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Serum YKL-40 level is correlated with apnea hypopnea index in patients with obstructive sleep apnea syndrome

L.C. MUTLU[1], F. TÜLÜBAŞ[2], R. ALP[3], G. KAPLAN[1], Z.D. YILDIZ[2], A. GÜREL[2]

[1]Pulmonary Medicine, Namık Kemal University, Medical School, Tekirdağ, Turkey
[2]Medical Biochemistry, Namık Kemal University, Medical School, Tekirdağ, Turkey
[3]Neurology, Namık Kemal University, Medical School, Tekirdağ, Turkey

Abstract. – OBJECTIVE: Obstructive sleep apnea syndrome (OSAS) has been associated with elevated biochemical markers of inflammation. Although the exact mechanism is unknown, both sleep deprivation and hypoxemia are believed to be important causative factors. YKL-40, also known as chitinase-like protein, has been shown to be related to various inflammatory conditions including atherosclerosis, diabetes, cancer and asthma. The present study aimed to evaluate the relationship between YKL-40 levels and the Apnea Hypopnea Index (AHI) in patients with obstructive sleep apnea syndrome.

PATIENTS AND METHODS: The study was conducted at the Sleep Unit of the Namik Kemal University Research Center. From January 2013 to December 2013, 120 patients diagnosed with OSAS by polysomnography and 40 subjects without OSAS were recruited. Patients in both groups were matched by age, sex, and body mass index (BMI). They were further divided into groups of mild, moderate and severe OSAS based on their AHI value. Serum YKL-40 concentrations were measured by the enzyme-linked immunosorbent assay (ELISA).

RESULTS: OSAS patients showed significantly elevated YKL-40 levels compared to the control group; 102,05 (23.14) pg/ml in the control group *vs*. 144.81 (65.53) pg/ml in the OSAS group. A Spearman correlation analysis showed that serum YKL-40 levels were significantly and positively correlated with AHI ($r = 0.434$, $p < 0.001$) and oxygen desaturation index ($r = 0.374$, $p < 0.001$).

CONCLUSIONS: The study demonstrated that high serum YKL-40 levels correlated with the severity of OSAS and might serve as a non-specific biomarker for prediction and progression of the disease.

Key Words:
Obstructive sleep apnea, YKL-40, Inflammatory cytokine.

Introduction

Obstructive sleep apnea syndrome (OSAS) is a clinical disorder characterized by complete or partial obstruction of the respiratory airways. When repeated overnight, this obstruction results in oxygen desaturation and sleep fragmentation. Studies show that OSAS increases the risk of hypertension, coronary artery disease, stroke and mortality[1-3]. Sympathetic activation, oxidative stress and inflammation have been implicated as the main underlying mechanisms[4]. Furthermore, hypoxemia and sleep deprivation may be associated with the release of several cytokines[5].

YKL-40, a glycoprotein consisting of 383 amino acids with a molecular weight of 40 kDa, shows increased levels in the presence of systemic inflammation[6]. Although it is structurally similar to bacterial chitinase, it has no enzymatic activity[7]. Its physiological effects and their mechanisms are unclear. However, several immunohistochemical studies have shown that cells with a high level of metabolic activity and/or proliferation express high levels of YKL-40[8,9]. Recent studies have also reported elevated serum YKL-40 levels in inflammatory diseases such as sarcoidosis, rheumatoid arthritis and various cancers[10]. Similarly, serum YKL-40 levels increase significantly in patients with severe asthma[11] and chronic obstructive pulmonary disease (COPD)[12]. A study of adults showed its important role in predicting the risk of all-cause mortality[13] and all-cause cardiovascular mortality[14]. However, how YKL-40 influences these outcomes is still unknown.

Systemic inflammation may play a role in the pathogenesis of OSAS. A recent study reported that serum YKL-40 levels are elevated in patients with OSAS and correlate with severity of the dis-

Corresponding Author: Levent Cem Mutlu, MD; e-mail: cemmutlu68@yahoo.co.uk

ease[15]. The present study investigated whether OSAS patients had higher serum YKL-40 levels than a control group and evaluated the relationship between YKL-40 levels and the Apnea Hypopnea Index (AHI), which measures disease severity. It also looked at how YKL-40 correlates with the desaturation index (ODI) and OSAS, using inflammatory markers such as fibrinogen and CRP as secondary endpoints.

Patiens and Methods

Patients

The study included 120 patients presenting to the Sleep Unit of the Medical Facility of Namik Kemal University between January 2013 and December 2013 with a preliminary diagnosis of obstructive sleep apnea as well as a diagnosis of OSAS by polysomnography. It also included 40 patients with a preliminary diagnosis of obstructive sleep apnea and normal polysomnographic results during the same period. We excluded 56 patients taking anti-inflammatory medication or suffering from systemic inflammatory diseases such as rheumatoid arthritis, osteoarthritis, cancer, or asthma; chronic lung diseases such as COPD; advanced renal or hepatic failure; heart failure or coronary artery disease. All patients gave informed consent at the beginning of the study, which was approved by the local Ethics Committee.

Study Protocol

The diagnosis of OSAS was based on clinical and standard polysomnographic assessments carried out during the night. Patients with an AHI value of ≥ 5 /hour were grouped as having mild (5 < AHI < 15, n: 40), moderate (15 < AHI < 30, n: 40) or severe (AHI ≥ 30 n:40) OSAS. Forty patients who complained of snoring but had polysomnographic results within normal values were included in the control group.

Polysomnography

Sleep scoring was performed according to the Rechtschaffen and Kales criteria. Both study and control groups underwent standardized full-night polysomnography including electroencephalography, electrooculography, electromyography, assessments of arterial oxygen saturation by pulse oximetry, measurement of oral and nasal airflows by thermistor, and tracking of thoracic and abdominal respiratory movements. Scoring was based on American Academy of Sleep Medicine guidelines[16].

Apnea was defined as an airflow interruption lasting at least 10 seconds and hypopnea as a greater than 30% reduction in nasal pressure signal for at least 10 seconds accompanied by a reduction in oxygen saturation by 4% or more. AHI was calculated as the total number of apnea and hypopnea events per hour of sleep. ODI was calculated as a blood oxygen level reduced 3% from baseline value during one hour of sleep. Polysomnographic parameters such as the percentage of sleep time with SaO_2 < 90% (T90%), the lowest O_2 saturation ($LaSO_2$) and mean nocturnal oxygen saturation were also recorded.

Biochemical Analyses

Blood samples were collected from all subjects between 6:00 a.m. and 7:00 a.m. after full overnight polysomnography. After clotting, the samples were immediately centrifuged and stored at 80°C until analysis. All subjects underwent laboratory analyses, including determination of fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) using a routine autoanalyzer. The YKL-40 concentrations were measured in duplicate with a commercially available enzyme-linked immunosorbent assay (ELISA) kit with an intra-assay and inter-assay CV of both < 10% (USCN LIFE, Houston, TX, USA).

Statistical Analysis

We analyzed the data using an SPSS (SPSS, version 18, Chicago, IL, USA) statistical program and examined the relationships between the variables by Spearman's correlation analysis. Continuous variables were expressed as mean ± standard deviation and categorical variables as percentages. The Student's *t*-test was used to compare parametric variables, and the Mann-Whitney U test and Kruskal-Wallis test were used for nonparametric variables. We used Tukey HSD test to validate ANOVA. A p value of < 0.05 was considered statistically significant.

Results

Baseline Clinical Characteristics

Patients' baseline characteristics are shown in Table I. No significant difference was found in age, gender, BMI, smoking rate, levels of fasting blood glucose (FBG), fibrinogen, triglycerides,

Table I. General characteristics of OSAS patients and control group.

	OSAS (N =120)	Control group (N = 40)	*p* value
Age (years)	49.90 ± 11.75	46.20 ± 10.61	0.431
Sex (F/M)	36/84	13/27	0.768
BMI (kg/m^2)	30.95 ± 4.65	29.44 ± 4i41	0.737
Smoking. n(%)	54 (%45)	18 (%45)	1.000
Fasting glucose	103.02 ± 24.32	96.59 ± 13.58	0.084
AHI	22.9 (24.3)	1.7 (2.4)	< 0.001
FBG	309.41 ± 75.64	308 ± 72.44	0.860
CRP	2.0 (3.6)	1.8 (3.3)	0.953
TC	206.05 ± 39.08	200.90 ± 35.37	0.522
TG	138 (87)	158 (120)	0.882
LDL	128.41 ± 33.29	122.85 ± 29.54	0.754
HDL	45 (14)	42.5 (18)	0.397
ODI	25.50 (29.6)	2.7 (3.3)	< 0.001
T 90%	1.72 ± 3.25	0.21 ± 0.90	< 0.001
YKL-40 (pg/ml)	144.81(65.53)	102.05(23.14)	< 0.001

Normally distributed data were expressed as mean±SD, skewed data (including AHI, ODI, CRP, TG, HDL, YKL-40) were presented as median (interquartile range). Categorical variables were expressed as numbers. AHI, apnea-hypopnea index; ODI, oxygen desaturation index; T90%, the percentage of total sleep time spent with SaO_2 < 90%; $LaSO_2$, lowest O_2 saturation; average SaO_2, average O_2 saturation.

total cholesterol, low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL) between the OSAS and control groups. However, ODI and T90% values were significantly higher in OSAS patients compared to the control group.

YKL-40 Levels in Control and OSAS Groups

OSAS groups showed higher YKL-40 levels than the control group (Table I). A multivariate logistic regression including all variables showed YKL-40 to be a significant and independent predictor of OSAS (odds ratio 1,037, 95% confidence interval (CI) 1.020-1.055, $p < 0.001$) (Table II).

Relationship Between YKL-40 Level and Severity of OSAS and Correlation Between AHI and ODI

The YKL-40 levels for the control group and mild, moderate and severe OSAS groups are shown in Figure 1. The YKL-40 levels were as follows: 102.05 pg/ml (IQR; 24.64) in the control group; 125,70 pg/ml (IQR:83,14) in the mild group; 145,37 pg/ml (IQR: 114.72) in the moderate group and 146,18 pg/ml (IQR: 69.08) in the severe group. The intergroup comparison of YKL-40 levels was performed using the Kruskal-Wallis test, which showed a statistically significant difference. The Mann-Whitney U test was used for dual comparisons. No significant difference was found between the control group and the mild OSAS group ($p = 0.022$) whereas the control group and the moderate and severe OSAS groups differed significantly ($p < 0.001$).

A Spearman correlation analysis showed serum YKL-40 levels to be significantly and positively correlated with AHI (r = 0.434, $p < 0.001$) (Figure 2), and similarly, serum YKL-40 levels were significantly and positively correlated with oxygen desaturation index (ODI) (r = 0,374, $p < 0.001$) (Figure 2).

Discussion

Recent studies have evaluated the relationship of YKL-40 levels to disease severity and prognosis for many systemic diseases. Our study

Table II. Multiple logistic regression analysis for the presence of OSAS.

Variables	OR (95% CI)	*p* value
Age (per year)	1.016 (0.963-1.071)	0.568
Male (yes)	0.705 (0.210-2.364)	0.571
Smoking (yes)	2.338 (0.779-7.018)	0.130
BMI (per kg/mm^2)	1.109 (0.977-1.261)	0.111
Fasting blood glucose	1.010 (0.974-1.048)	0.574
Fibrinogen	0.996 (0.989-1.003)	0.271
TC	1.004 (0.963-1.047)	0.856
TG	0.993 (0.984-1.003)	0.159
LDL	1.005 (0.956-1.056)	0.845
YKL-40 (pg/ml)	1.037 (1.020-1.055)	< 0.001

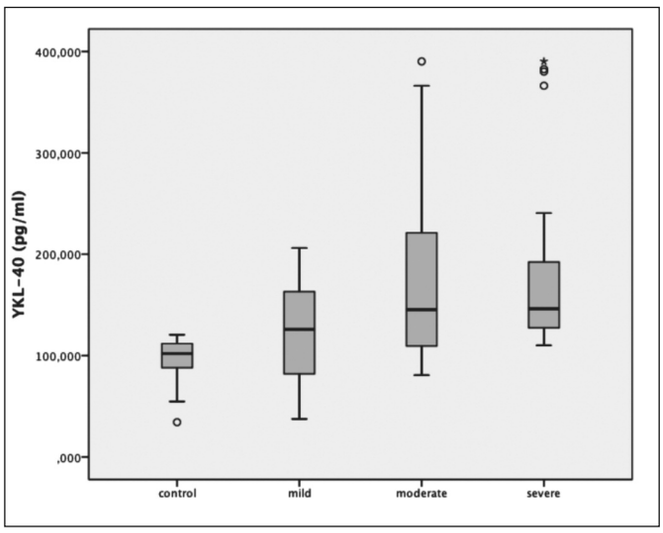

Figure 1. Box and whisker plot showing serum YKL-40 concentrations in mild (n=40), moderate (n=40), severe (n=40) obstructive sleep apnea syndrome (OSAS) patients and control (n=40) group.

showed that OSAS patients experienced significantly higher YKL-40 levels compared to the control group. The elevated YKL-40 levels correlated with the AHI value, which describes the severity of OSAS. Only one study has examined the correlation between YKL-40 levels and AHI, and, like us, they found a positive correlation between severity of the disease and YKL levels[15]. Alternatively, we also found a positive correlation between YKL-levels and ODI, which has not been studied before.

Although OSAS is characterized by repeat episodes of complete or partial obstruction in the upper respiratory airways, studies have shown systemic effects. Findings indicate a relationship between OSAS and arterial hypertension[17], and CPAP treatment reduces the vascular risk[18]. Similarly, two important studies have shown a relationship between OSAS and stroke, coronary artery disease and heart failure[19,20]. The underlying mechanism of systemic hypertension in OSAS patients is still unclear. It may be related to increased sympathetic activity as a result of overnight episodic desaturations[21] and systemic inflammation. A relationship also exists between elevated YKL-40 levels and coronary artery disease and its severity in OSAS patients[22]. YKL-40 might be a potential marker of increased cardiovascular mortality and morbidity in OSAS patients compared to healthy subjects.

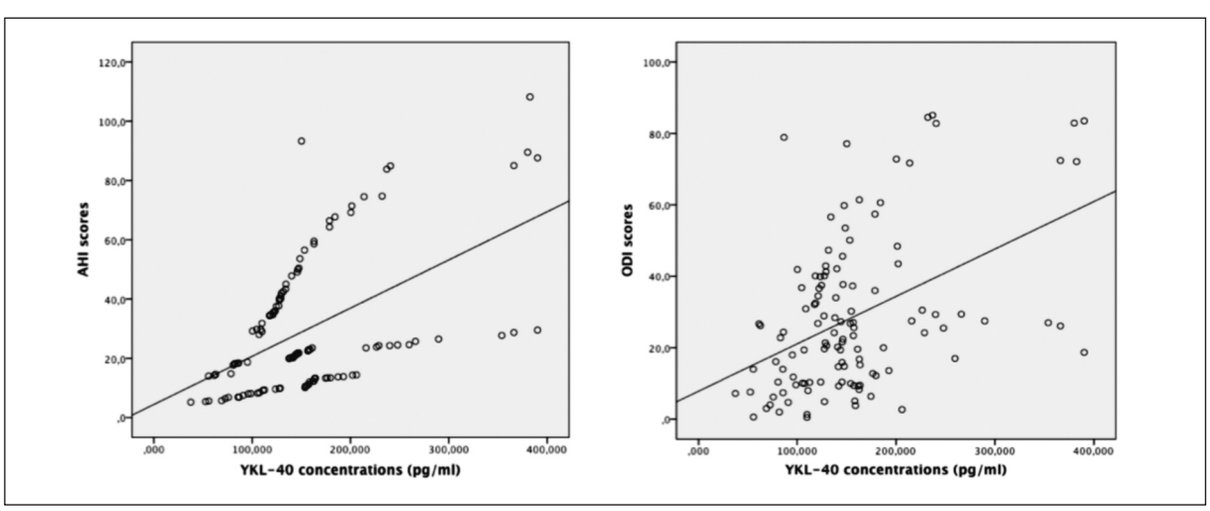

Figure 2. Correlation of serum YKL-40 levels with Apnea-hypopnea index (AHI) scores ($r = 0.434$, $p < 0.001$) and Oxygen desaturation index (ODI) scores ($r = 0.374$, $p < 0.001$) in OSAS patients.

Gumus et al[23] reported YKL-40 serum levels no higher in COPD patients than in the control group; such levels significantly correlated with low PaO2 values, suggesting that hypoxemia increased YKL-40 synthesis. The present study found a similar relationship between ODI, repeated cycles of hypoxemia and YKL-40 levels. Repeated cycles of hypoxemia probably increase the release of YKL-40.

Previous studies have shown elevated serum levels of systemic inflammatory markers such as CRP, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and fibrinogen in OSAS patients[5,24-26]. It is known that hypoxemia stimulates fibrinogen synthesis, enhancing serum levels. Of note, in the present study, we found no statistical difference in fibrinogen and CRP levels in OSAS groups compared to the control group. A recent study[27] examined the relationship between sleep-related respiratory disorders and systemic inflammatory markers and found a correlation only between IL-6 and OSA severity after adjustment for BMI, while no association was found between CRP and fibrinogen. Consistent with previous study, no statistical significance was found in fibrinogen and CRP levels between control and OSAS groups in the present study.

Conclusions

The present study showed that YKL-40 levels were elevated in OSAS patients compared to the control group and were associated with severity of the disease and ODI. Further studies using a larger patient population may show that serum YKL-40 levels can potentially predict the severity and progression of OSAS.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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