that the combination of high AHI, high BMI, and high leptin levels can be considered a phenotype in OHS, and it was the most important phenotype requiring CPAP treatment in the present study. We also found phenotypes in which the leptin levels were not elevated despite severe OSAS and obesity, making it necessary to examine the effectiveness of the CPAP treatment in these individuals. Just as obesity and metabolic syndrome[21] depend on the presence or absence of metabolic disorders, for example, evaluating whether apnea directly affects metabolic dysfunction is an important factor to increase the cardiovascular risk. If this can be evaluated, it will help to determine the effectiveness

Table 5 Comparison of results before and after six months of continuous positive airway pressure treatment.

		All clusters ($n = 97$)	Cluster 1 (n = 49)	Cluster 2 ($n = 6$)	Cluster 3 ($n = 11$)	Cluster 4 ($n = 31$)	*p*-value
AHI (events/h)	Before	57 ± 68	$40 \pm 15^{*}$	60 ± 20	72 ± 25	84 ± 30	< 0.0001
	After	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
	After	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	$39 \pm 8.1^{*}$	16 ± 7.0	< 0.0001
	After	13 ± 12	8.3 ± 8.3	8.0 ± 2.1	$27 \pm 17^{*}$	17 ± 9.0	< 0.0001
Adiponectin (ng/mL)	Before	15 ± 7.0	15 ± 7.0	64 ± 10	10 ± 4.1	16 ± 10	0.35
	After	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
	After	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	$26 \pm 2.6^{*}$	28 ± 5.8	34 ± 6.7	33 ± 3.5	< 0.0001
	After	28 ± 5.2	$25 \pm 2.7^{*}$	27 ± 5.5	34 ± 6.2	32 ± 4.0	< 0.0001

Abbreviations: AHI, apnea-hypopnea index; ESS, Epworth Sleepiness Scale; h-CRP, high-sensitivity C-reactive protein.
Notes: All data are shown as mean ± standard deviation values. The *p*-values are derived by analysis of variance (ANOVA) with Tukey post hoc tests.

of the CPAP treatment. However, evaluating it accurately is difficult and requires further study.

Subgroup-specific Links to Adipokines

The present study investigated whether adipokines, especially leptin, could be a biomarker for the efficacy of the CPAP treatment. Leptin is a hormone produced by fat cells, which suppresses appetite. It may play an important role in OHS because 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 the efficacy of CPAP because obesity could not be ruled out as a confounding factor. We attempted to eliminate any confounding effects by separating the clusters by BMI. Clusters 1 and 2 had normal leptin levels, and clusters 3 and 4 had high leptin levels. Clusters 3 and 4 had different leptin levels, but the same degree of obesity. The levels of adiponectin and leptin-to-adiponectin ratios were calculated, but the differences among the clusters were unclear.

Adiponectin is a protein hormone that plays an important role in regulating glucose levels as well as fatty acid breakdown. It is secreted by adipose tissue and has been shown to have anti-inflammatory and insulin-sensitizing properties. Studies[22] have shown that the CPAP treatment can increase adiponectin levels in OSA patients. However, our data showed no significant change before and after the CPAP treatment. The levels of adiponectin can vary among studies due to several factors, including sample population, method of measurement, health status of participants, and lifestyle factors.[23]

Prevalence of OSAS in Women

Leptin levels differ between men and women. However, our data did not take these differences into account because the main purpose was to determine changes before and after the use of CPAP in all patients, regardless of sex. Previous studies[24] have reported that the prevalence of OSAS in women is of 9% (and of 24% in men). In the present study, there was no significant difference in the number of men and women, but the number of participants was small. However, the proportion of women was higher in cluster 3 than in the other clusters. Studies[25,26] have reported that the frequency of OSAS suddenly increases in women after menopause and that postmenopausal hormone changes (particularly the decrease in progesterone) suppress respiratory stimulation. However, men are still more likely to experience OSAS, probably because of the shape of the throat and airways, the role of respiration-stimulating hormones, and because upper body obesity is more common in men (women are more likely to be obese in the lower body).[27]

However, OHS is more common in women (particularly in those who are postmenopausal) than in men.[28] It is also more likely to be associated with heart disease than OSAS, even in people with similar BMIs. One study[29] reported that the rate of mortality associated with OHS due to heart disease was of 46% (after a follow-up with a mean duration of 50 months). In the present study, clusters 3 and 4 may have contained more women because OHS was more common in these two clusters. Overall, the relationship between the conditions is complex and multifactorial because women are more likely to develop OHS because of a combination of factors, including higher body fat percentage, certain medical conditions, and hormonal changes. Further research is needed to better understand the mechanisms behind this relationship and to develop effective treatments.

Therapeutic effects of the CPAP Treatment

The therapeutic effect of CPAP on OSAS and the current treatment practice are on findings published many years

ago.[30] However, a previous randomized controlled trial[4] failed to find any therapeutic effect of CPAP on OSAS. It is therefore necessary to select and include more cases than it was possible using only the AHI. Noninvasive ventilation and CPAP are effective treatments for OHS, the most serious form of OSAS. However, blood gas analysis and invasive procedures are required to meet the diagnostic criteria.[31] Even if the criteria for OHS are not met, as was the case in the present study, grouping by phenotype would make it possible to divide the population by the likely effect of the CPAP treatment. Indeed, cluster 3 showed markedly improved leptin levels after the treatment, and clusters 3 and 4 showed a decline in h-CRP, although this was not significant. Conversely, even when patients have severe OSAS according to the AHI, those with moderate obesity and low leptin levels may have a low risk of presenting abnormal lipid metabolism.

Many studies have reported on the effect of CPAP on leptin levels. Some studies[32–36] have reported that it is effective in improving leptin levels. However, others[37–46] have found no improvement. The evidence regarding whether leptin alone is a biomarker for CPAP therapy is therefore mixed, but several studies[46] have suggested that gender and BMI may influence the results. This is the first time that patients have been classified by phenotype, suggesting that leptin may be a possible biomarker, especially in severely obese patients and women.[5,22,23]

In the group with moderate obesity and low leptin levels, CPAP may not decrease the risk of developing cardiovascular disease because of normal lipid metabolism. Several previous studies have reported that this treatment improves leptin levels, but others have not found this.[5] Assessing efficacy based on a single factor is difficult. However, it is possible to find a population for which treatment is effective using cluster classification and combining multiple factors. The present study was limited by the small sample size, cluster sizes, and narrow capacity for generalization. The risk of developing cardiovascular disease was low, but the number of patients was small; further studies need to include more cases. We also believe that the abnormal lipid metabolism in OSAS should be included as a factor in future cluster analyses. To generalize our results will require larger sample sizes, and we also recommend the use of non-hierarchical cluster classification. To establish whether leptin could be a biomarker for CPAP treatment, it will be necessary to examine the mechanisms of lipid metabolism, leptin levels, and ventilatory responses in more OSAS patients. A prospective study should examine whether leptin level is a predictor of the effect of CPAP on cardiovascular disease.

Conclusion

In the present study, when two factors each for OSA and obesity were classified into four clusters, we found that there was a group with very severe obesity but with differences in leptin levels before and after the CPAP treatment, regardless of changes in body weight. The population with changes in leptin levels included many women. In many previous studies, the authors have expressed their opinions on whether the levels of leptin and adiponectin are altered due to the therapeutic effects of CPAP, but no consensus has been reached. This time, by using cluster classification, we examined whether it is possible to resolve the discussion by dividing the sample into groups according to phenotypes. We believe that this cluster classification has implications in several fields and will influence future clinical practice and future research. However, the number of patients in each cluster was limited, and larger samples should be considered. In the future, therapeutic effects classified by phenotype, not just as a single item for the AHI, need to be examined.

Conflict of Interests

The authors have no conflict of interests to declare.

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