Journal of Clinical and Nursing Research, 2024, Volume 8, Issue 9
http://ojs.bbwpublisher.com/index.php/JCNR
Online ISSN: 2208-3693
Print ISSN: 2208-3685

The Relationship Between NLRP3 Inflammasome and Its Downstream Inflammatory Factors in Obstructive Sleep Apnea Patients with Carotid Atherosclerosis Under Cigarette Exposure

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Abstract: *Aim:* To study the relationship between NLRP3 (nucleotide oligomerization domain [NOD]-, leucine-rich repeats [LRR]-, and pyrin domain-containing protein 3) inflammasome and its downstream inflammatory factors in obstructive sleep apnea (OSA) patients with carotid atherosclerosis (CAS) under cigarette exposure, further exploring the risk factors of CAS in OSA patients. *Methods:* A total of 109 adult males who underwent polysomnography and carotid artery ultrasonography in our hospital from October 2019 to December 2021 were selected. According to the detection results, they were divided into the OSA group, the CAS group, and the OSA combined CAS group; additionally, 29 healthy subjects who underwent a physical examination were also included. According to whether they were smoking, the groups were further divided into smoking and non-smoking groups. The age, body mass index (BMI), blood pressure, apnea-hypopnea index (AHI), lowest blood oxygen saturation ($LSaO_2$), carotid intima-media thickness (CIMT), levels of blood sugar, blood low-density lipoprotein cholesterol (LDLc), and serum NLRP3, interleukin-1β (IL-1β), and interleukin-18 (IL-18) of all subjects were recorded. *Results:* The OSA combined CAS group had higher LDLc levels and AHI and lower $LSaO_2$ than the OSA group and CAS group. The levels of serum NLRP3, IL-1β, and IL-18 in the OSA group were higher than those in the normal control group ($P < 0.05$); and those in the OSA combined CAS group were higher than the OSA group and CAS group ($P < 0.05$), regardless of cigarette exposure. Considering cigarette exposure, serum NLRP3, IL-1β, and IL-18 levels were higher in the OSA, CAS, and OSA combined CAS smoking groups than those in the non-smoking group ($P < 0.05$). Under cigarette exposure, AHI, LDLc, NLRP3, IL-1β, and IL-18 were significantly positively correlated ($P < 0.05$), and $LSaO_2$ was negatively correlated with CAS in OSA ($P < 0.05$). AHI, $LSaO_2$, LDLc, NLRP3, and IL-1β are the risk factors for OSA combined with CAS. *Conclusion:* $LSaO_2$, AHI, LDLc, NLRP3, and IL-1β are the important risk factors for OSA combined with CAS under cigarette exposure, and their levels can be used to predict the occurrence of CAS in OSA.

Keywords: Obstructive sleep apnea; Smoking; NLRP3; Carotid atherosclerosis

Online publication: September 26, 2024

1. Introduction

Obstructive sleep apnea (OSA) is a sleep apnea disorder characterized by recurrent upper airway collapse, the incidence of which has been on the rise in recent years, affecting nearly 1 billion adult people worldwide [1]. OSA is an independent risk factor for a variety of cardiovascular and cerebrovascular diseases, and epidemiological studies have shown that OSA is closely associated with atherosclerosis (AS) [2,3], which is a major cause of many cardiovascular diseases. Cigarette smoking is an important risk factor for OSA and can also induce a variety of cardiovascular and cerebrovascular diseases. In recent years, it has been shown that the NLRP3 (nucleotide oligomerization domain [NOD]-, leucine-rich repeats [LRR]-, and pyrin domain-containing protein 3) inflammasome and its downstream inflammatory factors interleukin (IL)-1β and IL-18 are involved in the occurrence and development of macrovascular lesions [4]. In the past two years, studies on the relationship between the NLRP3 inflammasome and OSA have also gradually emerged, which shows that the NLRP3 inflammasome and its downstream inflammatory factors are correlated with OSA and AS, respectively, but whether there is still a correlation between AS that is coexistent with OSA has not been reported. This study aims to investigate the relationship between NLRP3 inflammasome and its downstream inflammatory factors in OSA patients combined with carotid atherosclerosis (CAS) under cigarette exposure, further exploring the risk factors of CAS in OSA patients.

2. Subjects and methods

2.1. Study subjects

138 adult males who underwent polysomnography (PSG) and carotid ultrasonography in our hospital from October 2019 to December 2021 were selected, and the inclusion criteria were (1) males ≥ 18 years old; (2) patients with PSG monitoring: apnea-hypopnea index (AHI) ≥ 15 beats/h, lowest blood oxygen saturation ($LSaO_2$) ≤ 85%; and patients who fulfilled the diagnostic criteria of OSA (OSA can be diagnosed as adult OSA if they satisfy the following A + B or C [4]. A: Presence of at least one of the following: (i) Daytime drowsiness, unrecovered energy upon awakening, fatigue, or insomnia. (ii) Wakefulness due to nighttime breathlessness, gasping, or choking. (iii) Habitual snoring, interrupted breathing. (iv) Hypertension, coronary heart disease, stroke, heart failure, atrial fibrillation, type 2 diabetes mellitus, mood disorders, and cognitive disorders. B: PSG monitoring: AHI ≥ 5 beats/h, predominantly obstructive events. C: None of the above symptoms; PSG monitoring: AHI ≥ 15 beats/h, predominantly obstructive events.); (3) not receiving OSA-related treatment; (4) all patients signed an ethically informed consent.

Exclusion criteria: (1) other types of sleep apnea syndrome; (2) the presence of other obstructive respiratory diseases such as chronic obstructive pulmonary disease and chronic hypoxic diseases; (3) the presence of hypertension, diabetes mellitus, coronary artery disease, fatty liver, severe hepatic and renal insufficiency, and malignant tumors; (4) acute and chronic infections and inflammatory diseases; (5) patients with autoimmune disorders; (6) rheumatic disease with rheumatic activities recently (within 3 months); (7) recent use of drugs that affect the immune response (e.g., corticosteroids); (8) consciousness or cognitive dysfunction. According to cigarette exposure, the groups were further divided into 13 cases of the normal smoking group and 16 cases of the normal non-smoking group; 23 cases of the OSA smoking group and 13 cases of the OSA non-smoking group; 19 cases of the CAS smoking group and 18 cases of the CAS non-smoking group; 22 cases of the OSA combined CAS smoking group and 14 cases of the OSA combined CAS non-smoking group. The study was approved by the Medical Ethics Committee of the hospital, and the patients signed an informed consent form.

2.2. Data and specimen collection

Patients' age, abdominal circumference, body mass index (BMI), blood pressure (including systolic and diastolic blood pressure), and smoking status were recorded. 5 ml of fasting peripheral venous blood was collected, with 3 ml put into an anticoagulation tube. The blood tube was centrifuged for 10 min at 3000 rpm/min under constant temperature (4°C); the serum was separated and stored at -80°C for measurement.

2.3. Polysomnography

All the subjects were monitored with the Somte (Kandi, Australia) polysomnography system. The monitoring items included: electroencephalogram (F3-M1, F4-M1, C3-M1, C4-M1, O1-M1, O2-M1), electrocardiogram (modified II-lead electrocardiogram), electromyogram (including chin electromyogram and lower limb electromyogram), electrooculogram (E1-M2, E2-M2), oronasal airflow, oxygen saturation ($LSaO_2$), chest respiration, abdominal respiration, snoring, body position, and limb movement, and so on. On the day of monitoring, no coffee, tea, alcohol, sleeping pills, or other drinks, food, or drugs that affect sleep were allowed. The monitoring results were interpreted and reviewed by a professional sleep physician who analyzed polysomnography according to the 2020 American Academy of Sleep Medicine Manual of Interpretation of Sleep and Related Events Rules, Terminology, and Technical Specifications, version 2.6.

2.4. Carotid plaque detection

Carotid ultrasonography was performed using a Philips IU22 color Doppler ultrasound machine with a 7.5–10 MHz probe. Diagnostic criteria for carotid atherosclerosis [5] are (1) thickening of carotid intima-media thickness: CIMT ≥ 1.0 mm; (2) the occurrence of plaque and its location (bilateral common carotid arteries, carotid sinus, and internal carotid arteries): limited CIMT ≥ 1.5 mm indicates plaque formation. Carotid atherosclerosis is recognized if one of the above two criteria is met.

2.5. Measurement of serum NLRP3, IL-1β, and IL-18 levels

Serum specimens were taken for the measurement of blood glucose and lipid (including triglyceride [TG], cholesterol [CHOL], high-density lipoprotein cholesterol [HDLc], and low-density lipoprotein cholesterol [LDLc]) levels using an automatic biochemistry analyzer; serum NLRP3, IL-1β, and IL-18 concentrations were determined by enzyme-linked immunosorbent assay (ELISA) kits. The test kits were purchased from Xin Bosheng Biotechnology Co., Ltd.

2.6. Statistical analysis

SPSS26.0 software was used to perform a *t*-test, a non-parametric (Kruskal–Wallis) test, and Spearman's correlation analysis of OSA combined with CAS.

3. Results

3.1. Comparison of baseline data between all groups

There was no statistically significant difference in the comparison of age, abdominal circumference, BMI, and blood pressure between the groups ($P > 0.05$), as shown in **Table 1**.

Table 1. Comparison of baseline data between all groups

Groups	Age (years)	Abdominal circumference (cm)	BMI (kg/m^2)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
Normal smoking group	47 (38–53)	89.2 ± 9.3	23.2 (21.4–25.1)	121.3 ± 13.7	79.3 ± 11.1
Normal non-smoking group	41 (36–52)	91.4 ± 11.6	23.6 (20.5–24.9)	118.6 ± 16.4	79.2 ± 10.3
OSA smoking group	48 (38–55)	93.5 ± 9.4	25.3 (23.5–28.7)	126.7 ± 12.4	81.7 ± 12.4
OSA non-smoking group	48 (34–55)	92.8 ± 9.2	26.5 (22.7–29.1)	122.1 ± 11.6	78.6 ± 11.5
CAS smoking group	46 (35–53)	90.7 ± 10.1	26.9 (23.8–28.2)	127.3 ± 9.2	76.9 ± 13.2
CAS non-smoking group	45 (28–59)	90.3 ± 8.8	25.4 (22.0–27.6)	123.5 ± 13.1	77.4 ± 12.8
OSA + CAS smoking group	42 (34–53)	92.8 ± 12.2	27.1 (23.4–29.8)	124.4 ± 12.8	81.2 ± 13.5
OSA + CAS non-smoking group	46 (36–53)	89.4 ± 11.5	23.2 (21.7–25.9)	121.2 ± 11.3	78.2 ± 12.7
P	0.876	0.247	0.133	0.631	0.275

3.2. Comparison of blood test indexes and PSG detection indexes between groups

3.2.1. Comparison of blood glucose, lipid, and PSG results between the OSA group, the CAS group, and the OSA combined CAS group

Comparing the OSA combined CAS group with the OSA group and CAS group, respectively, the patients in the OSA combined CAS group had significantly higher LDLc levels and AHI and lower $LSaO_2$ than those of the OSA group and CAS group ($P < 0.05$). The results are shown in **Table 2**.

Table 2. Comparison of blood glucose, lipid, and PSG results between the OSA group, the CAS group, and the OSA combined CAS group

Groups	OSA group	CAS group	OSA + CAS group	*P*
Blood glucose (mmol/L)	5.14 (4.70-5.99)	5.28 (4.65–5.67)	5.63 (5.12–5.98)	0.118
TG (mmol/L)	2.06 (1.64–2.76)	1.53 (1.17–2.50)	2.09 (1.38–2.76)	0.325
CHOL (mmol/L)	4.81 (3.85–5.33)	5.44 (4.79–6.19)	5.05 (4.38–5.71)	0.090
HDLc (mmol/L)	1.05 (0.85–1.21)	1.6 (1.06–2.17)	1.65 (1.05–2.0)	0.122
LDLc (mmol/L)	2.63 (2.38–3.11)	3.14 (2.92–3.25)	3.43 (2.87–3.66) [ab]	0.000
AHI (times/h)	52.6 (35.8–66.6)	3.3 (2.8–4.7)	70.1 (34.4–73.4) [ab]	0.000
$LSaO_2$ (%)	75.3 (71.6–82.2)	85.6 (79.4–93.4)	55.8 (66.4–80.5) [ab]	0.000

Note: [a]$P < 0.05$ compared with the OSA group; [b]$P < 0.05$ compared with the CAS group.

3.2.2. Expression levels of the NLRP3 inflammasome and its downstream inflammatory factors in OSA patients

Compared with the normal non-smoking group, serum NLRP3, IL-1β, and IL-18 levels were significantly elevated in the OSA non-smoking group ($P < 0.05$); compared with the normal smoking group, serum NLRP3, IL-1β, and IL-18 levels were significantly elevated in the OSA smoking group ($P < 0.05$), as presented in **Tables 3** and **4**.

Table 3. Comparison of NLRP3, IL-1β, and IL-18 levels between the OSA non-smoking group and the normal non-smoking group

Groups	NLRP3 (ng/mL)	IL-1β (pg/mL)	IL-18 (pg/mL)
Normal non-smoking group	0.83 (0.56–1.02)	14.41 (11.04–21.70)	28.99 (20.86–34.27)
OSA non-smoking group	2.82 (1.55–3.22)	36.25 (33.52–42.57)	63.90 (59.37–83.8)
P	0.000	0.000	0.000

Table 4. Comparison of NLRP3, IL-1β, and IL-18 levels between the OSA smoking group and the normal smoking group

Groups	NLRP3 (ng/mL)	IL-1β (pg/mL)	IL-18 (pg/mL)
Normal smoking group	1.38 (1.16–1.71)	27.10 (22.20–30.24)	53.90 (46.36–57.77)
OSA smoking group	4.22 (2.53–4.90)	59.72 (42.46–68.66)	91.04 (79.33–115.99)
P	0.000	0.000	0.000

3.2.3. Comparison of NLRP3 and its inflammatory factor levels between disease groups

Serum NLRP3, IL-1β, and IL-18 levels were elevated in the OSA combined CAS non-smoking group compared with the OSA non-smoking group and the CAS non-smoking group, respectively (**Table 5**); and compared with the OSA combined CAS smoking group, the serum NLRP3, IL-1β, and IL-18 levels were elevated in the OSA smoking group and CAS smoking group (**Table 6**). The differences were all statistically significant ($P < 0.05$).

Table 5. Comparison of NLRP3 and inflammatory factor levels among non-smoking disease groups

Groups	OSA non-smoking group	CAS non-smoking group	OSA + CAS non-smoking group	*P*
NLRP3 (ng/mL)	2.82 (1.55–3.22)	1.23 (1.0–2.10)	7.67 (7.51–8.01) [ab]	0.004
IL-1β (pg/mL)	47.6 (44.8–49.7)	35.99 (23.28–46.93)	78.07 (73.11–80.63) [ab]	0.000
IL-18 (pg/mL)	75.6 (70.4–80.0)	60.82 (42.51–72.56)	124.32 (113–129.11) [ab]	0.000

Note: [a] indicates a comparison with the OSA non-smoking group; [b] indicates a comparison with the CAS non-smoking group.

Table 6. Comparison of NLRP3 and inflammatory factor levels among smoking disease groups

Groups	OSA smoking group	CAS smoking group	OSA + CAS smoking group	*P*
NLRP3 (ng/mL)（ng/mL）（ng/mL）	5.3 (4.4–6.39)	6.8 (6.4–7.8)	8.7 (8.3–9.5) [ab]	0.000
IL-1β (pg/mL)	36.25 (33.52–42.57)	67.3 (63.2–74.7)	92.8 (87.1–95.9) [ab]	0.000
IL-18 (pg/mL)	63.90 (59.37–83.8)	92.7 (89.2–98.9)	153.1 (147.4–164.0) [ab]	0.000

Note: [a] indicates a comparison with the OSA smoking group; [b] indicates a comparison with the CAS smoking group.

3.2.4. Comparison of the levels of NLRP3 and its inflammatory factors between the smoking and non-smoking groups for each disease

Comparing the OSA smoking group with the non-smoking group, the OSA smoking group had elevated serum NLRP3, IL-1β, and IL-18 levels (**Table 7**); comparing the CAS smoking group with the non-smoking group,

the CAS smoking group had elevated serum NLRP3, IL-1β, and IL-18 levels (**Table 8**); comparing the OSA combined CAS smoking group with the non-smoking group, the OSA combined CAS smoking group had elevated serum NLRP3, IL-1β, and IL-18 levels (**Table 9**). The differences were all statistically significant ($P < 0.05$).

Table 7. Comparison of NLRP3 and inflammatory factor levels between the smoking group and the non-smoking group in OSA patients

Groups	OSA smoking group	OSA non-smoking group	*P*
NLRP3 (ng/mL)	5.3 (4.4–6.39)	2.82 (1.55–3.22)	0.000
IL-1β (pg/mL)	47.6 (44.8–49.7)	36.25 (33.52–42.57)	0.000
IL-18 (pg/mL)	75.6 (70.4–80.0)	63.90 (59.37–83.8)	0.000

Table 8. Comparison of NLRP3 and inflammatory factor levels between the smoking group and the non-smoking group in CAS patients

Groups	CAS smoking group	CAS non-smoking group	*P*
NLRP3 (ng/mL)	6.8 (6.4–7.8)	1.23 (1.0–2.10)	0.000
IL-1β (pg/mL)	67.3 (63.2–74.7)	35.99 (23.28–46.93)	0.000
IL-18 (pg/mL)	92.7 (89.2–98.9)	60.82 (42.51–72.56)	0.000

Table 9. Comparison of NLRP3 and inflammatory factor levels between smoking and non-smoking groups in patients with both OSA and CAS

Groups	OSA + CAS smoking group	OSA + CAS non-smoking group	*P*
NLRP3 (ng/mL)	8.65 (8.29–9.46)	7.67 (7.51–8.01)	0.000
IL-1β (pg/mL)	92.75 (87.11–95.96)	78.07 (73.11–80.63)	0.000
IL-18 (pg/mL)	153.06 (147.37–164.03)	124.32 (113–129.11)	0.000

3.3. Correlation analysis

3.3.1. Correlation of expression levels of the NLRP3 inflammasome and its downstream inflammatory factors with OSA

The correlation of NLRP3, IL-1β, and IL-18 with AHI and $LSaO_2$ was compared between the non-smoking OSA group and the normal control group, and the results in **Table 10** showed a significant positive correlation between NLRP3, IL-1β, and IL-18 and AHI ($r = 0.835$, 0.831, and 0.836, $P < 0.05$), and a negative correlation between NLRP3, IL-1β, and IL-18 and $LSaO_2$ ($r = -0.356$, -0.371, -0.212, $P < 0.05$).

Table 10. Correlation between NLRP3 inflammasome expression and OSA

	NLRP3		IL-1β		IL-18	
	r	*P*	*r*	*P*	*r*	*P*
AHI	0.835**	0.000	0.831**	0.000	0.836**	0.000
$LSaO_2$	-0.356	0.000	-0.371	0.000	-0.212	0.000

Note: * indicates a significant correlation at $P < 0.05$, ** indicates a significant correlation at $P < 0.01$.

3.3.2. Correlation between observation indicators and the occurrence of CAS in OSA

According to the results of Kruskal–Wallis analysis, the statistically different indicators were subjected to Spearman's analysis of the correlation between whether CAS occurred in OSA under cigarette exposure. The results in **Table 11** showed that AHI, LDLc, NLRP3, IL-1β, and IL-18 were significantly positively correlated with the occurrence of CAS in OSA under cigarette exposure (r = 0.426, 0.0575, 0.135, 0.379, 0.360, $P < 0.05$), and $LSaO_2$ was negatively correlated with the occurrence of CAS in OSA (r = -0.621, $P < 0.05$).

Table 11. Correlation between observation indicators and the occurrence of CAS in OSA

Indicators	AHI	$LSaO_2$	LDLc	NLRP3	IL-1β	IL-18
r	0.426	-0.621	0.575**	0.135*	0.379*	0.360*
P	0.000	0.000	0.000	0.028	0.012	0.018

Note: * indicates a significant correlation at $P < 0.05$, ** indicates a significant correlation at $P < 0.01$.

3.3.3. Correlation between cigarette exposure and OSA disease severity, NLRP3, IL-1β, and IL-18 levels

Cigarette smoking was significantly and positively correlated with AHI, serum NLRP3, IL-1β, and IL-18 expression levels (r = 0.448, 0.724, 0.915, and 0.823, all $P < 0.01$), and smoking was significantly and negatively correlated with $LSaO_2$ (r = -0.326, $P < 0.01$), as presented in **Table 12**.

Table 12. Correlation between smoking and OSA disease severity and NLRP3, IL-1β, and IL-18 levels

	Smoking	
	r	***P***
AHI	0.448**	0.006
$LSaO_2$	-0.326**	0.000
NLRP3	0.724**	0.000
IL-1β	0.915**	0.000
IL-18	0.823**	0.000

Note: ** indicates a significant correlation

3.4. Influencing factors of OSA combined with CAS

According to the results of correlation analysis, the indicators that were not correlated with the occurrence of CAS in OSA were excluded, and multifactorial logistic regression analysis was conducted with whether OSA was combined with CAS as the dependent variable, and smoking, AHI, $LSaO_2$, LDLc, NLRP3, IL-1β, IL-18 as the independent variables. Based on **Table 13**, $LSaO_2$, LDLc, NLRP3, and IL-1β were risk factors for CAS combined with OSA.

Table 13. Influencing factors of OSA combined with CAS

Factors	*B*	*SE*	Wald value	OR value	95% CI for OR		*P*
					Lower limit	Upper limit	
Smoking	2.522	1.076	5.495	12.458	1.512	102.654	0.019*
Age	-0.025	0.037	0.439	0.976	0.907	1.049	0.508
BMI	-0.060	0.128	0.224	0.941	0.733	1.209	0.636
AHI	1.322	0.043	0.064	0.989	2.908	1.077	0.028*
$LSaO_2$	-1.893	0.254	0.072	1.332	0.487	0.917	0.013*
Blood glucose	0.833	0.630	1.751	2.300	0.670	7.900	0.186
TG	0.854	0.547	2.438	2.349	0.804	6.859	0.118
CHOL	-0.555	0.547	1.030	0.574	0.197	1.676	0.310
HDLc	0.804	0.536	2.254	2.235	0.782	6.383	0.133
LDLc	1.497	0.753	3.950	4.467	1.021	19.542	0.047*
NLRP3	0.039	1.020	3.880	0.134	1.018	0.990	0.049*
IL-1β	0.251	0.124	4.080	1.286	1.007	1.641	0.043*
IL-18	0.009	0.061	0.020	1.009	0.895	1.137	0.888

Note: * denotes $P < 0.05$

3.5. ROC curve analysis of AHI, LSaO2, LDLc, NLRP3, and IL-1β and whether CAS occurs in OSA

The results of the ROC curve showed that the sensitivity of AHI influencing the occurrence of CAS in OSA patients was 78.3% and the specificity was 62.8% when AHI was greater than or equal to 52.6 beats/h; the sensitivity of $LSaO_2$ was 95.7% and the specificity was 72.1% when $LSaO_2$ was less than or equal to 60.4%; for LDLc greater than or equal to 2.82 mmol/L, the sensitivity was 75% and the specificity was 63.9%; for NLRP3 greater than or equal to 1.34 ng/ml, the sensitivity was 93.6% and the specificity was 13.9%; and for IL-1β greater than or equal to 43.3 pg/ml, the sensitivity was 94.4% and the specificity was 44.4%. The results are shown in **Table 14**.

Table 14. ROC curve of influencing factors in OSA combined with CAS

Factors	AUC	Cut-off value	Sensitivity	Specificity	*P*
AHI	0.681	52.6	0.783	0.628	0.006
$LSaO_2$	0.754	60.4	0.957	0.721	0.000
LDLc	0.712	2.82	0.75	0.639	0.002
NLRP3	0.655	1.34	0.936	0.139	0.024
IL-1β	0.785	43.30	0.944	0.444	0.000

4. Discussion

OSA is one of the most common sleep apnea disorders characterized by recurrent upper airway collapse, which

is mainly manifested as snoring with apnea during sleep. Patients also suffer from recurrent nocturnal hypoxemia, hypercapnia, and sleep structural disorders, which cause various pathophysiological changes in the body. The most important pathophysiological feature is chronic intermittent hypoxia (CIH), which is closely related to hypertension, diabetes mellitus and its complications, cardiovascular and cerebrovascular diseases, etc. [6,7]. Smoking can change the patient's original sleep structure and reduce sleep stability, and at the same time, nicotine in tobacco makes the muscles of the upper respiratory tract relax. The increased sleep arousal threshold leads to a weakened neural response, making the respiratory tract more prone to collapse. Repeated stimulation of the upper respiratory tract mucosa exacerbates inflammatory responses, which in turn contributes to the development of OSA [8]. Atherosclerosis is characterized by chronic progressive endothelial damage and inflammation-induced endothelial lesions [9], and arterial intima-media thickening and atherosclerosis formation are closely related to the expression of inflammatory factors. Chronic intermittent hypoxia in OSA induces oxidative stress and inflammation, as well as sleep structural disorders that can trigger atherosclerosis. Cigarette smoking can cause inflammation and oxidative stress, thus impairing the function of endothelial cells [10], which is closely related to the formation of atherosclerosis. Both OSA and cigarette smoking can trigger and aggravate the occurrence and development of atherosclerosis through inflammatory mechanisms and oxidative stress. Cigarette exposure induces an increase in the expression of the NLRP3 inflammasome in peripheral blood mononuclear cells and aortic tissues of rats and elevated levels of downstream inflammatory factors IL-1β and IL-18 in serum, thus causing vascular endothelial damage and leading to atherosclerosis [11], which is facilitated by the hypoxemia induced by OSA. In recent years, experiments have shown that the NLRP3 inflammasome and its downstream inflammatory factors IL-1β and IL-18 are involved in the development of large-vessel lesions. Thus, it is important to study the correlation between cigarette exposure, the NLRP3 inflammasome, and CAS in combination with OSA. It is also of great significance to further explore the risk factors of CAS in OSA patients, which is important for the clinical prevention and treatment of OSA target organ damage as well as for the research of cardiovascular diseases.

NLRP3 is a crucial component of the nucleotide-binding oligomerization-like receptor (NLR) family. It features a pyrin domain at its N-terminal region, which facilitates its interaction with Apoptosis-Associated Speck-Like Protein Containing CARD (ASC). This interaction recruits cysteine protease 1 (caspase-1), leading to the assembly of a multiprotein complex known as the inflammasome. Upon inflammasome activation, activated caspase-1 (P20 or P10) is cleaved, and pro-inflammatory cytokines such as IL-1β and IL-18, which play an important role in host resistance to infection, are subsequently released [12]. Inflammatory vesicles are an essential component of the innate immune response, and the NLRP3 inflammasome plays an increasingly important role in innate immune components [13]. Studies have shown the presence of oxidative stress activation and elevated levels of pro-inflammatory cytokines in patients with OSA, the presence of neutrophilic inflammatory infiltrates in the bronchi of patients with OSA, the correlation between IL-6 and IL-8 levels in pharyngeal lavage fluid and sputum levels of IL-8, and the severity of OSA [14,15]. The NLRP3 inflammasome has been widely associated with a wide range of human diseases, and therefore, we hypothesized that the NLRP3 inflammasome is associated with OSA.

In this study, by comparing the expression levels of the NLRP3 inflammasome and its downstream inflammatory factors between OSA patients and the normal population, we found that the expression of NLRP3, IL-1β, and IL-18 was increased in OSA patients, and the correlation analysis results showed that AHI and $LSaO_2$ were significantly correlated with NLRP3, IL-1β, and IL-18, which indicated that the expression

levels of the NLRP3 inflammasome and its downstream inflammatory factor expression levels are closely related to the occurrence of OSA. Cigarette exposure induces elevated levels of the NLRP3 inflammasome and its downstream inflammatory factors IL-1β and IL-18 in the peripheral blood mononuclear cells and aortic tissues of rats, causing vascular endothelial damage and leading to atherosclerosis [5]. In this study, NLRP3, IL-1β, and IL-18 levels were compared among smoking and non-smoking patients between disease groups and between smoking and non-smoking patients in the normal population, and the results showed that smokers had significantly higher levels of NLRP3, IL-1β, and IL-18 than non-smokers in all groups, which suggests that tobacco exposure may cause the activation of the NLRP3 inflammasome and downstream inflammatory factors. This suggests that tobacco exposure may cause NLRP3 inflammasome activation and downstream inflammatory factor expression, which promote atherosclerosis in OSA patients. Therefore, we conjecture that cigarette exposure may lead to the formation of atherosclerosis in OSA by activating the NLRP3 inflammasome pathway and that the activation of NLRP3 inflammasomes induced by tobacco exposure may be a potential mechanism of cardiovascular dysfunction triggered by OSA.

OSA is caused by chronic intermittent hypoxia due to repeated apneic events, which leads to oxidative stress and inflammation as well as disturbed sleep architecture. Smoking also alters the original sleep structure of patients, while nicotine in tobacco can aggravate the changes in the inflammatory response of the airway, leading to the development of OSA [8]. A foreign study found that smoking has an effect on the severity of OSA and daytime symptoms [16], and smokers have a significantly higher AHI than non-smokers, lower mean oxygen saturation during sleep, higher daytime sleepiness than non-smokers, and a significantly higher AHI in smokers with OSA. In this study, we also compared the correlation between smoking and AHI and $LSaO_2$ by correlation analysis, and the results showed that the AHI of smoking patients was significantly higher than that of non-smokers ($r = 0.448$, $P < 0.01$), and the $LSaO_2$ of smokers was significantly lower than that of non-smokers ($r = -0.326$, $P < 0.01$), and the effect of smoking on OSA should not be ignored.

In this study, we further investigated the risk factors for CAS in OSA patients using ROC curve analysis and found that $LSaO_2$, AHI, LDLc, NLRP3, and IL-1β were important risk factors for CAS in OSA under cigarette exposure. It has been demonstrated that *in vitro* stimulation of mouse and human macrophages to activate the NLRP3 inflammasome and subsequent IL-1β secretion plays an important role in the development and progression of atherosclerosis [16]. In this study, we analyzed whether CAS occurred in NLRP3, IL-1β, and OSA by ROC curve analysis, and the results showed that NLRP3 greater than or equal to 1.34 ng/ml had a sensitivity of 93.6% and a specificity of 13.9%, and IL-1β greater than or equal to 43.3 pg/ml had a sensitivity of 94.4% and a specificity of 44.4%. The above results show that NLRP3 and IL-1β are valuable in predicting the occurrence of CAS in OSA. It has been shown that elevated cholesterol levels, especially LDLc levels, are considered a major risk factor for atherosclerosis [17]. In this study, it was found that LDLc was significantly elevated in patients with CAS and patients with CAS and OSA relative to patients with OSA, whereas no significant differences were seen in TG, CHOL, and HDLc; multifactorial regression analysis showed that LDLc was a risk factor for combined CAS with OSA; and the results of the ROC curve analysis showed that, for the influence of patients with OSA and CAS, when LDLc was greater than or equal to 2.82 mmol/L, the sensitivity was 75%, and the specificity was 63.9%, suggesting that LDLc has a more important position in the formation of atherosclerosis relative to other lipid components. This may be due to the fact that atherosclerosis is mainly considered to be the result of passive lipid accumulation in the vessel wall, of which the major component is LDLc.

5. Conclusion

In summary, AHI, $LSaO_2$, LDLc, NLRP3, IL-1β, and IL-18 were significantly associated with CAS combined with OSA under cigarette exposure. $LSaO_2$, AHI, LDLc, NLRP3, and IL-1β were significant risk factors for CAS combined with OSA under cigarette exposure. AHI, $LSaO_2$, LDLc, NLRP3, and IL-1β levels can be used to predict the occurrence of CAS in OSA.

Funding

Guangxi Natural Science Foundation (2022JJA140014)

Disclosure statement

The authors declare no conflict of interest.

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