Clinical research

Cardiotrophin-1 and leptin as cardiovascular risk markers in male patients with obstructive sleep apnea syndrome

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Abstract

Introduction: Elevated cardiotrophin-1 (CT-1) and leptin levels are important risk factors for cardiovascular diseases (CVDs). Obstructive sleep apnea syndrome (OSAS) has also been reported to increase this risk. The aim of this study is to evaluate serum concentrations of CT-1 and leptin in patients with OSAS and whether there is a possible association between CT-1, leptin and OSAS severity.
Material and methods: Fifty newly diagnosed OSAS patients and thirty nonapneic snoring subjects participated in this study. Fasting serum lipid profile markers were evaluated. The measurements of serum CT-1 and leptin levels were carried out using human ELISA kits.
Results: Significant differences were found in the serum CT-1 and leptin levels between the two groups. Serum median CT-1 levels in patient and control groups, respectively, were 19.47; 8.23 pg/ml and leptin levels were 2.07; 1.29 ng/ml ($p < 0.001$). In the severe patient group, serum median CT-1 level was statistically significantly higher than the median level in the mild/moderate group. There was no correlation between patients' leptin and lipid profile parameters and CT-1 concentrations were not associated with triglyceride, cholesterol or LDL cholesterol levels except HDL cholesterol: CT-1 levels were positively correlated with HDL levels ($p = 0.02$).
Conclusions: Both CT-1 and leptin were significantly elevated in the patient group. Furthermore, CT-1 and leptin were associated with OSAS and CT-1 was associated with the disease severity.

Key words: cardiotrophin-1, cardiovascular disease, leptin, obstructive sleep apnea syndrome.

Introduction

Obstructive sleep apnea syndrome (OSAS) is a widespread disorder with a prevalence of 2% and 4% of women and men, respectively. The male:female ratio of OSAS is approximately 3 : 1 [1]. The OSAS is a complicated chronic disease which can be diagnosed by the frequency of apnea and hypopnea while performing polysomnography (PSG) and treated by continuous positive airway pressure (CPAP), oral orthodontic treatment, medications, etc. Performing polysomnography is considered to be the gold standard to diagnose the disease characterized by obstruction of the upper airway that can be followed by anoxia, snoring and daytime sleepiness [2]. The severity of OSAS is classified as the

apnea-hypopnea index (AHI), which is an indicator of apnea-hypopnea episodes per hour during sleep [3]. Obstructive sleep apnea syndrome (OSAS) has been reported to lead to metabolic abnormalities and increase the risk of cardiovascular diseases (CVDs) such as myocardial infarction, atrial fibrillation, congestive heart failure, cardiac sudden death or only systemic hypertension. Except systemic hypertension, the relation between OSAS and CVDs remains unclear. Intermittent hypoxemia secondary to repeated episodes of apnea/hypopnea may increase sympathetic activity. Sympathetic hyperactivation induces acute hemodynamic changes such as impairment of vasomotor reactivity, vascular inflammation, endothelial dysfunction, oxidative stress and metabolic disorders, and they all lead to complications during sleep in patients with OSAS [4–7]. Untreated OSAS may increase the incidence of non-fatal cardiovascular events, myocardial infarction and stroke. Despite all these explanations, the mechanisms how OSAS induces CVDs have not yet been fully elucidated.

Leptin, a 16 kDa protein, was discovered by Zhang *et al.* in 1994. Leptin is an adipose-tissue specific adipokine, a member of the interleukin-6 (IL-6) superfamily and involved in regulation of food intake, carbohydrate and lipid metabolism and hence energy hemostasis [8]. It also has multiple roles in the reproductive system as well as inflammatory and immune reactions [9]. Cardiotrophin-1 (CT-1) is a member of the IL-6 superfamily like leptin and shares glycoprotein (gp) 130 as a receptor. CT-1 is also known as a key regulator of energy homeostasis [10].

Body mass index (BMI) and gender are the baseline characteristic factors affecting serum leptin concentrations. Leptin levels in women are two to three times higher than men with the same BMI [11]. This situation can be explained by a higher ratio of adipose tissue, which is the main production tissue of leptin. But there are inconsistent results on the relationship between CT-1 and BMI. Obese subjects showed significantly lower CT-1 levels, and these levels were inversely correlated with BMI in a cohort of 81 patients with a high prevalence of diabetes, hypertension and metabolic syndrome [12]. In Rendo-Urteaga *et al.*' study, individuals with higher BMI had lower CT-1 levels [13]. In a study of overweight and normal weight adolescent subjects, CT-1 levels did not differ and BMI did not correlate with CT-1 [14]. In another study, compared with normal weight subjects, increased CT-1 levels were observed in obese patients [15]. In many studies the role of leptin in the pathogenesis of CVDs is reported, too [16–18]. Leptin exerts many atherogenic effects that are involved in atherosclerosis pathophysiology. Leptin provokes hypertension by promoting oxidative stress in endothelial cells. Leptin decreases vascular expandability by stimulating arterial smooth muscle cell migration and proliferation [19, 20]. It has been noted that for coronary heart disease, leptin is a novel risk factor [21]. However, the precise role of leptin has not been determined in the cardiovascular system, yet. As an originally identified factor, CT-1 could induce hypertrophy on cardiac myocytes *in vitro* [10], and also CT-1 has been identified as an active inducer of cardiomyopathies, cardiac hypertrophy, hypertension, atherosclerosis, valvular heart disease, congestive heart failure, and acute coronary syndrome [12–15, 22]. The aim of the present study is to evaluate the relationship between two risk factors for CVDs (CT-1 and leptin) and OSAS, and also the severity of the disease.

Material and methods

Study groups

Serum CT-1 and leptin concentrations were determined in patients who were suspected of sleep apnea and referred to the sleep unit at the Chest Diseases Clinic of Kayseri Education and Research Hospital, Turkey. The local ethics committee approved this study. All patients gave their written informed consent. Fifty newly diagnosed OSAS patients with excessive daytime sleepiness and thirty nonapneic controls participated in this study. In order not to effect leptin levels, we excluded the gender factor. So we chose only male participants for the patient and control groups in our study. Obstructive apnea was defined as a cessation of airflow for at least 10 s [23]. Hypopnea was defined as an arousal, or a reduction in respiratory airflow of ≥ 30% for at least 10 s plus an oxygen desaturation of > 4%, or a reduction of airflow of ≥ 50% for at least 10 s plus an oxygen desaturation of > 3%. AHI was defined as the mean number of hypopneic episodes plus apneic episodes per hour of sleep. According to their AHI, included subjects were grouped as mild/moderate (AHI between 5 and 29.9) and severe (AHI > 30). The control group consisted of patients with AHI < 5. AHI groups of patients are shown in Table I. Patient groups were created as group A (mild/moderate OSAS) and group B (severe OSAS), according to their AHI. There were 11 patients in group A (22%) and 39 patients in group B (78%) (Table II). Upper airway resistance, lung disease, heart failure, cardiovascular disease, cerebrovascular disease, chronic renal failure, systemic steroid therapy, hormone replacement therapy and central sleep apnea were the exclusion criteria in this study. By a standardized questionnaire, the participants' data (age, history of chronic and/or metabolic diseases, drugs, habits and cigarette smoking status)

Table I. Comparison of baseline characteristics and findings between OSAS patients and control group

Baseline characteristics and findings	OSAS	Control	*P*-value
N	50	30	–
Gender:			
Male	50	30	–
Age [years]	47.40 ±13.30[a]	43.23 ±10.50[a]	NS
AHI groups:			
0–4.9	–	30	< 0.001
5–14.9	1 (2%)	–	–
15–29.9	10 (20%)	–	–
> 30	39 (78%)	–	–
BMI [kg/m^2]:			NS
< 24.9	5 (10%)	3 (10%)	
25–29.9	20 (40%)	12 (40%)	–
30–34.9	15 (30%)	9 (30%)	–
35–39.9	5 (10%)	3 (10%)	–
> 40	5 (10%)	3 (10%)	–
Cigarette smoking (current)	50	30	–
Comorbidity:			
HT	1 (2%)	0 (0%)	–
Diabetes mellitus	4 (8%)	3 (10%)	
Triglycerides [mg/dl]	175.50[†] (130.75; 238.75)	166.0[†] (105.75; 192.75)	NS
Total cholesterol [mg/dl]	205.0[†] (178.0; 237.0)	195.0[†] (177.5; 217.5)	NS
LDL cholesterol [mg/dl]	128.34 ±31.72[a]	122.96 ±32.12[a]	NS
HDL cholesterol [mg/dl]	42.62 ±7.29[a]	43.20 ±6.33[a]	NS
Cardiotrophin-1 [pg/ml]	19.47[†] (10.34; 35.52)	8.23[†] (4.16; 22.37)	< 0.0001
Leptin [ng/ml]	2.07[†] (1.33; 4.59)	1.29[†] (0.7; 1.94)	< 0.0001

[†]*Mann-Whitney test; data are median and interquartile range (25%; 75%).* [a]*t-test; data are mean ± SD. NS – non-significant, OSAS – obstructive sleep apnea syndrome, AHI – apnea-hypopnea index, BMI – body mass index.*

Table II. Cardiotrophin-1 and leptin levels by OSAS classification of the patients

Parameter	Group A	Group B	*P*-value
OSAS severity	Mild/moderate	Severe	–
Number of patients	11	39	–
Cardiotrophin-1 [pg/ml]	10.34[†] (9.6; 19.72)	21.70[†] (12.81; 49.61)	0.013
Leptin [ng/ml]	2.07[†] (1.33; 2.89)	1.92[†] (1.33; 5.85)	NS

[†]*Mann-Whitney test. Data are median and interquartile range (25%; 75%). NS – non-significant, OSAS – obstructive sleep apnea syndrome.*

were recorded. Body mass index was calculated for each participant before the sleep study. All of the participants were current smokers. Four of the patients and three of the control subjects were diabetic. Only one participant in the patient group was hypertensive (Table I). Venous blood samples were drawn from all participants in the early hours on the morning after performing PSG.

Polysomnographic evaluation

Full night diagnostic PSG was applied to all subjects in a single-bedded room, in the Center for

Snoring and Sleep Disorders of the Chest Diseases Clinic of Kayseri Education and Research Hospital. During spontaneous sleep with a supervising technician, patients' electrocardiogram (ECG), electroencephalogram (EEG), right-left electrooculogram (EOG), submental and right/left tibial electromyogram (EMG), body position parameters, thoracic and abdominal respiratory movements, blood oxygen saturation with pulse oximetry and of course their nasal air flow were recorded throughout the night. Participants were divided into four groups according to their AHI severity (Table I). All patients slept at least 6 h, and at least 50% of their sleeping time was recorded.

Lipid profile

Fasting serum cholesterol (mg/dl), triglyceride (mg/dl), LDL cholesterol (mg/dl) and HDL cholesterol (mg/dl) levels were measured on an AU 2700 instrument (Beckman Coulter, California, USA). There was no statistically significant difference between the two groups in terms of lipid profile (Table I).

Cardiotrophin-1 and leptin measurement

On the morning after performing PSG, venous blood samples were drawn. They were centrifuged for 10 min at 2000 rpm, and serum was separated and stored at –80°C until the assays were determined. Serum CT-1 and leptin concentrations were measured by commercial enzyme linked immunosorbent assay kits (USCN Life Science Inc. and DIAsource ImmunoAssays S.A., respectively) based on the protocol provided by the manufacturers. The minimum detectable concentrations of CT-1 and leptin were 6.3 pg/ml and 0.04 ng/ml, respectively. The intra-assay and inter-assay coefficient of variation, respectively, were < 10% and < 10% for leptin and < 10% and < 12% for CT-1.

Statistical analysis

Statistical analysis was carried out using SPSS software version 23.0 for Windows (SPSS Inc., USA). The results of groups with normal distribution are presented as mean ± SD, and the median was used to present results that showed abnormal distribution. Categorical variables were presented as percentages. To determine significant differences between the groups, the *t*-test was used for data with normal distribution and the Mann-Whitney *U* test was used for data with non-normal distribution. To determine the relationship between the variables, for each group, Spearman's correlation coefficient was used. A receiver operating characteristic (ROC) analysis was performed to determine the best cut-off value. *P*-values < 0.05 were accepted as statistically significant.

Results

Patients' and control subjects' baseline characteristics and clinical findings are listed in Table I. Fifty male OSAS patients with a mean age of 47.40 ±13.30 years were enrolled in our study. The control group included 30 healthy male subjects, in terms of body mass index similar to the patient group, with a mean age of 43.23 ±10.50 years and without apneas during sleep or pulmonary diseases. No statistically significant difference in age was found between the two groups ($p > 0.05$). The median CT-1 level of the patients was statistically significantly higher than the median CT-1 level of the control group (19.47 pg/ml and 8.23 pg/ml respectively) ($p < 0.001$). The patients' serum leptin levels were found to be significantly higher than the levels of the control group. The difference between the median values of serum leptin in these two groups (2.07 ng/ml and 1.29 ng/ml respectively) was statistically significant ($p < 0.001$). The serum median level of CT-1 was statistically significantly higher in group B than the level in group A (21.70 pg/ml and 10.34 pg/ml respectively) but leptin levels were not different between the two OSAS groups (Table II). In order to evaluate the correlation between patients' lipid profile and CT-1 and/or leptin levels, we performed Spearman's correlation analysis. Although there was no correlation between patients' leptin and lipid profile parameters, their CT-1 levels were positively correlated with HDL levels ($p = 0.02$) (Table III). But CT-1 concentrations were not associated with triglyceride, cholesterol or LDL cholesterol levels. According to a ROC analysis of serum CT-1 and leptin, the ROC rederived cut-off level for CT-1 was 14.165 pg/ml (AUC: 0.736; 95% confidence inter-

Table III. *P* and *r* values of the Spearman's correlation analyses between cardiotrophin-1, leptin and lipoprotein levels in OSAS patients ($n = 50$)

Parameter	Triglycerides	Total cholesterol	LDL cholesterol	HDL cholesterol
Cardiotrophin-1	$p = 0.592$ $r = 0.078$	$p = 0.116$ $r = 0.225$	$p = 0.340$ $r = 0.138$	$p = 0.022^{*}$ $r = 0.323$
Leptin	$p = 0.622$ $r = –0.071$	$p = 0.734$ $r = –0.049$	$p = 0.955$ $r = 0.008$	$p = 0.332$ $r = –0.140$

**P < 0.05. OSAS – obstructive sleep apnea syndrome.*

val (CI): 0.619–0.854, 66% sensitivity, 70% specificity, $p < 0.001$) and the cut-off level for leptin was 1.74 ng/ml (AUC: 0.743; 95% CI: 0.636–0.850, 62% sensitivity, 63% specificity, $p < 0.001$) (Figure 1).

Discussion

The OSAS is a respiratory disorder characterized by recurrent episodes of apnea and hypopnea that results in the reduction or cessation of airflow [24]. In the literature there is evidence that overweight acts directly upon the pathophysiology of the narrowing of the upper airways and involvement of the oropharyngeal muscles, so obesity is a risk factor for OSAS [25]. The OSAS has a prevalence of approximately 40% in obese individuals, and about 70% of OSAS patients are diagnosed as obese [8]. In our study 50% of OSAS patients had BMI > 29.9 kg/m^2.

The OSAS has been reported to induce CVDs. The CT-1 and leptin have been found to play a significant role in obesity, and obesity clearly has an important role in OSAS. Leptin and CT-1 are both members of the IL-6 superfamily and described as risk factors of CVDs. In many studies the role of leptin in the pathogenesis of CVDs is reported [16–18]. But, in the cardiovascular system, the precise role of leptin has not been determined yet. The CT-1 was identified as an inducer of hypertrophy on cardiac myocytes *in vitro*. Its active inducer effects on cardiomyopathies, valvular heart disease, congestive heart failure, atherosclerosis, hypertension, cardiac hypertrophy and acute coronary syndrome have also been described [10, 12–15, 21]. Inflammation is an another potential risk factor in OSAS. Hypoxia leads to oxidative stress, activates the systemic inflammatory response, and increases proinflammatory cytokines and antioxidant activity. Inflammatory cytokines were found to be associated with OSAS [26]. Leptin and CT-1 are significant markers of inflammation as an indicator of a systemic inflammatory response. Inflammation is known to be associated with CVDs and elevated concentrations of leptin and CT-1 as an inflammatory agents were measured in our patients with OSAS. In the literature there is only one study evaluating CT-1 and IL-6 levels in OSAS patients [27]. In this study Kurt *et al*. found no significant difference in the plasma levels of CT-1 and IL-6 between the OSAS group and the controls. So to the best of our knowledge, this is the first report describing a significant difference in the serum levels of CT-1 between OSAS patients and controls, and a statistically significant difference between two OSAS groups. Our study showed that serum CT-1 and leptin levels were higher in OSAS patients compared with healthy controls. Serum CT-1 levels in severe OSAS patients were significantly higher than those in mild/moderate OSAS patients (Table II) and CT-1 levels were positively correlated with patients' HDL levels.

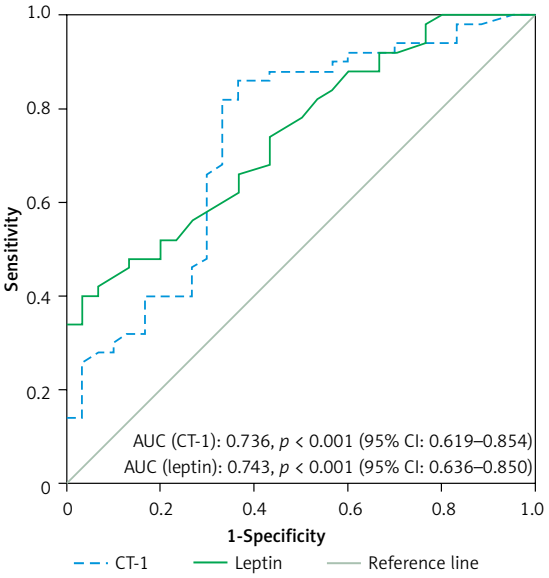

Figure 1. Receiver operating characteristics (ROC) curve analysis for cut-off value of 14.165 pg/ml and 1.74 ng/ml for CT-1 and leptin, respectively

AUC – area under the curve, CI – confidence interval.

In conclusion, our findings suggest that elevated serum CT-1 levels are associated with the presence and severity of OSAS. In our study we found that OSAS increases the serum concentrations of CT-1 and leptin. In our study cut-off values of serum CT-1 and leptin for OSAS were 14.165 pg/ml and 1.74 ng/ml, respectively (Figure 1). So, OSAS patients who have values above these concentrations should be evaluated in terms of CVD risk. The CT-1 and leptin can be used as early markers in OSAS patients with asymptomatic/or without known CVDs.

Our relatively small sample size and the evaluation of only two adipokines were the limitations of our study. Further studies with larger sample size and long-term follow-up after CPAP treatment are warranted to elucidate the predictive value of CT-1 and leptin in OSAS patients.

Conflict of interest

The authors declare no conflict of interest.

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