

Inflammatory cytokines tumor necrosis factor- α , interleukin-8 and sleep monitoring in patients with obstructive sleep apnea syndrome

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Abstract. The present study investigated the changes of tumor necrosis factor- α (TNF- α), interleukin-8 (IL-8) and sleep ability in patients with obstructive sleep apnea hypopnea syndrome (OSAHS). A total of 684 patients who were admitted to Xuzhou Central Hospital between June 2012 and June 2016 were enrolled to serve as the experimental group and 192 healthy subjects were selected as the control group. Polysomnography was performed on both groups, and serum TNF- α and IL-8 levels were measured by ELISA. Pearson's correlation analysis was used to analyze correlations between factors. Compared with control group, the levels of TNF- α and IL-8, the morning systolic and diastolic pressure in OSAHS group were significantly higher ($P < 0.01$). Furthermore, the mean oxygen saturation (MSaO₂) and lowest oxygen saturation (LSaO₂) of the OSAHS group were significantly lower compared with those in control group ($P < 0.01$). Results also indicated that TNF- α was positively correlated with apnea-hypopnea index (AHI), morning systolic and diastolic pressure ($r = 0.621, 0.464, 0.539$; $P < 0.05$), and negatively correlated with MSaO₂ and LSaO₂ ($r = -0.526, -0.466$; $P < 0.05$). Notably, IL-8 was positively correlated with AHI, morning systolic and diastolic pressure ($r = 0.337, 0.413$ and 0.629 ; $P < 0.05$), and negatively correlated with MSaO₂ and LSaO₂ ($r = -0.329$ and -0.417 ; $P < 0.05$). Therefore, it was concluded that TNF- α and IL-8 may be involved in the occurrence and development of OSAHS, are closely related to OSAHS and may be important risk factors for cardiovascular disease in patients

with OSAHS. The present findings suggest that TNF- α and IL-8 can be used to assess the degree of OSAHS.

Introduction

Obstructive sleep apnea hypopnea syndrome (OSAHS) is a potentially lethal respiratory sleep disorder that, in recent years, has attracted increasing attention. It mainly affects middle-aged obese men, and the main pathogenesis is the collapse of the upper airway during sleep. Symptoms of OSAHS such as snoring, daytime drowsiness and nocturia can be easily ignored by patients. The recurrence of the disease can cause coronary heart disease and cerebrovascular disease, and sudden deaths can even occur at night (1,2). It has been reported that OSAHS affects ~5% of male adults worldwide (3), and its incidence is still increasing. OSAHS is related to a variety of inflammatory factors. In OSAHS patients, the levels of proinflammatory cytokines are increased, and the level of anti-inflammatory factors are decreased, eventually causing endothelial dysfunction (4). Interleukin-8 (IL-8) is a multifunctional chemokine that causes neutrophils to leave the bloodstream and travel to lesions. IL-8 is significantly increased in patients with respiratory diseases such as pulmonary fibrosis, respiratory distress syndrome, bronchitis and bronchiectasis (5). Tumor necrosis factor- α (TNF- α), as a relatively common cytokine, is mainly secreted by adipocytes and mononuclear-macrophage system. TNF- α regulates the immune system, induces inflammation, and participates in the regulation of fat metabolism. TNF- α in the blood of OSAHS patients is usually increased compared with healthy subjects (6). IL-8 and TNF- α levels are increased in inflammatory response of various diseases, but their involvement in OSAHS has not been well studied. In this study, we investigated the changes of TNF- α , IL-8 and sleep ability in patients with OSAHS to investigate their correlations with OSAHS.

Patients and methods

Research subjects. A total of 684 OSAHS patients admitted to Xuzhou Central Hospital from June 2012 to June 2016 were enrolled in this study. Among them, 446 were males and 238 were females, with an average age of 51.34 ± 5.16 years

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Table I. Clinical data of 876 subjects [n (%)].

Basic information	Experimental group (n=684)	Control group (n=192)	χ^2	P-value
Sex			0.142	0.732
Male	446 (65.20)	128 (66.67)		
Female	238 (34.80)	64 (33.33)		
Age (years)			0.028	0.928
≥ 50	491 (71.78)	139 (72.40)		
< 50	193 (28.22)	53 (27.60)		
Ethnicity			0.471	0.452
Han	653 (95.47)	181 (94.27)		
Other	31 (4.53)	11 (5.73)		
Marital status			0.917	0.640
Married	639 (93.42)	181 (94.27)		
Divorced	41 (5.99)	9 (4.69)		
Unmarried	4 (0.58)	2 (1.04)		
Major changes in recent living habits			2.047	0.171
Yes	13 (1.90)	7 (3.65)		
No	671 (98.10)	185 (96.35)		
Lifestyle and habits			0.236	0.672
Closed	247 (36.11)	73 (38.02)		
Open	437 (63.89)	119 (61.98)		
Pittsburgh sleep quality index			36.612	< 0.001
≤ 7	237 (34.65)	113 (58.85)		
≥ 8	447 (65.35)	79 (41.15)		
BMI (kg/m ²)			1.979	0.165
≥ 27	367 (53.65)	92 (47.92)		
< 27	317 (46.35)	100 (52.08)		
Diabetes			2.413	0.131
Yes	59 (8.63)	10 (5.21)		
No	625 (91.37)	182 (94.79)		
Metabolic syndrome			1.699	0.276
Yes	18 (2.63)	2 (1.04)		
No	666 (97.37)	190 (98.96)		

BMI, body mass index.

(experimental group). A total of 192 healthy subjects were selected at the same period to serve as control group. Control group included 128 males and 64 females, with an average age of 52.18 ± 4.51 years (control group). The two groups had no significant difference in terms of sex, age, or other general data, but had significant difference in the Pittsburgh sleep quality index ($P < 0.001$) (Table I). Inclusion criteria: > 48 years old and body mass index (BMI) ranging from 24.1 to 30.5 kg/m². All patients with OSAHS met the diagnostic criteria of OSAHS diagnosis guideline set by the Respiratory Disease Branch of Chinese Medical Association. Exclusion criteria: patients with incomplete data or patients that could not cope with clinical diagnosis; patients with a history of cancer, nervous system diseases, other respiratory diseases, coronary heart disease, renal dysfunction, and other inflammatory diseases that could affect the experiment. The study was approved by the Ethics

Committee of Xuzhou Central Hospital (Xuzhou, China), and all the subjects or their relatives signed an informed consent.

Experimental reagents and equipment. TNF- α was detected using human TNF- α high sensitivity ELISA kit [Thermo Fisher (Shanghai) Co., Ltd., Shanghai, China]; IL-8 assay was performed using human IL-8 enzyme immunoassay kit [Thermo Fisher (Shanghai) Co., Ltd.]; immunoassay analyzer was UniCel DxI 800 automated chemiluminescence immunoassay analyzer [Beckman Coulter (Shanghai) Co., Ltd., Shanghai, China], and sleep monitoring was performed by using Alice LE multi-channel sleep monitor (Beijing Weikang Medical Devices, Beijing, China).

Experimental method. All subjects underwent polysomnography. Fasting venous blood (5 ml) was drawn from the subjects

Table II. Comparison of TNF- α and IL-8 levels between two groups (mean \pm SD).

Index	Experimental group (pg/ml)	Control group (pg/ml)	t	P-value
TNF- α	31.2 \pm 5.3	12.1 \pm 1.1	49.620	<0.001
IL-8	34.6 \pm 7.1	19.4 \pm 8.5	25.050	<0.001

TNF- α , tumor necrosis factor- α ; IL-8, interleukin-8.

Table III. Comparison of polysomnography results between two groups.

Index	Experimental group (n=684)	Control group (n=192)	t	P-value
Morning systolic blood pressure (mmHg)	139 \pm 9	119 \pm 8	27.860	<0.001
Morning diastolic blood pressure (mmHg)	88 \pm 7	75 \pm 6	23.430	<0.001
MSaO ₂ (%)	73.19 \pm 7.65	97.01 \pm 2.16	42.650	<0.001
LSaO ₂ (%)	50.87 \pm 9.24	84.71 \pm 3.84	49.540	<0.001
AHI (times/h)	31.15 \pm 9.12	4.34 \pm 2.01	40.440	<0.001

MSaO₂, mean oxygen saturation; LSaO₂, lowest oxygen saturation; AHI, apnea-hypopnea index.

after the second morning of monitoring. Serum TNF- α and IL-8 levels were measured by ELISA. All specimens were frozen and stored at -20°C.

Polysomnography. Patients were not allowed to take sedative drugs or food and drinks that could stimulate the nervous system 24 h prior to polysomnography detection. Sleep monitoring was performed in the hospital for >7 h using polysomnography. Index data were recorded after manual check at the end of monitoring. Indexes included early morning diastolic and systolic blood pressure, mean oxygen saturation (MSaO₂), lowest oxygen saturation (LSaO₂), and apnea-hypopnea index (AHI).

TNF- α and IL-8 detection methods. According to the manufacturer's instructions of ELISA kit, the reagent was removed from the refrigerator and placed at room temperature for 20 min before use, and the concentrated wash liquid was diluted with distilled water to a ratio of 1:20. Blank wells were set, and specimens or different concentrations of standard solution (100 μ l/well) were added into the corresponding well. The wells were sealed with plastic sheet and incubated at 37°C constant temperature for 90 min. Washing was performed every 25 sec for 4 times. After that, biotin mouse anti-human TNF- α and IL-8 monoclonal antibody (1:600; cat. nos. AHC3419 and M802B; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was added into each well (100 μ l/well), except the blank wells. After incubation at 37°C for 60 min, the plate was washed 4 times. Chromogenic reagent was added into each well (100 μ l/well), followed by incubation at 37°C for 20 min in the dark. Finally, stop solution was added into each well (100 μ l/well), and OD450 value was measured within 5 min. TNF- α and IL-8 content in serum was calculated according to the standard curve. The minimum detectable dose of human TNF- α level was 4 pg/ml, and intra- and inter-plate

coefficient of variation was <10%. The minimum detectable dose of human IL-8 was 3.9 pg/ml, and intra- and inter-plate coefficient of variation was <10%.

Statistical analysis. SPSS 18.0 (Tianjin Soft Network Technology Co., Ltd., Tianjin, China) software was used for all analyses. Enumeration data were expressed as n (%), and were compared by χ^2 test. Measurement data were expressed as (mean \pm SD) and were compared by t-test. Correlation analysis was performed using Pearson's correlation coefficient analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

Comparison of serum levels of TNF- α and IL-8 between two groups. Levels of TNF- α and IL-8 in the experimental group were 31.2 \pm 5.3 and 34.6 \pm 7.1 pg/ml, respectively, while those in the control group were 12.1 \pm 1.1 and 19.4 \pm 8.5 pg/ml, respectively (Table II). The levels of TNF- α and IL-8 in the experimental group were significantly higher than those in the control group (P<0.05).

Polysomnography results. Morning systolic blood pressure in experimental group was 139 \pm 9 mmHg, and in control group 119 \pm 8 mmHg. Morning systolic blood pressure in experimental group was significantly higher than that in control group (P<0.001). Morning diastolic blood pressure in experimental group was 88 \pm 7 mmHg, and in control group 75 \pm 6 mmHg. Morning diastolic blood pressure in experimental group was significantly higher than that in control group (P<0.001). MSaO₂ was 73.19 \pm 7.65% in experimental group and 97.01 \pm 2.16% in control group. MSaO₂ in experimental group was significantly lower than that in control group (P<0.001). LSaO₂ was 50.87 \pm 9.24% in experimental

Table IV. ELISA and polysomnography monitoring correlation analysis.

Index	TNF- α	IL-8
AHI		
r	0.621	0.337
P-value	<0.05	<0.05
Morning systolic blood pressure		
r	0.464	0.413
P-value	<0.05	<0.05
Morning diastolic blood pressure		
r	0.539	0.629
P-value	<0.05	<0.05
MSaO ₂		
r	-0.526	-0.329
P-value	<0.05	<0.05
LSaO ₂		
r	-0.466	-0.417
P-value	<0.05	<0.05

TNF- α , tumor necrosis factor- α ; IL-8, interleukin-8; AHI, apnea-hypopnea index; MSaO₂, mean oxygen saturation; LSaO₂, lowest oxygen saturation.

group, and $84.71 \pm 3.84\%$ in control group. LSaO₂ was significantly lower in experimental group than in control group ($P < 0.001$). AHI in experimental group was $31.15 \pm 9.12/h$, and in control group $4.34 \pm 2.01/h$. AHI in experimental group was significantly higher than that in control group ($P < 0.001$) (Table III).

Correlation analysis of ELISA and polysomnography monitoring results. Serum levels of TNF- α were positively correlated with AHI, morning systolic and diastolic pressure ($r = 0.621, 0.464$ and $0.539, P < 0.05$), but negatively correlated with MSaO₂ and LSaO₂ ($r = -0.526$ and $-0.466, P < 0.05$). There was a positive correlation of IL-8 with AHI, morning systolic and diastolic pressure ($r = 0.337, 0.413$ and $0.629, P < 0.05$), but negative correlation with MSaO₂ and LSaO₂ ($r = -0.329$ and $-0.417, P < 0.05$) (Table IV).

Discussion

Diagnosis of OSAHS is mainly based on the comprehensive evaluation of the patient's symptoms and the results of polysomnography (7,8), which may cause diagnostic errors. OSAHS symptoms of snoring and nocturia can be easily misdiagnosed as asthma, pharyngitis and other diseases (9-11), leading to delayed treatment and occurrence of cardiovascular disease, seriously affecting the quality of life of patients. Therefore, the development of more sensitive diagnostic markers for OSAHS is urgently needed.

Our results showed that the expression levels of TNF- α and IL-8 in experimental group were significantly higher than those in the control group, suggesting that TNF- α and

IL-8 may be involved in the occurrence and development of OSAHS. TNF- α is a small molecule protein secreted by macrophages. TNF- α has a variety of inflammatory biological functions (12), and most of OSAHS patients are affected by throat inflammation, which can cause changes in the levels of a series of inflammatory mediators, including the increased TNF- α release (13). Increased release of TNF- α may result in delipidation of cells, leading to procoagulant activity and the deposition of fibrin, which in turn increases the production of peroxide and damages the cardiovascular system (14). TNF- α may also promote neovascularization and formation of atherosclerosis (15), leading to the occurrence of cardiovascular complications and heart failure in OSAHS patients. Furthermore, TNF- α causes increased expression of adhesion molecules on the vascular endothelial cells and leukocytes, leading to accelerated activation of lymphocytes, causing severe inflammatory reactions in lesion area (16). Strengthened adhesion of endothelial cells and other cells may cause the formation of microcirculation channel blockage to affect blood supply to tissues, resulting in severe hypoxia in OSAHS (17). IL-8 has a strong influence on the activation, regulation and chemotactic effect of neutrophils. Akyol *et al* (18) have reported that IL-8 binds to surface-specific receptor of neutrophils, which will lead to cell deformation, degranulation and the increased generation of reactive oxygen species. This process may induce the production of lysosomes to activate arachidonic acid, leading to increased vascular permeability, plasma protein exudation, resulting in tissue damage, atherosclerosis and other diseases (19). Changes in the levels of TNF- α and IL-8 in patients with OSAHS are related to the activation of inflammatory reaction and abnormal production, and accumulation of these two factors may induce chemotactic infiltration of inflammatory cells and endothelial cell injury, which is an important risk factor for atherosclerosis.

Results of polysomnography showed that morning diastolic and systolic pressure were higher in experimental group than those in control group. These two indicators in the experimental group almost reached the critical point of international standard of hypertensive, indicating that patients in the experimental group had developed or tended to have symptoms of hypertension. MSaO₂ and LSaO₂ of the control group were higher than those in the experimental group. AHI of the control group was lower than that of the experimental group. Due to abnormal expression of TNF- α in patients with OSAHS, the microcirculation channel is blocked (20) and the upper airway collapses, resulting in increased frequency of low ventilation. Increased hypoventilation can also cause decreased MSaO₂ and LSaO₂, and decreased blood oxygen concentration, which in turn lead to the dormancy of functional cells (21). Correlation analysis showed that TNF- α and IL-8 were positively correlated with AHI, morning systolic and diastolic blood pressure, but were negatively correlated with MSaO₂ and LSaO₂. Elevated levels of TNF- α and IL-8 may cause cell deformation, increased adhesion of endothelial cells, which may lead to blockage of blood vessel wall and airway. Blocked blood vessel wall and airway aggregates hypoventilation, abnormal blood pressure and reduced blood oxygen levels, causing increased severity of the disease (22).

In summary, TNF- α and IL-8 may participate in the development of OSAHS. TNF- α and IL-8 may serve as important

markers for the diagnosis of OSAHS and the prediction of the severity of disease, and serve as an important basis for assessing the degree of disease in patients with OSAHS. A treatment focusing on these two factors can effectively prevent the occurrence of cardiovascular disease and improve the patients' quality of life.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HM wrote the manuscript. HM and AT recorded and analyzed the polysomnography data. HM, BL and YH treated the patients. CL and LC were responsible for ELISA. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Xuzhou Central Hospital (Xuzhou, China) and informed consents were signed by the patients or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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