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Relationship between inflammatory factors and arrhythmia and heart rate variability in OSAS patients

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Abstract. – OBJECTIVE: Obstructive Sleep Apnea Syndrome (OSAS) is a disorder characterized by recurrent upper airway obstruction, apnea, and hypopnea, associated with decreased oxygen saturation and disturbed sleep structure during sleep. It was found that OSAS was associated with a variety of arrhythmia and conduction disorders, but the relationship between multiple types of arrhythmia and the severity of OSAS, and its possible mechanism remain unclear. The purpose of this study was to observe the main types of arrhythmia and the condition of heart rate variability (HRV) in patients with OSAS, to detect the levels of multiple inflammatory factors in serum of OSAS patients, and to observe the correlation between polysomnographic parameters or inflammatory factors, and arrhythmia or HRV, as well as its possible mechanisms.

PATIENTS AND METHODS: 141 patients with suspected OSAS were collected in the Second Affiliated Hospital of Soochow University and Xinghua People's Hospital from February 2016 to February 2018. According to the sleep apnea hypopnea index (AHI), they were divided into control group (AHI <5, n = 34), mild-moderate OSAS group (5≤ AHI <30, n = 48), and severe OSAS group (AHI ≥30, n = 59). Clinical data such as gender and age were collected. All patients completed polysomnography (PSG), 24-hour Holter monitoring and blood routine, biochemical indexes and serum hs-CRP, TNF-α, IL-6, and IL-1β testing. The indicators in the three groups were compared, and the correlation between PSG parameters, HRV and inflammatory biomarkers was investigated.

RESULTS: Compared with control group, there were significant differences in age, sex ratio, BMI, uric acid, TC, and TG in the mild-moderate OSAS group (p<0.05), and in age, sex ratio, BMI, red blood cell count, hemoglobin, hematocrit, uric acid, FBS, TC, TG, LDL, and HDL in severe OSAS group (p<0.05). There were significant differences in gender ratio, BMI, red blood cell count, hemoglobin, hematocrit, uric acid, FBS, TC, TG, LDL, and HDL between mild-moderate OSAS group and severe OSAS group (p<0.05). Heart rate variability (HRV) parameters include SDNN, SDNN index, RMSSD, PNN50, LF, HF, and LF/HF. SDNN, PNN50, and HF in severe OSAS group and mild-moderate OSAS group were significantly lower than those in control group (p<0.05). LF/HF was significantly higher than that of control group (p<0.05). There was a significant difference in PNN50, HF, and LF/HF between severe OSAS group and mild-moderate OSAS group (p<0.05). In terms of inflammation, serum hs-CRP was significantly higher in mild-moderate OSAS group and severe OSAS group than that in control group (p<0.05). Serum IL-1β was significantly higher in mild-moderate OSAS group than that in severe OSAS group (p<0.05). There was no significant difference in other indicators (p>0.05). There was a significant positive correlation between hs-CRP and oxygen reduction index (ODI) (r=0.209, p=0.013) and a significant negative correlation with PNN50 (r=-0.188, p=0.025). There is no significant correlation between other indicators.

CONCLUSIONS: Systemic inflammatory reactions existed in patients with OSAS. With the increase of OSAS, inflammation was aggravated, especially serum hs-CRP. Hs-CRP was significantly and negatively correlated with PNN50 and positively correlated with ODI. The results suggested that the inflammatory response was involved in the occurrence of heart rate variability in OSAS patients.

Key Words:
Obstructive sleep apnea syndrome, Intermittent hypoxia, Arrhythmia, Heart rate variability, Cardiac autonomic nerve function.

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Introduction

Obstructive sleep apnea syndrome (OSAS) has been proved to be an independent risk factor for a variety of cardiovascular and cerebrovascular diseases, which seriously affects the quality of life and life of patients. OSAS could cause or aggravate heart and brain damage through various mechanisms such as endothelial cell injury, autonomic dysfunction, platelet aggregation, increased blood viscosity, enhanced fibrinolytic activity, oxidative stress, and chronic inflammation[1]. Concerning the heart, it was confirmed that OSAS is prone to arrhythmia, but the mechanism is not clear and may be related to hypoxemia and sleep deprivation triggering systemic inflammation. Serum levels of inflammatory factors such as High Sensitivity C-reactive Protein (hs-CRP), tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), and interleukin-1β (IL-1β) in OSAS patients may significantly increase. Moreover, it is correlated with apnea, hypopnea index (AHI), oxygen reduction index (ODI), and minimum blood oxygen saturation[2]. TNF-α has a strong pro-inflammatory effect, mainly secreted by the mononuclear macrophage system, which can up-regulate the expression of adhesion molecules by activating nuclear κ B, thereby inducing endothelial cell damage and apoptosis. In addition, TNF-α may cause inspiratory muscle dysfunction, leading to worsening of apnea during sleep. IL-6 can promote the release of interleukin-1 (IL-1), TNF-α and interferon γ (INFγ), and other pro-inflammatory cytokines from T cells and monocytes, and promote the expression of adhesion factors in vascular endothelial cells, chemotaxis of white blood cells, thus aggravating local inflammation. Previous studies have found that serum hs-CRP level in OSAS patients was significantly higher than that in healthy people, and it will continue to rise if the apnea of OSAS patients is not relieved. Hs-CRP could be used as a predictor of cardiovascular events[3]. Therefore, inflammatory factors might be involved in the occurrence of OSAS-induced arrhythmias, and attention should be paid to the relationship between inflammatory factors and arrhythmias. This study selected 141 patients with suspected OSAS admitted between February 2016 and February 2018, and explored the relationship between inflammatory factors and HRV, which helped us to pay more attention to the quantitative analysis of inflammatory factors in OSAS patients. This investigation aimed to guide the prevalence of the risk and complications of OSAS, and to help understand the role of inflammatory factors in the body, as well as to predict the occurrence of malignant arrhythmia. Then, the necessary intervention and treatment will be carried out.

Patients and Methods

Patients

From February 2016 to February 2018, 141 patients with suspected OSAS were collected from the Second Affiliated Hospital of Suzhou University (Suzhou) and Xinghua People's Hospital (Taizhou). According to the apnea hypopnea index (AHI), 141 patients with OSAS were divided into control group (AHI <5, n = 34), mild to moderate group ($5 \leq AHI < 30$, n = 48), and severe OSAS group ($AHI \geq 30$, n = 59). Height and weight were calibrated to calculate body mass index (BMI) for all participants. Informed consent was obtained from all subjects. This research has been approved by the Ethics Committee of Second Affiliated Hospital of Suzhou University and the Ethics Committee of Xinghua People's Hospital.

Inclusion Criteria

The patients were 21-76 years old, all of whom were diagnosed as primary patients without OSAS-related treatment. The main complaints were breath-holding at night or snoring during sleep, accompanied by daytime sleepiness. Overnight Polysomnography (PSG) and 24 h dynamic electrocardiogram were monitored. In addition, blood routine, biochemical indexes, and serum hs-CRP, TNF-α, IL-6, and IL-1β were tested.

Exclusion Criteria

Patients with cardiac pacemaker placement, atrial fibrillation, atrial flutter, and other abnormal heart rhythm affecting HRV detection; long-term use of digoxin, β blockers, and other drugs affecting autonomic nervous function; other diseases that seriously affect the quality of life, such as cancer, respiratory failure, severe physical disability, and mental illness.

Methods

General Information

Clinical data such as gender and age of patients were collected. All subjects were measured for

height, weight, and body mass index (BMI). The measurement unit of BMI is expressed in kg/m^2 and is calculated as body weight (kg)/height^2 (m^2).

PSG Detection

Polysomnography was recorded by using Alice 6 (Philips, Amsterdam, The Netherlands). Patients are required not to drink strong tea, alcohol, coffee, and other excitable beverages on the day of examination. The recording time is from 22:00 on the day to 6:00 the next day, and snoring, arterial oxygen saturation, heart rate, chest wall movement, and body position were recorded. Monitoring indicators include hypopnea and apnea. OSAS diagnostic criteria: hypothermia and apnea recurrence >30 times during sleep 7 hours per night, or AHI ≥ 5 times / h. AHI = (number of apneas during sleep + number of hypopneas during sleep) / total sleep time. The standard for hypopnea is that the pressure-type respiratory airflow intensity (amplitude) during sleep is reduced by more than 30% from the basal level and more than 10 seconds, accompanied by SaO_2 decreased by more than 4% from the basic level, or the intensity (amplitude) of the pressure-type respiratory airflow decreased by more than 50% from the basic level and more than 10 seconds, accompanied by SaO_2 decreased by more than 3% from the basic level. Apnea refers to the disappearance or significant reduction of the oral and nasal respiratory airflow during sleep (≥ 90% from baseline) and duration ≥10 seconds. Oxygen reduction index (ODI) is the number of times the oxygen saturation drops by ≥ 4% per hour. T90 refers to the percentage of blood oxygen saturation <90% of the total recording time. Arousals refer to changes in EEG frequency that last more than three seconds during non-rapid eye movement sleep (NREM), including θ waves, α waves, and/or brain waves with frequencies greater than 16 Hz (but excluding spindle waves). Diagnostic criteria for OSAS were light, moderate, and heavy (5≤AHI<30) and severe (AHI≥30).

24 h Dynamic Electrocardiogram Monitoring

A dynamic electrocardiogram (ECG) is a method of continuously monitoring cardiac dynamic electrical activity using Holter technology for a long time. As a portable recorder, the monitoring system of the dynamic electrocardiogram can continuously monitor the ECG information of the human body for 24-72 h, and use the computer to playback, analyze, process, and print the ECG information. The characteristics of the monitoring system of dynamic electrocardiogram are (1) the portable ECG recorder is not subject to the limitation of receptor position and activity, and is not affected by the monitoring distance; (2) the monitoring of ECG contains a large amount of information, which is more than 10,000 times of the ordinary. It can detect transient myocardial ischemia and capture transient arrhythmia, providing a unique diagnostic basis for coronary heart disease and arrhythmia. In this study, BI series dynamic ECG recorders made in Guangzhou and Shenzhen were used for 24 h dynamic ECG monitoring. Subjects were required to complete Holter record sheet on the day of the detection, to avoid excessive sweating caused by electrode loss, and avoid weightlifting, chest expansion movement, electric pulse therapy, bath. The recording time is 24 h. After the monitoring is completed, professional medical technicians removed electrode and recorder, and recorded 24 h dynamic electrocardiogram information into the computer for editing and analysis. Heart rate variability (HRV) spectrum index includes: the high-frequency component of HRV (HF): refers to the 0.15-0.4 hz frequency band; the low-frequency component of HRV (LF): refers to the 0.04-0.15 Hz frequency band; the very low-frequency component of HRV (VLF) : refers to the 0.01-0.04 Hz frequency band; LF/HF ratio: refers to the ratio of the low-frequency component to the high-frequency component. HRV time domain indicators include: mean square root of the difference between R-R interval (rMSSD), standard deviation of all normal sinusoids between R-R interval (SDNN), the percentage of the total number of R-R intervals in the total R-R interval, the difference between the adjacent R-R interval greater than 50 ms (proportion of NN50 divided by total number of NNs, pNN50).

Laboratory Inspection

Hs-CRP Detection

In this experiment, serum hs-CRP levels were measured by enzyme-linked immunosorbent assay (ELISA). The principle of this method is: C-reactive protein CRP in the sample covalent binds with latex particles to form CRP antibody, and then forms an immune complex with monoclonal mouse and multi-clonal sheep anti-human CRP antibody of spindle acid polystyrene

particles, showing an increase in turbidity. The concentration of hs-CRP was calculated by quantitative analysis at the wavelength of 500-600 nm. Venous blood 5 ml was extracted in a coagulation-promoting tube and centrifuged (4000 rpm×10 min) to obtain serum, which was then stored in a refrigerator at –70° C. The minimum hs-CRP concentration was <8 pg/ml according to the instructions of the kit.

TNF-α Detection

Double antibody sandwich ELISA was used in this experiment. The anti-human TNF-α monoclonal antibody was coated on the microtiter plate, TNF-α in the standard and the sample was combined with the monoclonal antibody, and the biotinylated anti-human TNF-α was added to form an immune complex attached to the plate. Horseradish peroxidase (HRP)-labeled Streptavidin was combined with biotin, and the enzyme substrate OPD was added to appear yellow. The stop solution sulfuric acid was added, the color became darker, and the OD value was measured at 492 nm. The TNF-α concentration was directly proportional to the OD value, which was determined by a standard curve. The detection steps are as follows:

1. Add 50 µl/well of the reagent diluent to the coated enzyme plate;
2. Add 50 µl of control group, standard substance, and sample to the enzyme plate, and mix well;
3. Cover with a seal film and incubate at room temperature for 2 h;
4. Discard the liquid, add 400 µl of washing solution, and repeat 5 times to completely absorb the liquid in the well;
5. Add the working solution of TNF-α antibody to the plate, cover it, and incubate it at room temperature for 2 hours;
6. Repeat step (4);
7. Add 100 µl of prepared chromogenic agent and incubate at room temperature for 30 min.
8. Add 100 µl of the reaction stop solution to terminate the reaction;
9. Measure the OD value at a wavelength of 450 nm;
10. Prepare a standard curve and calculate the concentration of the sample.

IL-6 and IL-1β Detection

Serum IL-6 or IL-1β level was measured by double antibody sandwich. The purified human blood IL-6 or IL-1β antibody was coated with microporous plates and made into solid phase antibody. IL-6 or IL-1β was added successively, and then combined with HRP-labeled antibody to form antibody-antigen-enzyme-labeled antibody complex, which was thoroughly washed, and the substrate TMB was added to make the color yellow. IL-6 or IL-1β in the sample is positively correlated with the depth of the color. The absorbance (OD value) was measured by a microplate reader at a wavelength of 450 nm, and serum IL-6 or IL-1β level was calculated by a standard curve. The detection steps were as follows:

1. Dilution of the standard: 10 wells of standard wells were placed on the enzyme-labeled plate, and 100 µL of the standard was added to the first and second wells. Then, 50 µL of the standard dilution was added, and thoroughly mixed; 100 µL of each of the first and second wells was added respectively to the 3rd and 4th wells, and then 50 µL of the standard dilution was added respectively to the 3rd and 4th wells, thoroughly mixed. Then, 50 µL was discarded from the 3rd and 4th wells, added to 5th and 6th wells, and so on;
2. Sample addition: blank control hole and sample hole to be tested were set respectively; the blank control hole was not added with enzyme standard reagent and sample, and the other steps were the same. Sample diluent 40 µL and sample to be tested 10 µL were successively added to the sample hole on the enzyme standard coating plate. Add the sample to the bottom of the hole of enzyme plate without touching the wall of the hole, and mix it thoroughly;
3. Incubation: plate membrane was used to seal the plate for incubation at 37° C for 30 min.
4. Dosing: 30 times concentrated washing solution is diluted with distilled water 30 times and saved;
5. Washing: remove the sealing film, discard the liquid, shake dry, fill it with the washing liquid, let stand for 30 seconds, then discard it, repeat this operation 5 times, pat dry;
6. Add enzyme: add 50 µL of enzyme labeling reagent;
7. Incubation: repeat step (3);
8. washing: repeat step (5);
9. Color development: add color developing agent A 50 µL and B 50 µL in sequence, mix them thoroughly, avoid light, and develop color at 37° C for 15 min.

10. Termination: add 50 μL of stop solution to terminate the reaction;

11. Measurement: the absorbance (OD value) of each well was measured at a wavelength of 450 nm of the microplate reader with a blank air conditioner zero, and the measurement should be performed within 15 min after the addition of the stop solution.

Other Biochemical Indicators Detection

Venous blood was collected 8 h after fasting on the second day of admission, and fasting blood glucose (FBG), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), and other biochemical indicators were measured by Beckman Coulter AU5800 automatic biochemical analyzer. Blood routine was measured on a fully automated blood analyzer.

Statistical Analysis

All statistical analyses were performed using SPSS 18.0. Statistical differences (SPSS Inc., Chicago, IL., USA) between the two groups were determined by the *t*-test. Linear regression was used to analyze the correlation between HRV indicators and PSG parameters. Statistical charts were made by GraphPad Prism 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). Correlation analysis was performed with Pearson correlation analysis. $p< 0.05$ indicated a statistically significant difference.

Results

General Information

Compared with control group, there were significant differences in age, sex ratio, BMI, uric acid, TC, and TG in mild-to-moderate OSAS group ($p<0.05$); there were significant differences in age, gender ratio, BMI, red blood cell count, hemoglobin, hematocrit, uric acid, FBS, TC, TG, LDL, and HDL in severe OSAS group ($p<0.05$). Compared with mild-to-moderate OSAS group, there were significant differences in gender ratio, BMI, red blood cell count, hemoglobin, hematocrit, uric acid, FBS, TC, TG, LDL, and HDL in severe OSAS group ($p<0.05$; Table I).

PSG Parameters

PSG parameters in control group, mild-to-moderate OSAS group, and severe OSAS group varied with OSAS levels. There were significant differences in the levels of ODI, AHI, and MI among the three groups ($p<0.05$; Table II).

HRV Parameters

Heart rate variability (HRV) parameters included SDNN, SDNN index, RMSSD, PNN50, LF, HF, and LF/HF. SDNA, PNN50, and HF in severe OSAS group and mild to moderate OSAS group were significantly lower than those in control group ($p<0.05$), while LF/HF was significantly higher than that in control group ($p<0.05$). There were significant differences in PNN50, HF, and LF/HF between severe and mild to moderate OSAS groups ($p<0.05$; Table III).

Table I. Comparison of general data of three groups of patients.

Project	Control group (AHI < 5, n = 34)	Light to moderate OSAS group (5 ≤ AHI < 30, n = 48)	Severe OSAS group (AHI ≥ 30, n = 59)
Age	34.74 ± 14.02	48.21 ± 14.01[a]	48.22 ± 11.73[a]
Gender: (male/female)	18/16	35/13[a]	52/7[ab]
BMI (kg/m^2)	23.80 ± 4.00	26.02 ± 3.39[a]	29.34 ± 4.00[ab]
Red blood cell count (×10^9/L)	4.74 ± 0.56	4.57 ± 0.47	4.98 ± 0.54[ab]
Hemoglobin (g/L)	141.06 ± 16.85	135.64 ± 16.80	148.73 ± 15.41[ab]
Hematocrit	0.43 ± 0.05	0.42 ± 0.05	0.45 ± 0.04[ab]
Uric acid (umol/L)	312.12 ± 78.92	358.56 ± 105.30[a]	399.27 ± 95.17[ab]
FBS (mmol/L)	5.28 ± 0.77	5.26 ± 0.87	6.25 ± 1.95[ab]
TC (mmol/L)	4.12 ± 0.61	4.72 ± 1.07[a]	5.12 ± 0.85[ab]
TG (mmol/L)	1.28 ± 0.75	2.32 ± 1.93[a]	2.81 ± 1.88[ab]
LDL (mmol/L)	2.21 ± 0.61	2.36 ± 0.86	2.65 ± 0.78[ab]
HDL (mmol/L)	1.34 ± 0.31	1.37 ± 0.33	1.26 ± 0.22[ab]
Hcy (umol/L)	13.07 ± 8.71	12.85 ± 8.70	11.10 ± 3.51

Note: BMI: body mass index; FBS: fasting blood glucose; TG: triglyceride; TC: total cholesterol; HDL-C: high density lipoprotein; LDL-C: low density lipoprotein; Hcy: homocysteine. A was compared with the control group, $p<0.05$; B was compared with mild to moderate OSAS group, $p<0.05$.

Table II. Comparison of PSG parameters in three groups of patients.

PSG parameters	Control group (AHI < 5, n = 34)	Light to moderate OSAS group (5 ≤ AHI < 30, n = 48)	Severe OSAS group (AHI ≥ 30, n = 59)
ODI (time/h)	1.77 ±2.81	10.58 ± 12.22[a]	41.76 ± 37.27[ab]
AHI (time/h)	2.23 ± 1.49	15.75 ± 7.92[a]	60.62 ± 16.56[ab]
MI (time/h)	10.62 ± 18.77	16.99 ± 23.45[a]	22.19 ± 21.84[ab]

Note: ODI: oxygen reduction index; AHI: apnea hypopnea index; MI: arousal index. A was compared with the control group, p<0.05; B was compared with mild to moderate OSAS group, p<0.05.

Table III. Comparison of HRV indicators in three groups of patients.

HRV parameters	Control group (AHI < 5, n = 34)	Light to moderate OSAS group (5 ≤ AHI < 30, n = 48)	Severe OSAS group (AHI ≥ 30, n = 59)
SDNN (ms)	150.67 ± 35.32	125.02 ± 41.45[a]	129.48 ± 60.98[a]
SDNN index (ms)	64.85 ± 13.79	52.60 ± 18.08	54.62 ± 15.52
RMSSD (ms)	39.09 ± 15.06	30.14 ± 11.60	28.39 ± 8.94
PNN50 (%)	17.09 ± 12.76	9.50 ± 8.52[a]	6.97 ± 4.98[ab]
LF (ms^2)	621.14 ± 258.16	437.89 ± 342.13	478.28 ± 247.18
HF (ms^2)	515.30 ± 469.94	267.25 ± 235.31[a]	198.78 ± 123.01[ab]
LF/HF (ms^2/ms^2)	1.80 ± 1.02	2.94 ± 1.82[a]	3.37 ± 1.69[ab]

Note: SDNN, all normal sinus R-R interval standard deviation; RMSSD, root mean square of the difference between adjacent R-R intervals; PNN50, in all R-R intervals, the difference between the adjacent R-R intervals greater than 50 ms as a percentage of the total R-R interval; LF, low frequency power (0.04-0.15 Hz); HF, high frequency power (0.15-0.4 Hz). A was compared with the control group, p<0.05; B was compared with mild to moderate OSAS group, p<0.05.

Serum Inflammatory Factor Levels

The serum hs-CRP levels in mild-to-moderate and severe OSAS groups were significantly higher than those in control group (p<0.05). The serum IL-1β in mild-to-moderate OSAS group was significantly higher than that in severe OSAS group (p<0.05). There was no significant difference in other indicators (p>0.05; Table IV).

Correlation Analysis

In Table IV, only hs-CRP and IL-1β were related to OSAS. The correlation between hs-CRP and IL-1β and PSG parameters and HRV was analyzed. The results showed that there was a significant positive correlation between hs-CRP and ODI (r=0.209, p=0.013), and a significant negative correlation with PNN50 (r=-0.188, p=0.025). There was no significant correlation between other indicators (Table V).

Discussion

The Relationship Between Inflammatory Factors and OSAS

Due to abnormal anatomical structure of the upper respiratory tract and excessive relaxation of the surrounding muscles during sleep, the respi-

Table IV. Comparison of serum inflammatory factors in three groups of patients.

Project	Control group (AHI < 5, n = 34)	Light to moderate OSAS group (5 ≤ AHI < 30, n = 48)	Severe OSAS group (AHI ≥ 30, n = 59)
hs-CRP (mg/l)	0.82 ± 1.21	2.44 ± 3.97[a]	3.14 ± 2.77[a]
TNF-α (pg/ml)	35.83 ± 87.32	56.47 ± 160.77	36.31 ± 91.83
IL-6 (pg/ml)	29.65 ± 78.49	29.99 ± 52.24	24.37 ± 47.49
IL-1β (pg/ml)	422.23 ± 913.53	626.33 ± 1154.12	318.50 ± 536.63[b]

Note: hs-CRP: hypersensitive C-reactive protein; TNF-α: tumor necrosis factor α; IL-6: interleukin-6; IL-1β: interleukin-1β. A was compared with the control group, p<0.05; B was compared with mild to moderate OSAS group, p<0.05.

Table V. Correlation analysis between hs-CRP and IL-1β and PSG parameters and HRV parameters.

Project		hs-CRP	IL-1β
ODI	R	0.209*	-0.047
	P	0.013	0.579
AHI	R	0.157	-0.108
	P	0.063	0.201
MI	R	0.117	-0.092
	P	0.192	0.314
SDNN	R	-0.078	-0.004
	P	0.358	0.959
SDNN index	R	-0.065	-0.008
	P	0.446	0.924
RMSSD	R	-0.151	0.072
	P	0.073	0.393
PNN50	R	-0.188*	0.111
	P	0.025	0.191
LF	R	-0.094	0.002
	P	0.266	0.979
HF	R	-0.149	0.045
	P	0.078	0.598
LF/HF	R	0.045	-0.145
	P	0.256	0.167

*There is a significant correlation, $p<0.05$.

ratory tract was partially or completely blocked during inhalation, resulting in increased resistance during breathing, slow breathing or suspension, and recurrent hypoxia and hypercapnia. The sympathetic and parasympathetic nerves were out of balance, and the intrathoracic pressure changed. The above pathophysiological changes can affect the multi-system function of the body, and even cause sudden death. The most important clinical manifestations of OSAS patients are excessive daytime sleepiness and inattention. In Western countries, approximately 5% of adult patients with OSAS have not been diagnosed[4]. In view of the pathological basis of OSAS, exogenous positive pressure ventilation support therapy is currently the main treatment of OSAS, and has achieved certain effects. At present, the exact pathogenesis of OSAS is not completely clear. As the respiratory tract is an open system, oxidative stress and inflammatory response have been proved to be the basis of various respiratory diseases. Therefore, the role of inflammatory response in the formation mechanism of OSAS has attracted the attention of medical workers.

Cardiovascular damage is one of the most prominent complications caused by OSAS. At present, although the exact mechanism of cardiovascular complication caused by OSAS has not been fully elucidated, many studies[5] have showed that multiple physiological pathways may be involved. The hypoxia of OSAS is a typical mode of frequent alternating hypoxia/reoxygenation (H/ROX), also known as Intermittent Hypoxia (IH). The long-standing H/ROX environment in patients with OSAS has proven to be a central factor in the pathological damage of OSAS. The IH of OSAS mode has the following characteristics: first, the IH frequency is higher, the frequency of OSAS patients can generally be more than 20 times/h; second, the degree of hypoxia is severe, with the minimum PaO_2 of some OSAS patients reaching up to 30 mmHg at night; third, the course of illness is long, and as long as OSAS are not treated, they will be exposed to IH overnight; fourth, the blood oxygenation changes greatly, and the process of hypoxia and reoxygenation occurs quickly. PaO_2 fluctuates rapidly between normal PaO_2 and very low PaO_2 in a sawtooth shape. Previous studies[6] have observed that IH is more likely to cause pathological damage than continuous hypoxia, making it more difficult for the body to adapt. Different from continuous hypoxia, IH is characterized by normal oxygen continuous irrigation after hypoxia, a process similar to hypoxia/reperfusion injury, which is the core mechanism of OSAS target organ injury. Studies[7] have shown that IH can cause systemic inflammation of the whole body and is closely related to cardiovascular and cerebrovascular diseases. The establishment of the IH model is of great significance for the study of cardiovascular diseases, especially hypertension, in patients with OSAS. The IH caused by OSAS is called intermittent hypoxia in obstructive sleep apnea mode.

Previous studies[8] have shown that IH can cause a large number of reactive oxygen species (ROS), reduce the removal of ROS, decrease the antioxidant capacity, increase ROS accumulation and release, and eventually lead to oxidative stress damage. In addition, ROS and its products can selectively activate sensitive signaling pathways and transcription factors, such as Activator Protein (AP-1) and Nuclear Transcription Factor κ B (NF-κ B) to activate reaction-sensitive transcription factors, but have little effect on the activation of hypoxic adaptive channel transcription factors. After the activation of AP-1 and NF-κ B, the expression level of relevant downstream inflammatory factors is up-regulated, leading to the production and release of a large number of adhesion molecules and cytokines, resulting in an inflammatory waterfall effect, causing systemic inflammation and damaging the functions of cells, tissues, and organs. Conversely, inflamma-

tory cells and inflammatory factors are important sources of ROS production. Oxidative stress products are not only the result of oxidative stress but also a medium that activates oxidative stress, which can cause the continuation of oxidative damage and the eternal inflammatory response[9]. It can be said that the inflammatory reaction and oxidative stress penetrate the target organ damage of IH, especially the process of cardiovascular disease. The proposal of intermittent hypoxic vascular endothelial dysfunction has opened up a new research field for studying the physiological mechanisms of OSAS-related diseases. The dysregulation of vasoactive substances secreted by endothelial cells (EC) is a major manifestation of vascular endothelial dysfunction, including NO, ET, and Vascular Endothelial Growth Factor (VEGF). Due to its special physiological characteristics and anatomical location, it is determined that EC is not only vulnerable to hypoxia and reoxygenation damage, but also the main site of oxygen free radical production[9]. VEGF is a permeability and mitogenic factor of endothelium, which is closely regulated by hypoxia. As a target gene expression product of the transcription factor HIF-1, VEGF is regulated by HIF-1 under hypoxia, and there is a HIF-1 Q binding site in the promoter region of the gene. HIF-1 has been confirmed to be associated with the increase of VEGF[10]. Serum level of VEGF in OSAS patients was significantly increased, and VEGF level was significantly correlated with sleep duration and respiratory disturbance index of $SpO_2 < 90\%$[11]. Therefore, the peripheral blood level of VEGF in OSAS patients is increased, and VEGF plays an important role in the regulation of oxygen transport. Teramoto et al[12] have found that VEGF levels are significantly elevated in OSAS with severe hypoxia, and VEGF levels are significantly associated with nocturnal oxygen saturation.

Inflammatory chemokines (IL-1 and TNF-α) have been widely demonstrated to be involved in sleep physiologic regulation[13]. At the onset of sleep, serum IL-1β levels were the highest, suggesting a periodic change in IL-1β during sleep. Previous researches have found that clonal IL-1 receptor antagonists have a short-term inhibition of the non-rapid eye movement sleep phase (NREM) in rabbits, while increased secretion of IL-1β by cytosyl acyl Endotoxin and TNF-α can improve non-eye-moving active sleep, but when IL-1β is decreased by glucocorticoid and prostaglandin E, sleep would be inhibited. Kataoka et al[14] have compared the expression levels of IL-1β, IL-6, and TNF-α in patients with sleeping sickness, hypersomnia, and OSAS. The results showed that the levels of TNF-α in OSAS and sleeping sickness patients increased significantly, and there is a correlation with sleep level. IL-6 was only increased in patients with OSAS and correlated with BMI to some extent. These results were also observed in obese individuals with OSAS and obese individuals alone. Entzian et al[15] reported that by comparing the changes in serum IL-10, IL-6, TNF-α, and γ interferon release in 10 patients with OSAS and healthy group, it was found that the secretion of TNF-α in OSAS patients was significantly different. TNF-α formed a rule of peak disappearance during daytime peak hours, while IL-1β, IL-6, and γ interferon did not find significant circadian rhythms. The TNF-α disorder persisted in most OSAS patients 3 months after CPAP treatment. If TNF-α is associated with lethargy and fatigue, TNF-α may play an important role in the development of OSAS. In addition, the contractile force of the diaphragm of mice exposed to TNF-α was significantly decreased. When the diaphragm and limb muscle specimens of mice were added to TNF-α, the maximum tonic tension was almost reduced by 20%. This muscle weakness can be reversed by N-acetylcysteine, suggesting that oxidative stress is one of the causes of muscle contractile dysfunction, so serum TNF-α levels are closely related to muscle weakness[16]. Therefore, the increase in circulating levels of TNF-α may be one of the causes of dysfunction of the pharyngeal muscles, which ultimately aggravates the asphyxia during sleep. As a sensitive marker of systemic inflammation, elevated serum CRP levels reflect the activation of inflammation in the body. CRP levels have been indicated to be closely related to the mortality of cardiovascular and cerebrovascular diseases, and studies have found that BMI has a certain correlation with CRP levels[17]. Kageyama et al[18] studied 22 patients with OSAS and 20 healthy controls, and found that both groups were matched in age and body mass index; CRP levels were increased in patients with OSAS group and related to the severity of OSAS. Yokoe et al[19] reported that by analyzing the changes of CRP and IL-6 levels in patients with OSAS and obese patients before and after nasal CPAP treatment, it was found that serum CRP and IL-6 markers significantly increased before treatment, but decreased after treatment, and BMI did not decrease due to treatment. Therefore, it is considered that there is systemic inflammation in

patients with OSAS. The core factor of OSAS physiology damage is the existence of a long-term intermittent hypoxia/reoxygenation environment. Similar to hypoxia/reperfusion injury, IH in sleep apnea mode can also lead to oxidative stress, activation of systemic inflammatory response, damage to endothelial cell function, increased sympathetic and renin-angiotensin system activity, and the occurrence of metabolic disorders, which may be the core mechanism of OSAS target organ damage[20]. IH promotes the massive release of ROS, which activates redox-sensitive transcription factors and inflammatory channels, leading to up-regulation of related downstream inflammatory factor genes. The production and release of a large number of adhesion molecules and cytokines lead to the formation of inflammatory waterfall effects, resulting in damage to cells, tissues, systemic inflammatory reactions, and organ dysfunction, while inflammatory cells and inflammatory factors can promote ROS production. The products of oxidative stress are not only mediators of oxidative stress but also results of oxidative stress, which can cause the continuation of oxidative damage and the eternal inflammatory response. The inflammatory response and oxidative stress together cause IH damage in the OSAS mode, resulting in clinical complications. The results of the present study showed that the serum hs-CRP level in the mild-to-moderate and the severe OSAS groups were significantly higher than that in control group ($p < 0.05$). The serum IL-1β in mild-to-moderate OSAS group was significantly higher than that in severe OSAS group ($p < 0.05$). There was no significant difference in other indicators ($p > 0.05$). Hs-CRP is a sensitive indicator for distinguishing low-level inflammatory reactions. IL-1β is a hormone-like peptide substance that promotes the production of other pro-inflammatory factors and can synergize with other pro-inflammatory factors to induce the production of an inflammatory cascade. The results of our study indicate that there is extensive systemic inflammation in patients with serum OSAS, especially serum hs-CRP. Therefore, it is not difficult to see that serum hs-CRP has certain clinical application value in the diagnosis of OSAS severity and prognosis.

The Relationship Between Inflammatory Factors and Arrhythmia and Heart Rate Variability

The role of autonomic neuropathy in the pathogenesis of OSAS-induced arrhythmias has been confirmed in the first part. Heart rate variability (HRV) can quantitatively analyze the autonomic regulation of the heart, and decreased HRV can be used to predict the death and prognosis of cardiovascular events in OSAS patients.

Chronic low-grade inflammatory response in the body is another important pathological mechanism of cardiovascular disease in OSAS patients. Most studies have shown that the body's inflammatory response is regulated by autonomic nerves. Cardiac autonomic activity is dominated by the sympathetic and parasympathetic two autonomic nerves, so the coordination of the two plays an important role in maintaining the daily activities of the heart. Once the balance of sympathetic and parasympathetic nerves is lost, cardiovascular disease may occur. Changes including cardiac rhythm, such as abnormally reduced HRV, are predictable independent factors of death from heart and sudden cardiac death. Inspiratory pauses occur frequently during sleep due to obstruction or stenosis of the upper airway in patients with OSAS[21]. Narkiewicz et al[22] observed the HRV of patients with OSAS in daytime wakefulness. Compared with healthy people and mild OSAS patients, the results showed that the RR interval variability was significantly reduced in the moderate and severe OSAS patients, and the RR interval was significantly shortened. At the same time, HF increased and LF decreased. Although there were differences in RR between healthy people and mild OSAS patients, there was no statistical significance, indicating that the change of HRV was related to the severity of OSAS. Mutlu et al[23] reported that there were significant differences in indicators such as SDNN, LF, LF/HF among patients both complicated with OSAS, and that there were significant differences in rMSSD, HF, and age between mild and severe OSAS, suggesting that the more severe OSAS patients were, the smaller HRV was, and the higher the risk of cardiac events. In this study, Holter combined with PSG monitoring also found that OSAS patients with recurrent apnea before or after bradycardia, slow heart rate, increased heart rate during awakening and even tachycardia, indicating that OSAS patients have significant autonomic dysfunction, which is also considered to be the pathological basis of OSAS leading to arrhythmia[24]. Patients with OSAS tend to be associated with various types of arrhythmias, the most common of which is slow arrhythmia. In 1983, Guilleminault et al[25] first discovered that the occurrence of slow arrhythmia in OSAS patients was up to 18%. It is concluded that slow

arrhythmia is one of the important factors for the cardiovascular risk of OSAS patients. As an important complication of arrhythmia in OSAS patients, slow arrhythmia is closely related to the severity of apnea. OSAS has also been shown to be closely associated with tachyarrhythmia such as premature contraction and atrial fibrillation. Pépin et al[26] observed that up to 80% of OSAS patients have a variety of arrhythmias, and arrhythmia has the following characteristics: (1) according to changes in breathing, hernia - sleep apnea - return to normal breathing; (2) paroxysmal changes are based on sinus rhythm changes.

OSAS-induced arrhythmias usually occur during nighttime sleep, but attacks in the daytime awake are rare. Therefore, no discomfort such as giddy and palpitate is common in patients with OSAS, but this does not mean that arrhythmia is not important, mainly because of malignant arrhythmias such as complicated arrhythmias are the main causes of sudden death in OSAS patients. It has been that the time of highest mortality in OSAS patients is from midnight to 6 a.m. It is speculated that the increased risk of myocardial ischemia and sudden cardiac death at night by OSAS is related to severe ventricular bradycardia and arrhythmia. At present, the exact mechanism of ECG changes in patients with OSAS is not very clear. The main considerations are related to the following factors[27]: (1) the structural remodeling of the heart and the repeated occurrence of increased intrathoracic negative pressure leads to the change of regional left ventricular load, and finally causes the corresponding structural changes of the heart. In OSAS patients with no significant symptoms of heart disease it was found with echocardiography that the inner diameter of right ventricle, pulmonary artery pressure, and ventricular septal thickness were significantly higher than those of healthy people, and the blood flow velocity of mitral and tricuspid valves, stroke output, and left ventricular ejection fraction were significantly reduced; (2) electrophysiological changes. Some studies on ventricular myocytes have found that hypoxia and hypercapnia in blood can repeatedly stimulate functional ion channels such as sodium, potassium, and calcium of cardiac muscle cells, leading to changes in excitation conduction, imbalance of sympathetic and vagus nerve, which is also the trigger of nocturnal paroxysmal atrial fibrillation; (3) in addition, oxidative stress, apoptosis, and other inflammatory mediators involved in process are very important. CRP and IL levels in OSAS patients are significantly higher than those in healthy people, and they are at a high level of inflammatory reaction. CRP is involved in the clearance of myocardial apoptotic cells, and accelerates the formation of fibrous tissue, while the formation of myocardial fibrosis is an important pathological basis for the occurrence of arrhythmia. There is a physiological link between the body's inflammatory response and the autonomic nervous system, and the vagus nerve can reduce the inflammatory response through the "cholinergic anti-inflammatory pathway"[28]. The effects of sympathetic nerves on inflammation are influenced by receptor subtypes and regulation by neurotransmitter concentrations, which have a two-way effect of inhibiting and promoting inflammation[29]. In the case of coronary heart disease, hypertension, the sympathetic nervous system is activated, which can increase the expression of inflammatory factors IL-1β, IL-2, and IL-6, and the inflammatory response is enhanced. Resection of sympathetic nerve can reduce the expression of inflammatory factors in the myocardium[30]. The regulation function of autonomic nerve can be quantitatively analyzed by HRV, in which SDNN can reflect the overall regulation ability of autonomic nerve. The SDNN<50 ms is significantly reduced, the SDNN<100 ms is moderately reduced, the SDNN>100 ms is normal, and the RMSSD reflects the vagus nerve tension. Frequency domain analysis index LF is affected by the vagus and sympathetic nerve. HF is mainly regulated by the vagus nerve, LF mainly reflects the sympathetic tone; the significance of VLF is still unclear and both TP and SDNN reflect the overall change of HRV[31]. Notably, there is a significant correlation between reduced HRV and the prevalence and mortality of cardiovascular events, and reduced HRV is an independent predictor of local atherosclerosis in the coronary arteries[32]. It has been found that there is a negative correlation between inflammatory markers such as hs-CRP, IL-6 and TNF-α and HRV in healthy people, and patients with hypertension, heart failure, coronary heart disease or diabetes[33]. Psychari et al[34] reported that 98 patients with myocardial infarction within 24 h had the strongest correlation between SDNN and CRP. Hamaad et al[35] found that there were significant negative correlations between hs-CRP, white blood cell count, and IL-6 and SDNN in 100 patients with acute coronary syndrome.

Hs-CRP

CRP is a non-specific marker of acute inflammation, which is mainly synthesized by the liver. Hs-CRP and CRP, proteins, are only differentiated in sensitivity. The former has higher sensitivity, with the lowest detection concentration of 0.01 mg/L and a half-life of 19 h. Hs-CRP is a sensitive indicator to distinguish low levels of inflammation. In 1930, hs-CRP was first discovered in the cell wall of infected *streptococcus pneumoniae*, which can precipitate with C-polysaccharide. It is a cyclic pentameric protein with strong anti-protease degradation and heat resistance.

Hs-CRP is mainly distributed in the form of glycoproteins in the blood. It can combine a variety of pathogenic microorganisms and fungal polysaccharides to form nucleic acid and lecithin complexes under the action of calcium ions in the body. The complex can enhance the functions of phagocytes and white blood cells, and activate the complement system of the body to eliminate infectious pathogens, presenting as the body's inflammatory response[36]. In addition, hs-CRP plays an important role in immunity and autoprotection *in vivo*, and is also a sensitive inflammatory marker for early response of acute inflammation and cardiovascular events in recent years. Hs-CRP increased significantly in the early stages of infectious diseases, such as CRP, leukocytes, and other inflammatory markers and elevated body temperature, but hs-CRP did not change significantly in viral infections. Therefore, hs-CRP has important value in the diagnosis of sepsis caused by bacterial, viral or fungal infections. Hs-CRP can be increased within 4-6 h after infection with pathogenic microorganisms, hs-CRP level reaches peak at 36-50 h, and hs-CRP content can be more than 100 times of normal value[37]. CRP usually exists in the form of pentapeptide. In acidic or alkaline environment, CRP can be decomposed into monomers, which induces inflammatory reaction, but CRP monomer is mainly distributed in cell membrane instead of serum, and CRP concentration mainly depends on liver production. Therefore, it is difficult to detect in the serum of patients with early inflammation. The traditional CRP detection method lacks sufficient sensitivity, and the range is 3-200 mg/L. In recent years, the development of immunoluminescence and immunoprojective turbidimetry and other hypersensitive technologies has reduced the minimum detection concentration of CRP to 0.005-0.10 mg/L. Hs-CRP is one of the most sensitive and important indicators reflecting non-specific inflammation and is currently recognized as the most valuable acute phase protein. As a part of non-specific immune mechanism, the increase of hs-CRP level can indicate many inflammatory events in the body, so it has long been included in the routine examination of infectious diseases in clinical practice[38]. The content of hs-CRP in human serum is extremely weak, and the hs-CRP can be significantly increased in response to trauma, burns, infection, ischemia, and inflammation. Ridker et al[39] found that the risk of coronary atherosclerotic heart disease was positively correlated with hs-CRP levels in healthy people with no significant symptoms.

Recent studies have found a significant increase in the risk of myocardial infarction, stroke, and peripheral vascular disease in people with high hs-CRP concentrations, and hs-CRP can be completely independent of other risk factors. Biasucci et al[40] found that 53 patients with unstable angina pectoris with hs-CRP increased, even after receiving standardized treatment; there was still a continuous increase in hs-CRP at discharge, indicating that hs-CRP is an independent predictor of risk. Bickel et al[41] found that hs-CRP can be used as a predictor of death in patients with coronary heart disease. Tahrani et al[42] reported that serum hs-CRP levels of OSAS patients were significantly higher than those of the control group without OSAS, indicating that serum hs-CRP levels were significantly higher in patients with coronary heart disease with OSAS. In our study, the level of hs-CRP in OSAS patients was significantly higher than that in control group, and the hs-CRP levels were significantly higher in patients with severe OSAS than the mild and moderate OSAS. The results of our study indicate that OSAS has significant chronic inflammation, and the more severe the OSAS is, the more severe the inflammation is. The relationship between CRP and OSAS is highly controversial, and different studies draw different conclusions. The correlation between obesity and CRP may be one of the reasons for the inconsistent results. Different conclusions have been drawn from the previous two large OSAS cross-sectional studies. A study of 316 Japanese men showed a clear association between CRP and sleep-disordered breathing, but the Delhi Sleep Cohort Study found that 231 adults did not find an independent relationship between CRP and OSAS after BMI correction[43]. Many studies have come to different conclusions in OSAS, some authors have found CRP lev-

els increased, others have not found significant changes patients. In addition, whether CPAP ventilation therapy affects CRP levels remains unclear. A recent randomized trial found that effective CPAP treatment did not affect IL-6 and CRP levels. PN50 and RMSSD mainly reflect the effect of vagus nerve tension on heart rate regulation. As age increases, vagus nerve tension decreases, and the vagus nerve tension decreases significantly in the elderly. Madsen et al[44] on middle-aged healthy people found that nocturnal vagus nerve regulation was dominant, and RMSSD was negatively correlated with CRP, HF, and IL-6. Thayer et al[45] reported that RMSSD was significantly negatively correlated with white blood cell count and CRP in 611 healthy subjects. In our study, hs-CRP was significantly negatively correlated with PNN50 ($r=-0.188$, $p=0.025$). Therefore, we speculated that due to the decreased vagus nerve function and sympathetic hyperactivity in OSAS patients, the sympathetic nerve has an increased influence on the inflammatory response, while the vagus nerve has a reduced influence on the inflammatory response. AHI reflects the number of respiratory disturbance events per hour, and its value reflects the severity of OSAS, but AHI cannot reflect the frequency of hypoxia throughout the night and the degree of hypoxia per event. In 2005, many experts pointed out that it was not appropriate to divide OSAS by AHI alone, and T90, average SpO_2, and other indicators should be considered. In addition, inattention and daytime sleepiness are also important factors affecting the severity of hypoxia, and hypoxia at night can lead to decreased myocardial contractility and increased myocardial injury, which may be an important mechanism for OSAS-related cardiovascular events. Therefore, it is necessary and important to increase the indicators of hypoxia for assessing the severity of OSAS. To explain the hypoxia in our study, the relevant indicators in sleep monitoring were compared. OSAS patients had higher AHI and ODI than control group. AHI and ODI increased with OSAS, and hs-CRP was significantly positively correlated with ODI ($r=0.209$, $p=0.013$). OSAS is a process of repeated hypoxia/re-ventilation. After falling asleep, the soft tissue of the upper respiratory tract relaxes, which leads to airway obstruction, hypoxia, and lower oxygen saturation. Hypoxia further stimulates the respiratory center, which makes the respiratory muscles contract and breathing recover. ODI refers to the decrease of oxygen saturation by 4% per hour, which can be used to evaluate the condition of OSAS. The higher the ODI, the more serious the condition of OSAS is. Scholars have confirmed that repeated hypoxia/re-aeration is one of the important factors leading to inflammation in the body. In this process, repeated hypoxia/re-aeration leads to oxidative stress and further activates NF-κB. Ryan et al[46] used IH-treated HeLa model to simulate the repeated hypoxia/reopening process of OSAS. The results confirmed that ROS secretion level of Hela cells increased significantly after IH treatment, and the transcription factor NF-κ B was activated. The activation of NF-κ B promotes up-regulation of downstream inflammatory factors IL-6, hs-CRP, TNF-α, and mRNA, leading to up-regulation of inflammatory factors in circulating blood. Therefore, it is speculated that the more times repeated hypoxia/reventilation occurs in the pathophysiological process of OSAS, the more activation of NF-κB, the higher expression of downstream inflammatory factors and the higher ODI.

TNF-α

TNF-α, a cytokine with many biological functions is secreted mainly by monocyte-macrophage system and also by muscle and adipose tissue. As a pivot of inflammatory response, TNF-α can cause neovascularization, cell necrosis, and thrombosis, and is one of the important factors of endothelial dysfunction, which can lead to the formation of atherosclerotic plaque. Studies[47] have found that TNF-α levels in circulating blood are closely related to atherosclerosis, hypertension, and various complications in middle-aged men, and are predictors of congestive heart failure, hypertension, and coronary heart disease. The continued rise in TNF-α levels in patients with myocardial infarction predicts future coronary events. Neutrophils in the blood, macrophages, and monocytes in atherosclerotic plaques can synthesize TNF-α, which can lead to the release of TNF-α in case of plaque ruptures or ulcers or arterial damage. TNF-α has a direct cytotoxic effect on vascular endothelial cells, damaging the structural and functional integrity of endothelial cells, leading to large scale shedding of endothelial cells, damaging vascular endothelial cells, breaking the balance of endothelial cells secreting active substances, and increasing the level of endothelin synthesis and release[48]. TNF-α can rapidly up-regulate cell adhesion molecules

(CD11b/CDl8) and intercellular adhesion molecule-1 on leukocytes on endothelial cells, enhance the release of platelet-activating factor and endothelial cell IL-8, so as to activate white blood cells and endothelial cells and promote the release of inflammatory transmitters and the aggregation of inflammatory cells[49]. Ryan et al[50] disclosed that in patients with OSAS, the gene dependent on NF-κB, especially the significant increase in TNF-α might be related to cardiovascular disease, and TNF-α was also associated with excessive daytime sleepiness. The level of TNF-α was significantly increased in OSAS patients, and TNF-α was associated with EP score, hypoxia index, and cholesterol content. Steiropoulos et al[51] confirmed that TNF-α levels were also elevated in patients who developed sleepiness and snoring without apnea. CPAP treatment helps reduce TNF-α levels. After excluding the effects of obesity, TNF-α levels were significantly elevated in OSAS patients compared with controls, and monocytes and T cells were potential sources of TNF-α, which decreased significantly after treatment with effective CPAP. Also Minoguchi et al[48] detected that serum TNF-α levels in patients with moderate to severe OSAS were significantly higher than those in obese patients and control groups, and TNF-α levels were significantly decreased after 1 month of nCPAP treatment. However, some studies have not reached a relevant conclusion. A large prospective study found that after excluding confounding factors such as age, lethargy, and body mass index, no significant correlation was found between TNF-α levels and male OSAS patients with cardiovascular disease[52]. The results of our study found that serum TNF-α levels in OSAS patients were higher than those in non-OSAS patients, but there was no significant difference in serum TNF-α level. At present, the correlation between TNF-α and HRV has not been determined. It has been reported that TNF-α levels are not associated with HRV in patients with decompensated heart failure, whereas IL-6 levels are negatively associated with HRV[53]. A similar study also confirmed a significant negative correlation between TNF-α levels and HRV in patients with mild-to-moderate heart failure and healthy subjects. Our study did not observe the correlation between TNF-α and HRV, which may be related to the following factors: (1) it is well known that inflammatory factors are closely related to BMI, and adipose tissue can produce inflammatory factors. The higher the BMI, the higher the level of inflammatory factors. The BMI in our study is different from previous studies. (2) Patients with severe OSAS had the longest oxygen reduction time, and it was speculated that the severe OSAS might be partly due to continuous hypoxia and decreased activation of NF-κ B, and the TNF- alpha produced may not be significantly higher than those with mild OSAS. The presence of persistent hypoxia does not necessarily produce more inflammatory cytokines than mild group. (3) Factors such as TNF-α are regulated by many other physiological processes, and there are many interference factors. (4) The number of samples is limited. However, our study is a small sample control study. The small sample size may also be one of the reasons that lead to different experimental results from previous studies.

IL-6 and IL-1β

IL-6 is mainly produced by monocytes, macrophages, activated T cells, endothelial cells, tumor killer cells, and fibroblasts. Its functions are complex. It can stimulate liver cells to produce acute phase proteins, promote B cell , and T cell activation; promote the growth of plasma cell tumor and hybrid tumor; induces the formation of granulocytes or T cell/macrophage clones and stimulates their growth[54].

Human IL-6 gene is located on chromosome 7 and consists of 212 amino acids with a molecular weight of 21-30 kd. IL-6 is composed of α-chain and β-chain, with an α-chain molecular weight of 80 kd and a β-chain molecular weight of 130 kd. The difference is due to the difference in phosphorylation and glycosylation of peptide chains. The α chain can only bind to IL-6 with low affinity, and its complex with IL-6 can bind rapidly to the high affinity β chain, allowing information to be transmitted intracellularly. IL-6 expression is increased and released into the blood circulation during the occurrence of internal environmental disorder. Studies have indicated that endocrine cells can also synthesize and secrete IL-6. Under normal circumstances, IL-6 can affect the secretion of hormones in the hypothalamic-pituitary axis and other endocrine glands, while the synthesis and secretion of IL-6 are regulated by hormones[55]. Papanicolaou et al[56] reported that catecholamines have the effect of regulating the synthesis and secretion of IL-6. When OSAS occurs, the catecholamines increase due to apnea and hypoxia, and the excitability of sympathetic nerve increases, eventually leading to the increase of IL-6 secretion.

Yokoe et al[19] reported in 2003 that IL-6 was positively correlated with nocturnal hypoxia levels. The expression of IL-6 mRNA in peripheral blood mononuclear cells of OSAS patients increased significantly, and its expression level increased gradually with the severity of OSAS, indicating that OSAS hypoxia/reoxygenation affects peripheral blood IL-6 production. The mechanism of repeated hypoxia/reoxygenation leading to elevated IL-6 levels may be related to NF-κB. As a nuclear factor that initiates the expression of downstream inflammatory factors, NF-κB activates downstream IL-6 mRNA and increases IL-6 levels in body fluids. IL-1 is a cytokine discovered in 1972. IL-1 has two different molecular forms, IL-1α and IL-1β. IL-1α is composed of 159 amino acids, and IL-1β is composed of 153 amino acids. The sequence of IL-1α and IL-1β amino acids has about 26% homology, which has the same affinity with IL-1 receptor binding and plays the same biological role. IL-1 is widely distributed in the brainstem, hypothalamus, and other parts, and is distributed less in the brain, mainly exerting physiological effects in the form of IL-1β. IL-1β, an activating factor of endothelial cells, belongs to hormone-like peptides, mainly involved in immune regulation. IL-1β is mainly produced by activated monocyte-phagocytic cells, and also acts as a pro-inflammatory cytokine, which has the effect of promoting the production of other pro-inflammatory factors, and can synergize with other pro-inflammatory factors to induce the production of an inflammatory cascade. IL-1β is also distributed in the hippocampus and cortex, amygdala, paraventricular nucleus, and locus nucleus. It has been shown that long-term and intermittent hypoxia in OSAS patients leads to oxidative stress reaction in the brain, resulting in increased peripheral IL-1 level and entering the central center through the blood-brain barrier. High concentration of IL-1 can aggravate the inflammatory reaction in the brain and affect the release of neurotransmitters and the transmission process of synaptic neurotransmitters. Using OSAS rat model to simulate the pathophysiological process of hypoxia/reoxygenation of OSAS, has been found that the level of IL-1β in serum of rats in hypoxia/reoxygenation group was significantly higher than that of healthy control group. Therefore, it was speculated that IL-1β is involved in the pathological process of OSAS. Repeated intermittent hypoxia in OSAS patients can induce inflammatory reaction in the body, resulting in increased secretion of IL-1β and a series of clinical symptoms. In our study, no significant correlation was found between IL-6, IL-1β, and OSAS, which may be consistent with the fact that TNF-α is not associated with HRV.

Conclusions

In summary, local and systemic inflammation may cause abnormalities in the upper airway reflexes, collapse of upper airway, stenosis of upper respiratory tract anatomy, and dysfunction of inspiratory muscle, which forms a vicious cycle and aggravates the severity of OSAS. Activation of monocytes, neutrophils, and pro-inflammatory factors, particularly hs-CRP, may play an important role in the pathogenesis of OSAS. This research explored the correlation between hs-CRP, TNF-α, IL-6, IL-1β, and OSAS. The results showed that there were systemic inflammatory reactions in OSAS patients. With the increase of OSAS, the inflammatory reactions were aggravated, especially in serum hs-CRP. Hs-CRP was significantly negatively correlated with PNN50 and positively correlated with ODI. The results suggest that inflammatory response is involved in heart rate variability in OSAS patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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