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ORIGINAL RESEARCH

Correlation Analysis of Serum Lipopolysaccharide, Nuclear Factor Erythroid 2-Related Factor 2 and Haem Oxygenase 1 Levels and Cognitive Impairment in Patients with Obstructive Sleep Apnoea

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Objective: To investigate the correlation between the levels of serum lipopolysaccharide (LPS), nuclear factor erythroid 2-related factor 2 (Nrf2), haem oxygenase 1 (HO-1) and cognitive impairment in patients with obstructive sleep apnoea (OSA).

Methods: Serum LPS, Nrf2, HO-1 levels and cognitive impairment were measured using the Montreal Cognitive Assessment (MoCA) score in 56 patients in the "severe" group, 67 patients in the "mild-to-moderate" group and 100 healthy people in the "control" group. The differences in general conditions and serological indexes between the three groups were compared, the correlation between the MoCA scores and the serological indexes was explored and the independent predictors of the MoCA scores were analysed.

Results: Serum LPS, Nrf2 and HO-1 levels were higher in the severe group than in the mild-to-moderate group and the control group ($p < 0.05$). A total of 71 patients with OSA had combined cognitive impairment, accounting for 57.7%, and the MoCA scores were lower in the severe group than in the mild-to-moderate group and the control group ($p = 0.018$). Serum LPS, Nrf2 and HO-1 levels were significantly higher in the severe group and mild-to-moderate group than in the control group ($p < 0.05$) and were negatively correlated with the MoCA scores. Lipopolysaccharide ($p < 0.001$) and HO-1 ($p = 0.002$) could be considered independent predictors of the MoCA score.

Conclusion: Serum LPS and HO-1 levels are closely related to cognitive impairment in patients with OSA and have potential clinical value in the diagnosis.

Keywords: obstructive sleep apnoea, cognitive impairment, lipopolysaccharide, nuclear factor erythroid 2-related factor 2, haem oxygenase 1

Introduction

Obstructive sleep apnoea (OSA) is a sleep-breathing disorder that can cause intermittent hypoxia, hypercapnia and disruption of sleep architecture.[1] Reduced cognitive function is a common disorder in patients with OSA, manifested by reduced attention and memory.[2] Cognitive impairment mainly includes perceptual impairment, memory impairment and thinking impairment. Patients often experience symptoms such as delayed sensation, memory loss or errors and delusions.[3] Obstructive sleep apnoea is an independent risk factor for cognitive impairment and it is important to identify OSA when assessing patients with such impairment.[4] Repeated courses of airway collapse and obstruction in patients with OSA induces recurrent apnoea and periodic arousal during sleep, leading to intermittent hypoxia and excessive daytime sleepiness and contributing to the occurrence and development of neuroinflammation and consequent neurocognitive impairments.[5] Nuclear factor erythroid 2-related factor 2 (Nrf2) is an important signalling pathway in the oxidative stress response,[6] and Nrf2 activation is important in promoting anti-inflammation.[7] High haem oxygenase 1

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(HO-1) expression is closely associated with impaired cognitive function.[8] Kohman et al[9] demonstrated that intraperitoneal injection of lipopolysaccharide (LPS) caused activation of toll-like receptors in rats, resulting in increased expression of the inflammatory factors, interleukin (IL)-1β, IL-6 and tumour necrosis factor-alpha (TNF-α).

In recent years, oxidative stress and inflammatory response and their interrelationship have become the main direction of research on the mechanism of cognitive impairment in patients with OSA. This study investigates the relationship between serum LPS, Nrf2 and HO-1 levels and cognitive impairment in patients with OSA. Comparisons are made between the groups with normal cognition and cognitive dysfunction to provide a reference value for clinical diagnosis or prediction of cognitive impairment in patients with OSA.

Research Participants and Methodology

Study Participants

A convenience sampling method was used to select patients with OSA and medical examiners at our hospital between January 2020 and December 2022 as the study population. The case group comprised 123 patients who underwent sleep monitoring and were diagnosed with OSA; the group included 75 men, and the overall mean age was 39.32 years. The control group comprised 100 healthy people who were examined at the health screening centre; the group included 56 men, and the overall mean age was 41.12 years. Age, sex, alcohol consumption, body mass index (BMI) and prevalence of diabetes and hypertension were collected from all patients by reviewing their medical records and physical examination information. The study was approved by the hospital's ethics committee and the study participants or their families signed informed consent forms (the approved family members for signing the informed consent forms included spouses, parents and children).

The inclusion criteria were as follows: (1) adults aged >18 years and <60 years with independent cognitive behaviour; (2) patients with OSA conforming to the American Academy of Sleep Medicine's *International Classification of Sleep Disorders* (3rd edition);[10] and (3) patients who were informed, consenting and able to cooperate in completing the cognitive function assessment. The exclusion criteria were as follows: (1) patients with severe cardiovascular disease, liver and kidney failure or recent stroke and dementia; (2) patients with severe infectious diseases or mental disorders; (3) patients who were alcoholics or had been drinking alcohol for many years, or who were taking sedative or hypnotic drugs; (4) patients with serious diseases, such as malignant tumours, and related drug treatment; (5) patients with OSA who were previously undergoing any treatment (including weight reduction, medication, oral orthodontic appliances and continuous positive airway pressure ventilation); and (6) patients with long-term insomnia. The participant screening process is shown in Figure 1.

Research Methods

The present study was a case-control study in which the participants were divided into a case group and a control group according to whether they had OSA. The case group was then divided into "severe" and "mild-to-moderate" according to the severity of OSA. The serum LPS, Nrf2 and HO-1 levels and the incidence of cognitive dysfunction were compared between the groups. The correlation between serum LPS, Nrf2, HO-1 levels and cognitive dysfunction and the diagnostic value of combined cognitive dysfunction in patients with OSA were analysed in the case group.

Obstructive Sleep Apnoea Diagnosis and Grading

The polysomnographic (PSG) diagnostic OSA criterion was as follows: the PSG confirmed that respiratory events occurred >15 times/h during monitoring, including OSA, mixed sleep apnoea, hypoventilation and respiratory effort-related micro awakening.[11] All subjects undergoing sleep monitoring were subjected to PSG monitoring by trained physicians for more than 7 h continuously in the professional sleep monitoring room. The patients stopped taking sleep-regulating drugs and avoided drinking alcohol, tea, coffee and other sleep-affecting drinks 1 day before monitoring. The apnoea–hypopnea index (AHI), lowest oxygen saturation ($LSaO_2$), oxygen desaturation index (ODI) and oxygen saturation ≤90% of the total monitoring time were recorded and monitored. The ODI is used to measure the level of blood oxygen in the human body, which is related to the severity of OSA.[12] Based on the Multidisciplinary Guidelines for the Treatment of Obstructive Sleep Apnea in Adults,[12] OSA was diagnosed when the AHI ≥5 times/h, with clinical symptoms such as snoring, daytime

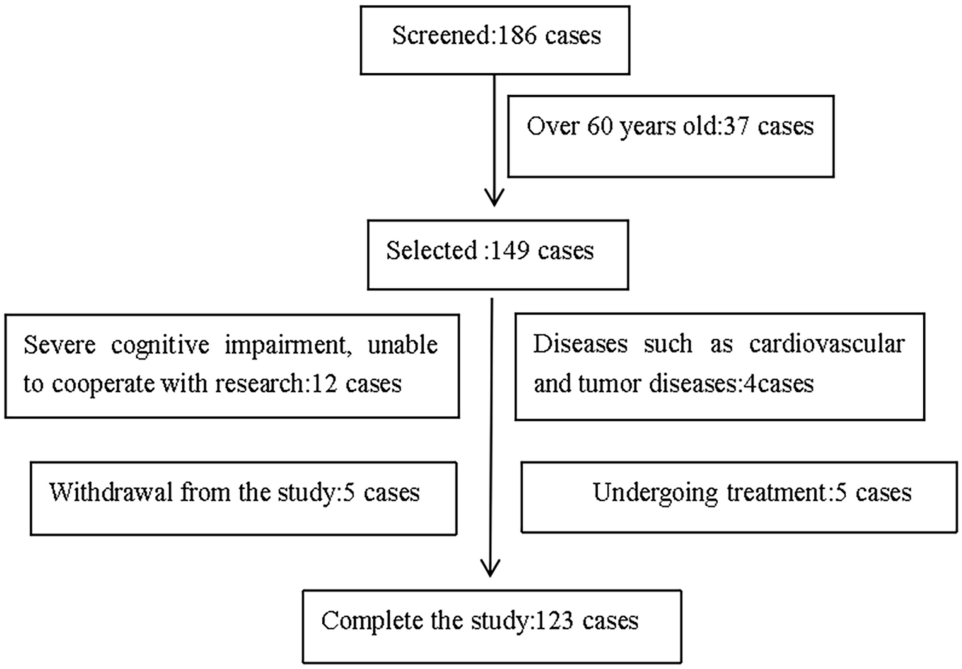

Figure 1 Participant screening flowchart.

sleepiness and fatigue. The OSA was classified as mild ($5 \geq AHI \leq 14$, $85 \geq LSaO_2 \leq 90$), moderate ($15 \geq AHI \leq 30$, $80 \geq LSaO_2 \leq 84$) and severe ($AHI > 30$, $LSaO_2 < 80$), where AHI was the main basis and $LSaO_2$ was the auxiliary basis.

Measurement of Serum Lipopolysaccharide, Nuclear Factor erythroid 2-Related Factor 2 and Haem Oxygenase 1 Levels

All participants had two tubes of 5 mL of fasting venous blood collected simultaneously in the early morning of the second day after the end of monitoring. One tube of 5 mL of fasting venous blood was centrifuged at 3000 r/min for 10 min, and the serum was kept at −80°C. When detecting Nrf2 and HO-1, the samples were thawed at room temperature for 20 min, and the enzyme-linked immunosorbent assay kit (Nanjing Jiancheng Biotechnology Co. Ltd, Jiangsu, China) was placed at room temperature for 30 min. When detecting LPS, another tube of 5 mL of fasting venous blood was taken and centrifuged at 3500 r/min for 10 min, and the serum was kept at −70°C for measurement; the serum LPS level was measured by a DL-ET 32 microbial dynamic detection system (Deere Bioengineering Co., Ltd., Zhuhai, China) using the quantitative dynamic turbidity method.[13] For the determination, the samples were thawed for 30 min, placed in a 75°C-water bath for 10 min, the endotoxin quantification instrument was preheated and the samples were then measured after adding the Limulus amoebocyte lysate reagent.

Assessment of Cognitive Impairment

Two researchers conducted two independent evaluations on the same day using the Montreal Cognitive Assessment (MoCA) scale (Version 7.1); the researchers did not interfere with each other's work. The mean score was calculated when the difference between the assessments of the two investigators was <3 points. The mean value was calculated after reassessment by one additional investigator when the difference was >3 points. The MoCA scale consists of 11 items in eight cognitive domains: attention and concentration; executive function; memory; language; visual structure skills; abstract thinking; computation; and orientation. The total score is 30 points and a score ≥26 points is normal;[14] 1 point is added to the total score if the subject has <12 years of education.

Statistical Methods

The study data were analysed using the SPSS 26.0 software package. Quantitative data were described by mean ± standard deviation, and the *t*-test was used for comparison between two groups of quantitative data conforming to normal distribution. The comparison of the three groups was performed using variance analysis, and the pairwise comparison of the three groups was performed using Tukey's honestly significant difference test. The non-parametric rank-sum test was used for comparison between groups of quantitative data not conforming to normal distribution. The qualitative data were described by rate or composition ratio *n* (%), and the sampling chi-squared test was used to compare the composition ratio or rate of two groups. Pearson correlation analysis was used to explore the correlation between the MoCA scores and serological indicators, and multiple linear regression was used to analyse the independent predictors of the MoCA scores, with a test level of $\alpha = 0.05$. The box view was used to identify outliers in the data. The abnormal value was set to a null value. Bonferroni's test was not used to correct for the test level because the analysis involved a comparison between groups of two or three.

Results

Basic Clinical Information

There were no significant differences in gender, age, BMI, history of alcohol consumption, history of hypertension and history of diabetes mellitus among the three study groups, and the demographic information of the three groups was considered to be comparable. The differences in serum LPS, Nrf2 and HO-1 levels among the three groups were significant ($p < 0.05$) and showed that the severe OSA group had higher levels than the mild-to-moderate OSA group and the control group, and that the mild-to-moderate OSA group had higher levels than the control group. The MoCA scores in the severe OSA group were lower than those in the mild-to-moderate OSA group and the control group, but the differences between the mild-to-moderate OSA group and the control group were not statistically significant, as shown in Table 1.

Serum Lipopolysaccharide, Nuclear Factor erythroid 2-Related Factor 2 and Haem Oxygenase 1 Levels and Cognitive Impairment

According to the MoCA scores, there were 71 patients with OSA with cognitive impairment, accounting for 57.7% of the total number of patients. There were no statistical differences in age, gender, BMI, history of alcohol consumption,

Table 1 Comparison of General Information, Serologic Indicators and Cognitive Status Among Groups

Indicators	Severe OSA Group (n=56)	Mild to Moderate OSA Group (n=67)	Control Group (n=100)	$F/x^2/H$	P
Male	36(64.3%)	39(58.2%)	56(56%)	1.041	0.594
Female	20(35.7%)	28(41.8%)	44(44%)		
Age (years)	40.38±9.77	38.43±7.69	41.12±8.61	1.273	0.163
BMI $(kg/m)^2$	26.86±2.31	26.75±2.75	25.83±1.97	0.671	0.712
History of alcohol consumption	19(33.9%)	21(31.3%)	32(32%)	0.100	0.951
History of hypertension	11(19.6%)	11(16.4%)	14(14%)	1.155	0.561
History of diabetes	7(12.5%)	9(13.4%)	12(12%)	0.075	0.963
LPS (pg/mL)	346.21±76.71[ab]	257.39±52.28[a]	61.81±13.74	29.341	<0.001
Nrf2 (ng/mL)	4.16±0.79[ab]	3.08±0.67[a]	1.07±0.19	8.371	0.003
HO-1 (ng/mL)	6.97±0.97[ab]	5.62±1.07[a]	3.03±0.56	5.078	0.007
MoCA Total Score	19.38±5.71[ab]	25.67±6.47	28.03±7.17	3.677	0.018
AHI	54.25±19.15[ab]	33.97±20.41[a]	4.36±5.14	18.14	<0.001
LSaO2(%)	64.78±12.61[ab]	83.67±11.12	89.61±9.21	9.172	0.002
WBC	5.30(4.90, 5.90)	5.40(5.20, 5.68)	5.32(4.90, 5.52)	1.193	0.087
RBC	5.82(5.61, 6.12)[ab]	4.85(4.53, 5.13)	4.91(4.51, 5.24)	5.317	0.005

Notes: [a]A significant difference compared with the control group (P < 0.05); [b]A significant difference compared with the mild to moderate OSA group (P < 0.05).

history of hypertension or history of diabetes between the group with cognitive impairment and the group with normal cognition, and the serum LPS, Nrf2 and HO-1 levels in the group with OSA with cognitive impairment were significantly higher than those in the group with normal cognition ($p < 0.05$), as shown in Table 2.

Serum Lipopolysaccharide, Nuclear Factor erythroid 2-Related Factor 2 and Haem Oxygenase 1 Levels and the Montreal Cognitive Assessment Scores

The Table 3 showed the results of correlation analysis of serum LPS, Nrf2, HO-1 levels and MoCA scores. Serum LPS ($r = -0.571$, $p < 0.001$), Nrf2 ($r = -0.313$, $p = 0.042$) and HO-1 ($r = -0.407$, $p = 0.003$) were significantly negatively correlated with MoCA scores in the patients with OSA.

Multifactor Analysis of the Montreal Cognitive Assessment Scores

In patients with OSA, MoCA score was included as the dependent variable, and OSA severity and serum LPS, Nrf2 and HO-1 levels were included as independent variables in a multiple linear regression model. The results showed that LPS and HO-1 could be considered as independent predictors of MoCA scores and that the serum LPS ($t = -4.809$, $p < 0.001$) and HO-1 ($t = -3.924$, $p = 0.002$) levels increased, suggesting a decrease in MoCA score, as shown in Table 4.

Table 2 Comparison of Indicators Between OSA with Cognitive Impairment Group and Normal Cognitive Group

Indicators	OSA with Cognitive Impairment Group (n=71)	OSA Cognitive Normal Group (n=52)	t/x^2	P
Male	45(63.4%)	30(57.7%)	0.408	0.523
Female	26(36.6%)	22(42.3%)		
Age (years)	37.12±11.03	41.34±10.87	1.087	0.221
BMI $(kg/m)^2$	25.37±3.43	26.05±3.56	1.001	0.315
History of alcohol consumption	23(32.4%)	17(32.7%)	0.001	0.972
History of hypertension	10(14.1%)	12(23.1%)	1.653	0.199
History of diabetes	7(9.9%)	5(9.6%)	0.002	0.964
LPS (pg/mL)	335.18±84.35	267.94±66.78	13.440	<0.001
Nrf2 (ng/mL)	3.68±0.64	3.42±0.73	2.725	0.027
HO-1 (ng/mL)	7.87±1.03	6.12±1.12	3.142	0.014

Table 3 Correlation Analysis of Serum LPS, Nrf2, HO-1 Levels and MoCA Scores

Projects	LPS		Nrf2		HO-1	
	r value	*P*	r value	*P*	r value	*P*
MoCA	−0.571	<0.001	−0.313	0.042	−0.407	0.003

Table 4 Multiple Linear Regression Model of Factors Influencing MoCA Scores

Model	Nonstandard Coefficient	Standard Error	Standard Coefficient	t	*P*
OSA severity (1 mild to moderate, 2 severe)	−0.981	0.465	−0.884	−2.352	0.018
LPS	−1.884	0.412	−1.837	−4.809	<0.001
Nrf2	−0.812	0.103	−0.804	−1.351	0.183
HO-1	−1.132	0.355	−1.183	−3.924	0.002

Discussion

According to the results of the MoCA scale scores, the rate of combined cognitive impairment in patients with OSA was 57.7%, and the MoCA scores of patients with severe OSA were significantly lower than those of the patients with mild-to-moderate OSA and the healthy controls. Some studies have found that in patients with OSA there is a significant correlation between elevated serum TNF-α, IL-1, IL-6, IL-10, IL-23 and other inflammatory cytokines and impairment of intellectual, executive and cognitive function.[15] However, the mechanisms mediating cognitive impairment are complex, and the relevance and specificity of serological indicators, such as LPS, Nrf2 and HO-1, need to be explored.

The results of the current study showed that the serum LPS, Nrf2 and HO-1 levels were negatively correlated with the MoCA scores, and the regression model fitting results suggested that serum LPS and HO-1 levels are independent predictors of cognitive impairment status, and that higher serum levels of both predict more severe cognitive impairment. Zhu Hongxia et al found elevated serum LPS levels in patients with OSA and higher LPS levels in those with more severe OSA, suggesting an association, consistent with the results of the current study.[16] Studies involving rat models have found that systemic LPS may contribute to memory cognitive impairment by inducing reactive oxygen species, increased nitrogen oxides and lipid peroxidation to produce peripheral inflammation with brain inflammation and oxidative damage.[17] Therefore, patients with OSA may have elevated serum LPS levels due to repeated hypoxic states, which are involved in the onset and progression of inflammatory responses, particularly specific activation of toll-like receptor 4, leading to neuronal damage and resulting in reduced cognitive function.

Nuclear factor erythroid-2-related factor 2 plays a key role in the response to oxidative stress, inducing the expression of several antioxidant genes, including HO-1.[18] Haem oxygenase 1 is generally underexpressed in vivo. It is highly induced by pro-oxidant and pro-inflammatory stimuli, such as ischemia/hypoxia, hormones and nitrogen oxides, and it exerts anti-inflammatory and antioxidant effects and is involved in regulating important physiological processes, such as cell proliferation, apoptosis and autophagy.[19,20] The Nrf2/HO-1 signalling pathway plays an important role in antioxidant stress and inflammatory responses in the body, and activation of the Nrf2/HO-1 signalling pathway enhances the antioxidant defence system and improves cognitive deficits in rats.[21] Similar to the findings obtained by Mueller et al,[22] we suggest that patients with OSA may upregulate their serum Nrf2/HO-1 levels to play a defensive role against the damage caused to cognitive function in order to resist oxidative stress and inflammatory responses, and there is a correlation between the upregulated levels and the severity of cognitive impairment.

This study has several limitations. First, this was a single-centre study and the results may not represent all patients. Second, although this study explored the correlation between cognitive impairment and the levels of serum LPS, Nrf2 and HO-1, further research is needed to determine whether these indicators are potential diagnostic indicators. Third, the detection indicators of the participants may have been biased due to the influence of the first-night effect. Finally, this study did not exclude the influence of certain confounding factors, such as sleep deprivation.

Conclusion

In summary, the serum LPS, Nrf2 and HO-1 levels are higher in patients with OSA than in the normal population and correlate with the degree of cognitive impairment. Lipopolysaccharide dysregulation can be involved in the occurrence of neuronal inflammatory response and is one of the pathways mediating the development of cognitive impairment in patients with OSA. The Nrf2/HO-1 signalling pathway may play a protective role in the process of cognitive impairment due to oxidative stress and inflammatory response being upregulated. Serum LPS and HO-1 levels have the potential to aid in the diagnosis of cognitive impairment in patients with OSA with comorbid cognitive impairment. Further research is needed to determine whether these indicators are potential diagnostic indicators.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Jinhua central Hospital (No.: Y2020-058-001). Written informed consent was obtained from all participants.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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