Xizhong Sui MD¹
Changqing Gao MD²

¹ Department of Cardiovascular Surgery, The Medical School of Chinese People's Liberation Army, General Hospital of Chinese People's Liberation Army

² Department of Cardiovascular Surgery, General Hospital of Chinese People's Liberation Army

Association of serum YKL-40 levels with the presence and severity of coronary artery disease in patients with obstructive sleep apnea syndrome

Abstract

Purpose: Current evidence supports a robust association between obstructive sleep apnea syndrome (OSAS) and the risk of coronary artery disease (CAD). YKL-40, a 40 kDa heparin- and chitin-binding glycoprotein, is found to be associated with the presence of CAD. This study aims to examine the association of serum levels of YKL-40 with the presence and severity of CAD in patients with OSAS.

Methods: A total of 246 patients with OSAS who underwent coronary angiography for the evaluation of CAD (134 patients with CAD and 112 patients without CAD) were enrolled in this study. The severity of CAD was assessed using the coronary atherosclerosis index (CAI). Serum levels of YKL-40 were determined using enzyme-linked immunosorbent assay.

Results: Serum YKL-40 levels were significantly higher in OSAS patients with CAD compared with those without CAD. Multivariable logistic regression analysis revealed that serum YKL-40 levels were an independent determinant of the presence of CAD in patients with OSAS. In addition, Spearman correlation analysis showed that serum YKL-40 levels were positively correlated with CAI in OSAS patients with CAD. Patients with statin treatment showed significantly lower levels of serum YKL-40 compared with those without.

Conclusions: Elevated levels of serum YKL-40 are associated with the presence and severity of CAD in patients with OSAS.

Manuscript submitted 27th July, 2013
Manuscript accepted 27th October, 2013

Clin Invest Med 2013; 36 (6): E306-E311.

Correspondence to:
Changqing Gao, M.D.
Department of Cardiovascular Surgery
General Hospital of Chinese People's Liberation Army
28 Fuxing Road
Beijing, 100853, P. R. China
e-mail: bjgaochangqing@126.com

Obstructive sleep apnea syndrome (OSAS) is characterized by recurrent episodes of complete or partial obstruction of the upper airway during sleep, accompanied by an oxygen desaturation and terminated by brief arousals which result in marked sleep fragmentation [1]. OSAS has a relatively high prevalence in the developed world: 4% in men and 2% in women [2]. OSAS has been reported to significantly increase the risk of cardiovascular disease, including hypertension, heart failure, arrhythmia, coronary artery disease (CAD) and stroke [3]. OSAS patients have a high morbidity and mortality due to cardiovascular disease, particularly CAD.

YKL-40, also named cartilage glycoprotein-39 or chitinase-3-like-1, is a heparin- and chitin-binding glycoprotein and a member of the mammalian chitinase-like protein family [4]. A variety of human cell types, including activated macrophages, neutrophils, chondrocytes, vascular smooth muscle cells and cancer cells, are found to produce and secrete YKL-40 [5]. YKL-40 is considered an inflammatory marker associated with both acute and chronic inflammation and with an established role in extracellular remodeling and angiogenesis [6]. Inflammation is implicated in the pathogenesis of both OSAS and CAD [7]. In addition, YKL-40 is closely related to both the early and late phases of the atherosclerotic process [8]. Recent studies have demonstrated elevated levels of serum YKL-40 in CAD patients [9]; therefore, it has been hypothesized that serum YKL-40 levels may be associated with the development of CAD in OSAS patients.

Previous studies have reported on serum YKL-40 levels in CAD patients; however, no study on the association of serum YKL-40 levels with the presence and severity of CAD in OSAS patients has been performed. In this study, serum levels of YKL-40 in OSAS patients, with and without CAD, were examined in order to assess its role in the pathophysiology of CAD in OSAS patients.

Materials and Methods

Study population

A consecutive population of 246 subjects with clinical symptoms of sleep apnea, which was examined by polysomnography (PSG) from August 2010 to October 2012 in General Hospital of People's Liberation Army, was enrolled in this study. OSAS was established according to clinical and polysomnographic criteria. Apnea-hypopnea index (AHI) was defined as the frequency of apnea and hypopnea per hour. Subjects with AHI ≥ 5 were considered to have OSAS. Patients were excluded if they had a history of arterial hypertension, chronic obstructive pulmonary diseases (COPD) or other pulmonary

disorders, infectious upper airway disorders, diabetes mellitus, and malignancy. The study protocol was approved by the Research Ethics Committee of the General Hospital of People's Liberation Army, and all patients gave their informed consent before commencement of the study.

Coronary angiography

CAD was defined as subjects who had angiographic evidence of stenosis $>50\%$ in at least one major coronary artery. OSAS patients were then divided into two groups, with or without CAD, 134 and 112 patients, respectively, based on visual angiographic results. The severity of CAD was assessed by assigning scores to each of the 15 segments. The coronary atherosclerosis index (CAI) was defined as the sum of these scores: grade 0 was defined as no significant stenosis; grade 1 was defined as stenosis of less than 25%; grade 2 was defined as 25 to 49% stenosis; grade 3 was defined as 50 to 74% stenosis; and, grade 4 was more than 75% stenosis. Thus, the highest possible score was 60 [10]. CAI was graded by two evaluators who were blinded to the results.

Laboratory examinations

Venous blood was collected after a minimum of 10 hours of overnight fasting. Serum samples were stored at -80°C until assessment was carried out. Serum YKL-40 levels were determined by a commercially available enzyme immunoassay kit (Quidel, San Diego, CA, USA) according to the manufacturer's instructions. Body Mass Index (BMI) was calculated as weight in kilograms divided by height squared in meters (kg m^{-2}).

Statistical analysis

Statistical analysis was carried out using SPSS version 13.0 software program (SPSS Inc, Chicago, Illinois, USA). Data are displayed as means $\pm$ SD or median (interquartile range). The Kolmogorov-Smirnov test was used to analyze the data normality. Unpaired t-test or Mann-Whitney U test were utilized to compare the characteristics between OSAS patients with and without CAD. Univariate analysis was performed and the variables with a $P < 0.20$ were then entered into a backward stepwise multivariate logistic regression model to calculate the odds ratio values (OR) and 95% confidence intervals (CI) for the presence of CAD in OSAS patients. Spearman correlation analysis was performed to evaluate the correlation between serum YKL-40 levels and CAI. Differences were considered significant at $P < 0.05$.

Results

Baseline clinical characteristics

Increased levels of LDL-C were found in OSAS patients with CAD compared with those without CAD. No significant differences were observed in other characteristics between the two groups. The clinical characteristics of the two groups are presented in Table 1.

Serum YKL-40 levels in relation to CAD

OSAS patients with CAD had significantly elevated levels of serum YKL-40 compared with those without CAD (Table 1). Simple logistic regression analysis indicated that BMI (OR 1.055, 95% CI 0.982 to 1.132; $P=0.143$), TC (OR 1.208, 95% CI 0.975 to 1.497; $P=0.085$), LDL-C (OR 1.492, 95% CI 1.145 to 1.945; $P=0.003$) and YKL-40 levels (OR 1.022, 95% CI 1.013 to 1.032; $P<0.001$) showed a trend ($P<0.20$) toward an association with the presence of CAD in OSAS patients (Table 2). All these parameters were then entered into a multivariate logistic regression model. YKL-40 levels were associated with the presence of CAD in OSAS patients (OR 1.021, 95% CI 1.012 to 1.031; $P<0.001$) (Table 2). As the variables in Table 2 have different ranges of distribution, then OR per standard deviation was then calculated to enable a true comparison. Only serum YKL-40 levels were found to be associated with the presence of CAD in OSAS patients (OR 4.131, 95% CI

FIGURE 1. Correlation of serum YKL-40 levels with CAI in OSAS patients with CAD; $r=0.417$, $P<0.001$.

2.734 to 6.242; $P<0.001$) after simple logistic regression analysis.

The association of serum YKL-40 levels with CAI

Spearman correlation analysis showed that serum YKL-40 levels were positively correlated with CAI ($r=0.417$, $P<0.001$) in OSAS patients with CAD (Fig. 1).

TABLE 1. Clinical and biochemical characteristics of OSAS patients with and without CAD

	OSAS patients without CAD	OSAS patients with CAD	P value
N	112	134	
Age (years)	55.98±8.76	56.65±8.12	0.536
Gender (M/F)	80/32	99/35	0.667
BMI (kg/m ²)	25.89±3.73	26.56±3.46	0.142
SBP (mmHg)	138.48±20.60	139.81±19.81	0.607
DBP (mmHg)	82.01±9.85	83.40±9.71	0.266
TC (mmol/L)	5.17±1.03	5.43±1.32	0.083
TG (mmol/L)	1.78±0.52	1.91±0.63	0.454
LDL-C (mmol/L)	3.34±0.95	3.73±1.04	0.002
HDL-C (mmol/L)	1.42±0.30	1.43±0.34	0.809
FPG (mmol/L)	5.76±0.77	5.71±0.75	0.602
AHI	23.50 (17.00-29.75)	24.00 (17.00-32.00)	0.539
YKL-40 (ng/mL)	115.19 (87.43-143.13)	136.12 (107.22-157.16)	<0.001
Smoking, n (%)	26 (23.21%)	34 (25.37%)	0.695
History of CAD, n (%)	8 (7.14%)	14 (10.45%)	0.366
Statins, n (%)	32 (28.57%)	45 (33.58%)	0.399
ACEI/ARB, n (%)	38 (33.93%)	52 (38.81%)	0.429

TABLE 2. Logistic regression analysis for the presence of CAD in OSAS patients

	Simple regression		Multiple regression	
	OR (95% CI)	P	OR (95% CI)	P
Age (years)	1.010 (0.980-1.040)	0.535		
Gender (M/F)	0.884 (0.503-1.551)	0.667		
BMI (kg/m ²)	1.055 (0.982-1.132)	0.143	1.037 (0.962-1.118)	0.342
SBP (mmHg)	1.003 (0.991-1.016)	0.605		
DBP (mmHg)	1.015 (0.989-1.042)	0.266		
TC (mmol/L)	1.208 (0.975-1.497)	0.085	0.811 (0.517-1.273)	0.363
TG (mmol/L)	1.076 (0.888-1.304)	0.454		
LDL-C (mmol/L)	1.492 (1.145-1.945)	0.003	1.774 (1.039-3.030)	0.036
HDL-C (mmol/L)	1.101 (0.506-2.396)	0.808		
FPG (mmol/L)	0.915 (0.656-1.277)	0.601		
AHI	1.011 (0.981-1.041)	0.487		
YKL-40 (ng/mL)	1.022 (1.013-1.032)	<0.001	1.021 (1.012-1.031)	<0.001
Smoking, n (%)	1.125 (0.626-2.021)	0.695		
History of CAD, n (%)	1.517 (0.612-3.758)	0.368		
Statins, n (%)	1.264 (0.733-2.179)	0.399		
ACEI/ARB, n (%)	1.235 (0.732-2.084)	0.429		

The influence of statin treatment on serum YKL-40

In OSAS patients without CAD, patients undergoing statin treatment showed significantly lower levels of serum YKL-40 compared with those without [100.24 (82.30-121.15) *vs.* 120.96 (95.48-147.42) ng/mL, $P=0.008$]. Furthermore, in OSAS patients with CAD, patients undergoing statin treatment also showed significantly lower levels of serum YKL-40 compared with those without [117.20 (102.57-144.02) *vs.* 143.40 (114.93-164.55) ng/mL, $P=0.001$].

Discussion

This study provides the first report of the association of serum YKL-40 levels with the presence and severity of CAD in patients with OSAS. These results indicate that serum levels of YKL-40 were significantly elevated in OSAS patients with CAD compared with those without CAD. Increased serum levels of YKL-40 were significantly associated with the development of CAD in patients with OSAS. In addition, Spearman correlation analysis showed that serum YKL-40 levels were correlated with CAI.

Recent evidence revealed that OSAS patients showed a significantly increased risk of developing cardiovascular disease, in particularly CAD. In 40–70 year-old males, subjects with severe OSAS (AHI $\geq$ 30 events/hour) were nearly 70% more likely to develop CAD compared with those without OSAS [11]. Sleep Heart Health Study (SHHS) provide epidemiol-

ogic evidence for a causal role of OSAS in the incidence of CAD and CAD-related mortality, which indicated the risk of CAD-related death in the male subset of this cohort tracked with all-cause mortality and was 70% greater in those with AHI $\geq$ 15 events/hour compared with unaffected individuals over an eight year follow-up of more than 6000 individuals [12]. Another study reported an increased risk of CAD with increasing AHI, even when adjusted for hypertension and BMI [13]. These studies provide supportive evidence that OSAS is a risk factor for incident CAD and for CAD-related mortality; therefore, it is essential that the risk of developing CAD in OSAS patients be evaluated and that strategies to prevent CAD for patients with OSAS be developed.

Biomarkers in serum are now considered an important method for predicting the presence and progression of different diseases. YKL-40, a new inflammatory marker, promotes chemotaxis, cell attachment and migration of vascular smooth muscle cells and the formation of branching tubules, indicating that YKL-40 plays a role in angiogenesis [14]. Kucur *et al.* reported that serum YKL-40 levels were significantly elevated in CAD patients compared with healthy controls [9]. In addition, serum YKL-40 concentration is found to be an independent predictor of 10 year all-cause and cardiovascular mortality in subjects with type 2 diabetes [15]. This study was undertaken in order to test the hypothesis that there are significant differences in the levels of serum YKL-40 between OSAS patients with CAD and those without CAD. Our results indicated that

the levels of serum YKL-40 were significantly elevated in OSAS patients with CAD compared with those without CAD. These data provide important evidence to support the addition of serum YKL-40 to the list of traditional risk factors for the assessment of cardiovascular risk in the primary prevention of CAD. The current study also revealed that serum levels of YKL-40 were associated with the severity of CAD which is assessed using CAI in patients with OSAS. Similar results were found in another study that found that increased serum YKL-40 levels were associated with angiographic lesion progression in patients with CAD [16]. In contrast, a study performed in patients with stable ischemic heart disease suggested that levels of serum YKL-40 did not correlate with the degree of CAD, assessed using different scoring systems [17]. The explanation for these conflicting data is unclear but may be attributable to differences in disease advancement, populations or assays applied, or incomplete control for confounding variables. YKL-40 may play a role in the development and progression of CAD in OSAS patients. Serum YKL-40 could be employed as an independent risk predictor for the presence and severity of CAD in OSAS patients. Further investigations will be needed to explain the exact role of YKL-40 in the presence or progression of CAD in OSAS patients.

Our studies also found that patients with statin treatment showed lower levels of serum YKL-40, indicating that statin could reduce serum YKL-40 levels. This is consistent with a study by Mygind *et al.* that reported that serum YKL-40 was significantly higher in non-statin-treated CAD patients compared with statin-treated ones [18]. YKL-40 was found in several studies to be associated with prognosis in patients with stable CAD [19] [20] [21]. Thus, YKL-40 may be utilized as a biomarker to predict the prognosis of CAD patients.

This study has several potential limitations. First, the sample size was not large enough to reach definitive conclusions and further studies with great numbers are warranted. Secondly, our study is of cross-sectional design, and the causative relation must be confirmed by future longitudinal studies. Last, the differences in serum YKL-40 levels between OSAS patients and healthy controls were not assessed. Serum YKL-40 levels were elevated in obese subjects [22] and patients with inflammation-related with disease, such as diabetes [23] and rheumatoid arthritis [24], compared with healthy controls. OSAS is associated with obesity and inflammation [7]; therefore, serum YKL-40 is hypothesized to be elevated in OSAS patients without CAD compared with healthy controls. Further studies are needed to determine this hypothesis.

In conclusion, OSAS patients with CAD had significantly elevated serum levels of YKL-40 compared with those without

CAD. Serum levels of YKL-40 were positively correlated with the severity of CAD. Serum levels of YKL-40 may serve as a new biomarker and may be useful, in addition to traditional diagnostic methods, for assessing the presence and severity of CAD in OSAS patients.

References

- Guilleminault C, Tilkian A, Dement WC. The sleep apnea syndromes. *Annu Rev Med* 1976; 27: 465-84.
- Young T, Palta M, Dempsey J, et al. The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med* 1993; 328: 1230-5.
- Weiss JW, Launois SH, Anand A, et al. Cardiovascular morbidity in obstructive sleep apnea. *Prog Cardiovasc Dis* 1999; 41: 367-76.
- Hakala BE, White C, Recklies AD. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. *J Biol Chem* 1993; 268: 25803-10.
- Rathcke CN, Vestergaard H. YKL-40--an emerging biomarker in cardiovascular disease and diabetes. *Cardiovasc Diabetol* 2009; 8: 61.
- Rathcke CN, Vestergaard H. YKL-40, a new inflammatory marker with relation to insulin resistance and with a role in endothelial dysfunction and atherosclerosis. *Inflamm Res* 2006; 55: 221-7.
- Levy P, Tamiier R, Arnaud C, et al. Sleep deprivation, sleep apnea and cardiovascular diseases. *Front Biosci (Elite Ed)* 2012; 4: 2007-21.
- Michelsen AE, Rathcke CN, Skjelland M, et al. Increased YKL-40 expression in patients with carotid atherosclerosis. *Atherosclerosis* 2010; 211: 589-95.
- Kucur M, Isman FK, Karadag B, et al. Serum YKL-40 levels in patients with coronary artery disease. *Coron Artery Dis* 2007; 18: 391-6.
- Tatami R, Mabuchi H, Ueda K, et al. Intermediate-density lipoprotein and cholesterol-rich very low density lipoprotein in angiographically determined coronary artery disease. *Circulation* 1981; 64: 1174-84.
- Punjabi NM, Caffo BS, Goodwin JL, et al. Sleep-disordered breathing and mortality: a prospective cohort study. *PLoS Med* 2009; 6: e1000132.
- Gottlieb DJ, Yenokyan G, Newman AB, et al. Prospective study of obstructive sleep apnea and incident coronary heart disease and heart failure: the sleep heart health study. *Circulation* 2010; 122: 352-60.
- Shah NA, Yaggi HK, Concato J, et al. Obstructive sleep apnea as a risk factor for coronary events or cardiovascular death. *Sleep* 2010; 14: 131-6.
- Rathcke CN, Vestergaard H. YKL-40, a new inflammatory marker with relation to insulin resistance and with a role in en-

- dothelial dysfunction and atherosclerosis. *Inflamm Res* 2006; 55: 221-7.
15. Lin CH, Li HY, Der Jiang Y, et al. Plasma YKL-40 Predicts 10-Year Cardiovascular and All-cause Mortality in Individuals with Type 2 Diabetes. *Clin Endocrinol (Oxf)* 2012 ;79: 185-91.
 16. Zheng JL, Lu L, Hu J, et al. Increased serum YKL-40 and C-reactive protein levels are associated with angiographic lesion progression in patients with coronary artery disease. *Atherosclerosis* 2010; 210: 590-5.
 17. Mathiasen AB, Harutyunyan MJ, Jørgensen E, et al. Plasma YKL-40 in relation to the degree of coronary artery disease in patients with stable ischemic heart disease. *Scand J Clin Lab Invest* 2011; 71: 439-47.
 18. Mygind ND, Harutyunyan MJ, Mathiasen AB, et al. The influence of statin treatment on the inflammatory biomarkers YKL-40 and HsCRP in patients with stable coronary artery disease. *Inflamm Res* 2011; 60: 281-7.
 19. Harutyunyan M, Götze JP, Winkel P, et al. Serum YKL-40 predicts long-term mortality in patients with stable coronary disease: a prognostic study within the CLARICOR trial. *Immunobiology* 2013; 218: 945-51.
 20. Mygind ND, Iversen K, Køber L, et al. The inflammatory biomarker YKL-40 at admission is a strong predictor of overall mortality. *J Intern Med* 2013; 273: 205-16.
 21. Kastrup J, Johansen JS, Winkel P, et al. High serum YKL-40 concentration is associated with cardiovascular and all-cause mortality in patients with stable coronary artery disease. *Eur Heart J* 2009; 30: 1066-72.
 22. Hempen M, Kopp HP, Elhenicky M, et al. YKL-40 is elevated in morbidly obese patients and declines after weight loss. *Obes Surg* 2009; 19: 1557-63.
 23. Nielsen AR, Erikstrup C, Johansen JS, et al. Plasma YKL-40: a BMI-independent marker of type 2 diabetes. *Diabetes* 2008; 57: 3078-82.
 24. Kazakova M, Batalov A, Deneva T, et al. Relationship between sonographic parameters and YKL-40 levels in rheumatoid arthritis. *Rheumatol Int* 2013; 33: 341-6.