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Associations of OSAHS complicated by cerebral infarction with intestinal flora, inflammatory factors, homocysteine and adiponectin expression

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Abstract. – OBJECTIVE: The aim of this study was to investigate the associations between obstructive sleep apnea hypopnea syndrome (OSAHS) complicated by cerebral infarction and intestinal flora, inflammatory factors, homocysteine, and adiponectin expression.

PATIENTS AND METHODS: A total of 30 healthy volunteers (control group), 28 patients with simple cerebral infarction (cerebral infarction group) and 28 patients with OSAHS complicated by cerebral infarction (OSAHS + cerebral infarction group) were enrolled as research objects. The fecal bacterial DNA of research objects was extracted and subject to 16S ribosomal ribonucleic acid sequencing. Furthermore, the levels of inflammatory factors, homocysteine and adiponectin in the peripheral blood were detected.

RESULTS: Compared with control group, cerebral infarction group exhibited significantly higher levels of interleukin-4 (IL-4), tumor necrosis factor-beta (TNF-β), IL-1β and C-reactive protein (CRP) (p<0.05). However, the levels of TNF-β, IL-1β and CRP in OSAHS + cerebral infarction group were notably higher than those in cerebral infarction group (p<0.05). The levels of myeloperoxidase (MPO) and malondialdehyde (MDA) were remarkably higher in cerebral infarction group than those in the control group (p<0.05). However, they were significantly higher in OSAHS + cerebral infarction group than cerebral infarction group (p<0.05). Compared with control group, cerebral infarction group exerted a noticeably higher level of homocysteine (p<0.05). However, homocysteine level was markedly higher in OSAHS + cerebral infarction group than that in cerebral infarction group (p<0.05). Adiponectin level was significantly lower in cerebral infarction group than that in the control group (p<0.05). Meanwhile, it was evidently lower in OSAHS + cerebral infarction group than that in the cerebral infarction group (p<0.05). Control group had the highest abundance of Actinobacteria, and cerebral infarction group exhibited the highest abundance of *Coriobacteriales, Vagococcus, Sphingobacteriales* and *Adlercreutzia*. However, OSAHS + cerebral infarction group exhibited the highest abundance of *Bifidobacterium, Parascardovia, Metascardovia* and *Anaerostipes caccae*. There was a strong positive correlation between *Proteobacterium* and *Ruminococcus* (r=0.9, p=0.000) and between *Firmicutes* and *Bacteroidetes* (r=0.72, p=0.004). However, there was a significant negative correlation between *Firmicutes* and *Enterobacteriales* (r=-0.45, p=0.009).

CONCLUSIONS: OSAHS complicated by cerebral infarction is significantly associated with intestinal flora, inflammatory factors, homocysteine and adiponectin expression.

Key Words:
OSAHS, Cerebral infarction, Intestinal flora, Adiponectin.

Introduction

Obstructive sleep apnea hypopnea syndrome (OSAHS) refers to the apnea and shallow breathing caused by airway obstruction during sleep, accompanied by snoring. As a result, it leads to the reduction of ventilation and even repetitive hypoxia and carbon dioxide aggradation, severely affecting the sleep quality of patients[1,2]. In addition to sleep structure disturbance and poor mental state during the day, OSAHS is also a causative factor of many diseases, such as metabolic diseases and cardiovascular diseases[3,4]. OSAHS is an independent risk factor of cerebral infarction, greatly threatening the life of patients[5,6]. Hence, investigating the clinical features of patients with OSAHS complicated by cerebral infarction is of significant importance for determining its patho-

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genesis of the disease and formulating corresponding treatment countermeasures.

Intestinal flora, important organisms colonizing the digestive tract of human body, play a critical role in maintaining normal life activities[7,8]. Disturbance of intestinal flora affects the physiological functions of the digestive tract. It can also change the systematic status of the body, such as the level of inflammation, thereby triggering multiple diseases[9]. The alteration of intestinal microorganisms will influence the metabolism of substances in the body, which may be associated with the occurrence of OSAHS complicated by cerebral infarction[10].

Therefore, in this study, the composition and abundance of intestinal microorganisms were compared among 30 healthy volunteers, 28 patients with simple cerebral infarction, and 28 patients with OSAHS complicated by cerebral infarction. Meanwhile, the features of intestinal microorganisms were investigated in patients with simple cerebral infarction and in patients with OSAHS complicated by cerebral infarction. Clinical indexes, such as inflammatory factors, homocysteine, and adiponectin were compared among groups as well. Finally, the clinical features of patients with OSAHS complicated by cerebral infarction were summarized.

Patients and Methods

Patients

A total of 30 healthy volunteers (control group), 28 patients with simple cerebral infarction (cerebral infarction group) and 28 patients with OSAHS complicated with cerebral infarction (OSAHS + cerebral infarction group) treated in our hospital were selected as research subjects. The selection of patients was based on the guideline of the International Classification of Sleep Disorders. There were no statistically significant differences in general data, such as gender, age, height and body weight among the three groups. Diagnostic criteria for OSAHS were as follows: such symptoms of drowsiness, lack of sleep, snoring and repeated apnea, low ventilation during the sleep found *via* polysomnography monitoring, and abnormal upper airway structure shown in imaging examination. Cerebral infarction was diagnosed based on the imaging examination, that was low-density shadow in CT. This investigation was approved by the Ethics Committee of The Affiliated Jiangsu Shengze Hospital of Nanjing Medical University.

Analysis of Intestinal Flora

Fresh mid-posterior-segment fecal samples (3-5 g) were collected from each group, and stored in a liquid nitrogen container within 2 h. Subsequently, total bacterial DNA was extracted. The variable region 4 of 16S rRNA was amplified *via* Polymerase Chain Reaction (PCR), purified and sequenced. Finally, the diversity of intestinal microorganisms was analyzed.

Detection of Homocysteine

Plasma homocysteine was detected *via* enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA) in each group. Briefly, 5 mL of peripheral blood was drawn from each object using EDTA (ethylenediaminetetraacetic acid) anticoagulant tubes and stored *via* ice water bath. The plasma was then separated within 30 min and stored at -80°C for use.

Detection of Inflammatory Factors and Oxidative Stress Molecules

The levels of inflammatory factors interleukin-4 (IL-4), tumor necrosis factor-beta (TNF-β), IL-1β and C-reactive protein (CRP), and serum oxidative stress indexes superoxide dismutase (SOD), myeloperoxidase (MPO) and malondialdehyde (MDA) were detected according to the instructions of ELISA kit, with 3 replicates in each group. Optical density (OD) value at 450 nm was detected using a micro-plate reader, and converted into the actual concentration of each index using standard curves.

Detection of Adiponectin Level

The level of serum adiponectin in each group was detected in strict accordance with the adiponectin quantitative assay kit. After the serum was diluted with buffer, it was added into wells and shaken with a shaker, followed by incubation and washing. Subsequently, enzyme-conjugated solution, substrate and stop buffer were added. OD value at 450 nm was finally determined using a micro-plate reader.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Unpaired *t*-test was used for the comparison of difference between two groups. $p<0.05$ was considered statistically significant.

Results

Basic Data of Objects in Each Group

As shown in Table I, there were no statistically significant differences in general data, such as gender, age distribution and body mass index (BMI) among the three groups ($p>0.05$).

Comparison of Levels of Inflammatory Factors in Each Group

As shown in Table II, the levels of IL-4, TNF-β, IL-1β and CRP were significantly higher in cerebral infarction group than those in control group ($p<0.05$). However, they significantly increased in OSAHS + cerebral infarction group compared with cerebral infarction group ($p<0.05$).

Comparison of Levels of Oxidative Stress Molecules in Each Group

As shown in Table III, the levels of MPO and MDA were remarkably higher in cerebral infarction group than those in control group ($p<0.05$). Meanwhile, they significantly rose in OSAHS + cerebral infarction group compared with cerebral infarction group ($p<0.05$).

Differences in Homocysteine Level in Each Group

Cerebral infarction group exhibited a markedly higher level of homocysteine than control group ($p<0.05$). In addition, OSAHS + cerebral infarction group had a significantly higher level of homocysteine than cerebral infarction group ($p<0.05$) (Figure 1).

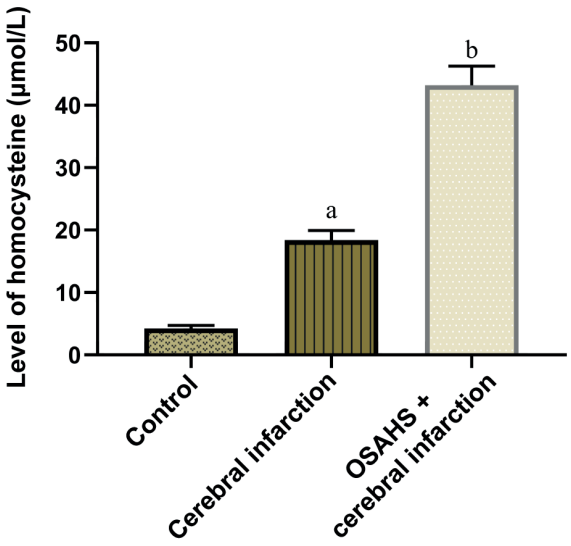

Figure 1. Level of homocysteine in each group ([a]$p<0.05$ *vs.* control group, [b]$p<0.05$ *vs.* cerebral infarction group, unpaired *t*-test).

Level of Adiponectin in Each Group

Cerebral infarction group had an evidently lower level of adiponectin than control group ($p<0.05$). Moreover, OSAHS + cerebral infarction group exhibited a remarkably lower level of adi-

Table I. Basic data of objects in each group.

	Control group	Cerebral infarction group	OSAHS + cerebral infarction group	*p*
n	30	28	28	
Gender (n)				>0.05
Male	14	15	14	
Female	16	13	14	
Age (Y)				>0.05
>60	21	20	18	
≤60	9	8	10	
BMI	23.32±2.45	23.19±3.18	22.98±3.64	>0.05

Table II. Comparison of levels of inflammatory factors in each group.

Group	n	IL-4 (ng/L)	TGF-β (ng/L)	IL-1β (ng/L)	CRP (ng/L)
Control group	30	2.09±0.11	2.41±0.25	1.48±0.34	1.34±0.21
Cerebral infarction group	28	3.98±0.34[a]	5.42±0.38[a]	5.98±0.84[a]	4.38±0.58[a]
OSAHS + cerebral infarction group	28	4.05±0.31	7.84±0.47[b]	7.22±1.04[b]	6.43±0.81[b]

Note: [a]$p<0.05$ *vs.* control group, [b]$p<0.05$ *vs.* cerebral infarction group, unpaired *t*-test.

Table III. Comparison of levels of oxidative stress molecules in each group.

Group	n	SOD (U/mL)	MPO (mg/L)	MDA (nmol/mL)
Control group	30	2.02±0.18	2.16±0.20	0.98±0.06
Cerebral infarction group	28	2.31±0.31	4.87±0.28[a]	3.22±0.15[a]
OSAHS + cerebral infarction group	28	2.27±0.23	6.85±0.62[b]	5.31±0.35[b]

Note: [a]$p<0.05$ *vs.* control group, [b]$p<0.05$ *vs.* cerebral infarction group, unpaired *t*-test.

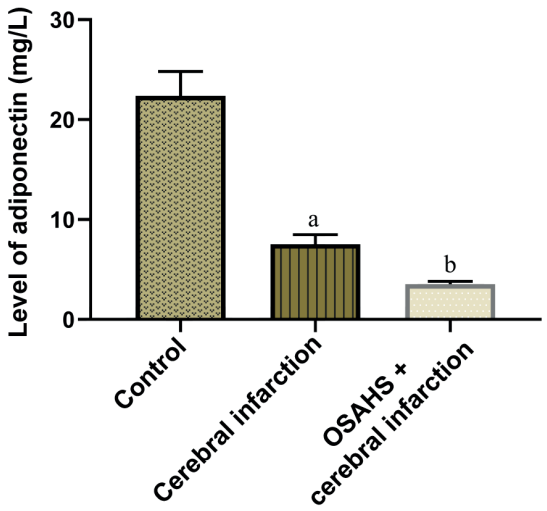

Figure 2. Level of adiponectin in each group ([a]$p<0.05$ *vs.* control group, [b]$p<0.05$ *vs.* cerebral infarction group, unpaired *t*-test).

ponectin than cerebral infarction group ($p<0.05$) (Figure 2).

Analysis of Intestinal Flora in Each Group

As shown in Figures 3 and 4, control group had a significantly higher abundance of Actinobacteria ($p<0.05$). The abundance of *Coriobacteriales, Vagococcus, Sphingobacteriales* and *Adlercreutzia* was significantly higher in cerebral infarction group ($p<0.05$). Furthermore, the abundance of *Bifidobacterium, Parascardovia, Metascardovia* and *Anaerostipes caccae* was evidently higher in OSAHS + cerebral infarction group ($p<0.05$).

Correlation Analysis of Intestinal Flora

According to the correlation analysis of intestinal flora (Figure 5), there was a significant positive correlation between *Proteobacterium* and *Ruminococcus* ($r=0.9$, $p=0.000$), and between *Firmicutes* and *Bacteroidetes* ($r=0.72$, $p=0.004$).

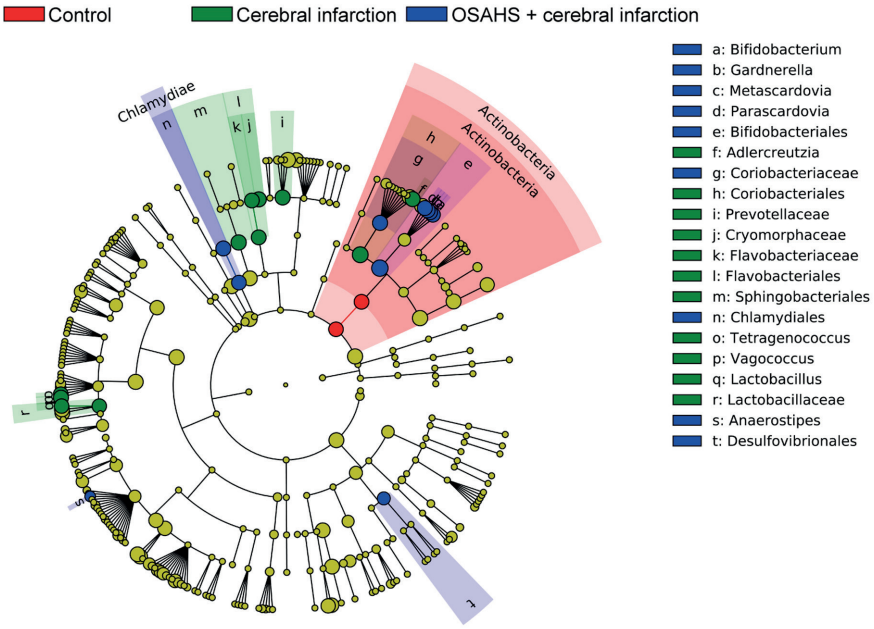

Figure 3. LEfSe of intestinal flora in each group.

However, there was a significant negative correlation between *Firmicutes* and *Enterobacteriales* (r=-0.45, p=0.009).

Discussion

Repetitive apnea of OSAHS patients during sleep will drive changes in blood oxygen level of the body, leading to brief episodes of hypoxemia and inducing a series of diseases[11,12]. OSAHS may promote the pathological processes of diseases through changes in the levels of systematic inflammation, immunity, and metabolism caused by hypoxia[13,14]. Cerebral infarction is a kind of blood supply disorder in local cerebral tissues induced by multiple factors. It may cause ischemia, hypoxia and necrosis of tissues, eventually leading to neuropsychiatric symptoms and even multiple organ dysfunction[15]. The occurrence of cerebral infarction is closely related to OSAHS, and about 50% of patients will suffer from OSAHS[16]. OSAHS may further decrease oxygen supply to local cerebral tissues by reducing systematic blood oxygen content and causing microangiopathy, thereby aggravating cerebral infarction[17,18]. However, the specific mechanism of the interaction between the two diseases has not been fully elucidated. Therefore, investigating the clinical features of patients with OSAHS complicated by cerebral infarction is important for clarifying the biological mechanism of OSAHS in promoting the occurrence of cerebral infarction. Meanwhile, this will also provide a new theoretical basis for its symptomatic treatment.

Intestinal flora is important for maintaining normal physiological status of the body. It mainly participates in the absorption, digestion and decomposition of substances in the digestive tract. Meanwhile, it also indirectly influences systematic inflammation, metabolism and other status[19]. Disturbance of intestinal flora will change the levels of some cytokines and metabolites in blood circulation, thus leading to various diseases[20]. Previous studies have shown that changes in intestinal flora promote or inhibit multiple diseases, such as arthritis and spondylarthritis[21]. As a disease affecting systematic blood oxygen content, OSAHS may lead to changes in intestinal flora, thus regulating the systematic status of the body. In this process, intestinal flora plays a vital role in inducing cerebral infarction. In this study, the composition and abundance of intestinal microorganisms were first compared among the three

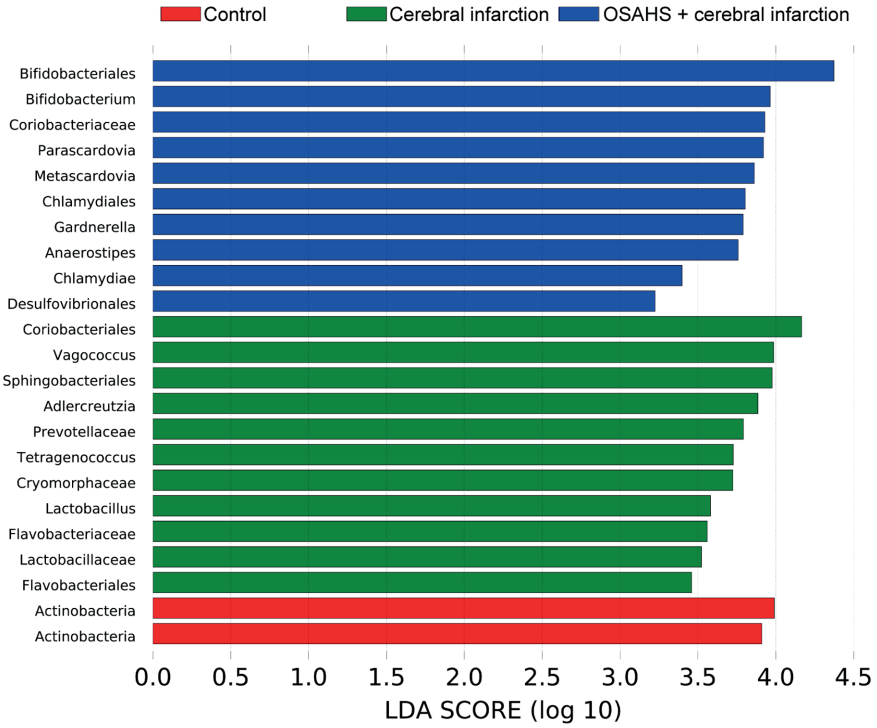

Figure 4. LDA score of intestinal flora in each group.

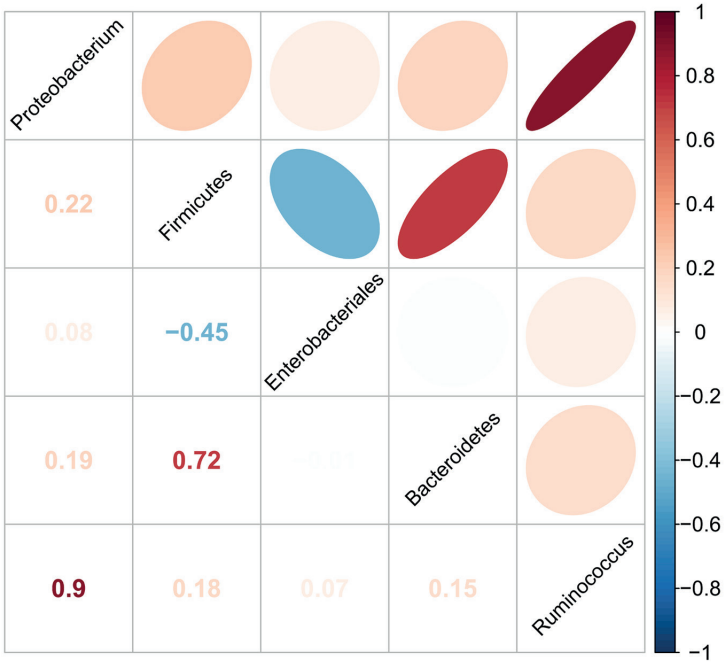

Figure 5. Correlation analysis of intestinal flora.

groups. It was found that control group had the highest abundance of *Actinobacteria*, cerebral infarction group exhibited the highest abundance of *Coriobacteriales, Vagococcus, Sphingobacteriales* and *Adlercreutzia*, while OSAHS + cerebral infarction group exerted the highest abundance of *Bifidobacterium, Parascardovia, Metascardovia* and *Anaerostipes caccae*. Moreover, there was a strong positive correlation between *Proteobacterium* and *Ruminococcus* (r=0.9, p=0.000), and between *Firmicutes* and *Bacteroidetes* (r=0.72, p=0.004). However, *Firmicutes* was negatively correlated with *Enterobacteriales* (r=-0.45, p=0.009). The above results indicate that there is an evident association between OSAHS complicated by cerebral infarction and intestinal flora disturbance.

In the present study, it was found that there was a certain degree of changes in inflammatory factors and oxidative stress molecules in patients with OSAHS complicated by cerebral infarction. The levels of IL-4, TNF-β, IL-1β and CRP were remarkably higher in cerebral infarction group than those in the control group (p<0.05). Meanwhile, the levels of TNF-β, IL-1β and CRP in OSAHS + cerebral infarction group were notably higher than those in cerebral infarction group (p<0.05). Besides, the levels of MPO and MDA were markedly higher in cerebral infarction group than those in control group (p<0.05). Meanwhile, they were significantly higher in OSAHS + cerebral infarction group than those in cerebral infarction group (p<0.05). These results suggest that the levels of inflammation and oxidative stress have an important impact on the development of OSAHS complicated by cerebral infarction. Homocysteine, a kind of sulfur-containing amino acid, is an important intermediate product of methionine metabolism. Adiponectin, a bioactive polypeptide secreted by adipocytes, is involved in the regulation of nutrient metabolism, immunity, vascular endothelial function, etc. It has been observed that both homocysteine and adiponectin are closely associated with cardiovascular and cerebrovascular diseases. Moreover, they may affect the occurrence of diseases by regulating the level of metabolism of the body. In this study, the results revealed that homocysteine level was evidently higher in cerebral infarction group than that in the control group (p<0.05). Meanwhile, it was noticeably higher in OSAHS + cerebral infarction group than that in cerebral infarction group (p<0.05). Besides, the level of adiponectin significantly decreased in cerebral infarction group than that in control group (p<0.05). It was significantly lower in OSAHS + cerebral infarction group than that in cerebral infarction group (p<0.05). The above findings indicate that homocysteine and adiponectin may be involved in the development of OSAHS complicated by cerebral infarction.

Conclusions

In summary, the novelty of this study was that OSAHS complicated by cerebral infarction is significantly associated with intestinal flora, inflammatory factors, homocysteine and adiponectin expression.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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