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ORIGINAL RESEARCH

Correlation Analysis of Characteristics of Intestinal Microbiota and Cytokine Levels in Patients with Obstructive Sleep Apnea-Hypopnea Syndrome

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Objective: The aim of this study was to analyze the relationship between the characteristics of the intestinal microbiota and cytokine levels in individuals with different degrees of obstructive sleep apnea-hypopnea syndrome (OSAHS) as well as to investigate intestinal microbiota imbalances in patients with OSAHS and the associated mechanisms.

Methods: Based on their sleep apnea hypopnea index (AHI), a total of 37 adults were assigned to a control group, a mild OSAHS group, or a moderate-to-severe OSAHS group. Fecal samples were collected to characterize the intestinal microbiota using metagenomic next-generation sequencing (mNGS), while blood samples were collected to detect levels of interleukin-17a (IL-17a), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) in each group.

Results: 1. There was no significant difference in the Shannon index among the three groups ($P > 0.05$). The three groups showed significant difference in the relative abundance of *Faecalibacterium prausnitzii* and *Bifidobacterium adolescentis* (with F values of 3.955 and 7.24, respectively, $P < 0.05$), while showed no significant difference in the relative abundance of *B. pseudocatenulatum, Bifidobacterium longum, Klebsiella pneumoniae*, and *Haemophilus parainfluenzae* ($P > 0.05$). 2. The three groups showed significant difference in the expression of serum IL-17A and TNF-α levels (with F values of 18.119 and 10.691, respectively, $P < 0.05$), while showed no significant difference in the expression of IL-10, IL-6, and CRP levels ($P > 0.05$). 3. Multiple linear regression analysis revealed that the relative abundance of *F. prausnitzii* was correlated with changes in BMI and AHI (with β values of 2.585 and −0.157, respectively, $P < 0.05$), while the relative abundance of *B. adolescentis* was correlated with changes in IL-17a (with β value of −0.161, $P < 0.05$).

Conclusion: The study revealed a significant correlation between intestinal microbiota abundance and cytokine levels, suggesting that gut microbiota disruption in OSAHS patients may be linked to systemic chronic inflammation.

Keywords: chronic inflammation, chronic intermittent hypoxia, cytokines, intestinal microbiota, OSAHS

Introduction

Obstructive sleep apnea-hypopnea syndrome (OSAHS) is the most common sleep apnea related breathing disorder. It is characterized by recurrent hypoventilation or interruption of breathing due to the collapse of the upper airway during sleep. This, in turn, leads to chronic intermittent hypoxia (CIH) and hypercapnia.[1] The overall prevalence of OSAHS in China is estimated to be 3.93%.[2]

In recent years, there has been a surge in research on the relationship between OSAHS and the intestinal microbiota of patients.[3–6] These studies have generally observed dysbiosis of gut microbiota in OSAHS. However, the current research is limited by the lack of a clear understanding of the role of gut microbiota in the development of OSAHS, particularly the association between gut microbiota and biochemical indicators has not been thoroughly elucidated. OSAHS is mainly characterized by CIH, which induces oxidative stress and leads to systemic, chronic, low-level

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inflammation.[7] Consequently, OSAHS triggers a systemic, chronic, inflammatory response involving various inflammatory mediators and immune cells. Serum biomarkers associated with inflammation were found to be activated in patients with OSAHS, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6).[8] These inflammatory mediators may lead to endothelial dysfunction, thereby causing various end-organ diseases and impaired neurocognitive function. The precise pathophysiological mechanisms responsible for the disruption of the intestinal microbiota in patients with OSAHS remain poorly understood, particularly in relation to inflammatory transmitters.

In this study, we analyzed the characteristics of the intestinal microbiota and serum cytokine levels in patients with different degrees of OSAHS. We investigated the correlation between intestinal microbiota and cytokines in patients with OSAHS, clarified the disorder of intestinal flora in OSAHS patients may be related to chronic inflammation, and explored the possible pathophysiological mechanisms of intestinal microbiota disorders in patients with OSAHS. Our aim is to provide insights and a theoretical basis for the intestinal-targeted treatment of OSAHS and its associated complications.

Materials and Methods

Study Participants

Source and Grouping of Study Participants

Patients with OSAHS who underwent polysomnography (PSG) monitoring and were diagnosed at the First Affiliated Hospital of Suzhou University from June 2021 to June 2023 were selected for the current study. They were categorized into a mild OSAHS group (10 cases) and a moderate-to-severe OSAHS group (17 cases) based on the international classification of sleep disorders using the apnea hypopnea index (AHI). In addition, individuals who were found not to have OSAHS by PSG monitoring during the same period were selected as the control group (10 cases). A total of 37 participants were enrolled in the study. The sample size was estimated using SPSS software, with a significance level (α) set at 0.05 and a statistical power (1-β) of 0.90. Based on the calculations, a sample size of 8 individuals per group was required, resulting in a total sample size of 16 individuals. Therefore, the sample size included in this study meets the requirements for the trial. The experimental grouping design refers to Ko's study.[9] Inclusion criteria: (1) Aged 18 years or older; (2) Voluntary participation in this study; (3) Ability for normal communication and active cooperation to complete polysomnography (PSG); (4) Availability of complete clinical data.

Exclusion Criteria

(1) patients with co-existing malignant tumors, hematologic diseases, severe liver and renal insufficiency, chronic obstructive pulmonary disease, and other chronic diseases; (2) those who underwent surgery or received ventilator treatment for OSAHS; (3) individuals who were administered antibiotics in the past three months, and those had used intestinal probiotic products within the last two weeks; (4) individuals who had diarrhea, constipation, or dysentery within the last three months, as well as those with chronic gastritis, chronic gastric ulcers, and other intestinal diseases; (5) patients with infectious diseases, rheumatic connective tissue systemic diseases, and so on; (6) pregnant women or minors aged under 18 years; and (7) patients with incomplete clinical information.

All participants adhered strictly to the inclusion and exclusion criteria. The selected subjects had no acute infections, malignant tumors, or diseases affecting the gut microbiota, and had not taken medications that could interfere with the gut microbiota. This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University (Approval No: (2023) Lun Yan Pi No. 005), and all participants provided written informed consent.

Research Methodology

Data Collection

Basic details pertaining to age, gender, body mass index (BMI), history of smoking and alcohol use, hypertension, diabetes mellitus, and Epworth Sleepiness Scale (ESS) scores were collected and recorded.

PSG Monitoring

A German polysomnography system (Weinmann SOMNOlab 2) was used for overnight monitoring (more than seven hours). AHI, mean oxygen saturation (SpO_2mean), minimum oxygen saturation (SpO_2min), and the longest time of sleep apnea (TAmax) were recorded.

Metagenomic Next-Generation Sequencing (mNGS) for the Detection of Intestinal Microbiota

Approximately 1 g of fecal samples from the study participants were collected in the morning of the second day after PSG. The samples were then placed in sterile, dry collection tubes and transported in ice packs within eight hours to the laboratory for storage in a refrigerator at −80°C.

(1) Nucleic Acid Extraction, Library Construction, and Online Sequencing: Metagenomic DNA in the fecal samples was extracted using the magnetic bead method and enzyme digestion, while metatranscriptomic RNA was extracted using the column method. The extracted DNA and RNA nucleic acids were quantified and underwent quality control assessments, and the total amount of DNA and RNA was determined using Qubit.

(2) Library Construction and Quality Control: A specified quantity of the extracted and qualified DNA and RNA were used for library construction. The library construction process involved fragment purification, end repair, junction joining, fragment size selection, PCR amplification, product purification, and quality control (QC) of library products for the fragmented DNA samples in a sequential manner. The process of RNA library construction incorporated the steps involved in DNA library construction as well as additional reverse transcription to generate double-stranded DNA.

(3) On-Board Sequencing: Following successful completion of the quality control checks, the library was subjected to sequencing as per the kit instructions, employing the Illumina NovaSeq high-throughput sequencer. The sequencing was conducted based on specified data volumes, with 3G for DNA-seq and 15G for RNA-seq.

(4) Down-Sequencing Data Analysis: FASTQ was used for quality control of the down-sequencing data to filter raw reads and obtain higher-quality clean reads. Bowtie2 software facilitated the comparison of non-human sequences with the human genome to filter out genome and mitochondrial DNA sequences of human origin. The remaining non-human sequences were then comprehensively analyzed using software such as Kraken, Bracken, and Humann2, among others, to investigate the microflora composition.

(5) Sequencing Data Processing: This phase involved analyzing the Shannon index of intestinal microorganisms, bacterial abundance, and other related factors. This analysis was conducted by the Nanjing Dinfectome Medical Testing Laboratory.

Serological Testing

A sample of 10 mL of fasting venous blood was collected in the early morning of the day following the completion of polysomnography. An enzyme-linked immunosorbent assay (ELISA) was utilized to measure the levels of serum cytokines interleukin-17a (IL-17a), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) in each group.

Statistical Analysis

SPSS 27.0 medical statistical software was used for the analysis and processing of data in this study. Measurement data that satisfied normal distribution and homogeneity of variances were expressed as $\bar{x} \pm s$, while measurement data that were not normally distributed were expressed as M (P_{25}, P_{75}). Counting data were expressed as percentages. For measures that met the normal distribution, one-way analysis of variance (ANOVA) tests were used.

Statistically significant information was then compared between each pair of groups using post-hoc multiple comparisons and Bonferroni tests. The Wilcoxon signed-rank sum test was used for non-normally distributed measures, and the Kruskal-Wallis H test was employed to compare the two groups for statistically significant data. Categorical data were compared using the $\chi 2$ test. Pearson correlation analysis was utilized to investigate the degree and direction of correlation between the two factors. For non-normally distributed measures, the Spearman correlation analysis was employed to examine the correlation between them. Multiple linear regression analysis was used for multifactorial analysis. A P value < 0.05 was considered statistically significant.

Results

Comparison of Basic Information Among the Three Groups

Basic Information of Study Participants in the Three Groups

Our study sample consisted of 37 participants, consisting of 23 males and 14 females aged between 18 and 75 years. The differences in gender, age, history of smoking, alcohol use, history of diabetes, history of hypertension, and BMI were not statistically significant overall when the three groups were compared ($P > 0.05$). ESS scores were significantly higher in patients in the moderate-to-severe OSAHS group than in those in the control group when the two groups were compared ($P < 0.001$). Patients in the moderate-to-severe OSAHS group had significantly higher AHI compared to those in the mild OSAHS group and the control group ($P < 0.001$). SpO2 mean was significantly lower in patients in the moderate-to-severe OSAHS groups than in both the mild OSAHS and the control groups ($P < 0.001$). SpO_2min was also significantly lower in patients in the moderate-to-severe OSAHS group compared to the other two groups ($P < 0.001$). Patients in the moderate-to-severe OSAHS groups had significantly higher TAmax when compared to the mild OSAHS and the control groups ($P < 0.001$). Details are given in Table 1.

Characterization of Intestinal Microbiota Among the Three Groups

Comparison of the Shannon Diversity of Intestinal Microbiota and Relative Abundance of Species at the Microbiota Level Among the Three Groups

The Shannon index refers to the richness and evenness of a community, reflecting the evenness of the microbiota (the extent of differences in richness among its members). A higher Shannon index corresponds to greater microbiota diversity. The difference in the Shannon index values among the three groups was not statistically significant ($P > 0.05$, Figure 1).

Comparison of the Relative Abundance of Intestinal Microbiota Species at the Species Level Among the Three Groups

There was a statistically significant difference in the relative abundance of *Faecalibacterium prausnitzii* and *Bifidobacterium adolescentis* when the three groups were compared (with F values of 3.955 and 7.24, respectively, $P < 0.05$). The relative abundance of *F. prausnitzii* was significantly lower in the mild OSAHS group and the moderate-to-severe OSAHS group compared to the control group when analyzing the groups pairwise ($P < 0.05$). *B. adolescentis* was significantly lower in samples from the moderate-to-severe OSAHS group in comparison with the control group ($P < 0.05$).

Table 1 Comparison of Basic Information of the Study Participants in the Three Groups

Indicator	Control Group (n=10)	Mild OSAHS Group (n=10)	Moderate-to-Severe OSAHS Group (n=17)	F(X^2)	*P* value
Male [case (%)]	5(50.0%)	6(60%)	12(70.5%)	1.616	0.446
Age, years [case (%)]	33.86±10.54	39.83±9.37	43.60±17.31	0.951	0.399
Smoking [cases (%)]	2(20%)	6(60%)	10(58.82%)	4.507	0.105
Alcohol use [case (%)]	2(20%)	6(60.0%)	4(23.52%)	4.788	0.091
Diabetes mellitus [case (%)]	1(10.0%)	3(30.0%)	4(23.52%)	1.248	0.536
Hypertension [case (%)]	2(20.0%)	2(20.0%)	8(47.06%)	3.070	0.215
BMI(Kg/m^2)	25.06±2.97	23.70±3.26	24.60±2.73	0.565	0.574
ESS (points)	3.70±2.21	7.00±.3.23	9.88±4.82[a]	8.109	<0.001*
AHI (times/h)	2.29±1.52	11.50±3.67	47.33±18.58[ab]	46.331	<0.001*
SpO2min(%)	82.40±4.35	76.10±11.30	61.47±13.51[ab]	12.444	<0.001*
SpO2mean(%)	96.11±0.91	95.16±1.36	90.00±4.76[ab]	13.428	<0.001*
TAmax (seconds)[f]	36.(29,65)	53(42,58)	106(96,117)[ab]	18.415	<0.001*

Notes: $F(X^2)$ denotes F-statistic or Chi-square value; * denotes overall comparison among the three groups ($P < 0.05$); [a] denotes comparison with the control group ($P < 0.05$); [b] denotes comparison with the mild OSAHS group ($P < 0.05$); [f] is expressed as $M(P_{25}, P_{75})$; BMI: body mass index; ESS: Epworth score; AHI: apnea hypopnea index; SpO_2mean: mean oxygen saturation ; SpO_2min: lowest oxygen saturation; TAmax: the longest time of sleep apnea.

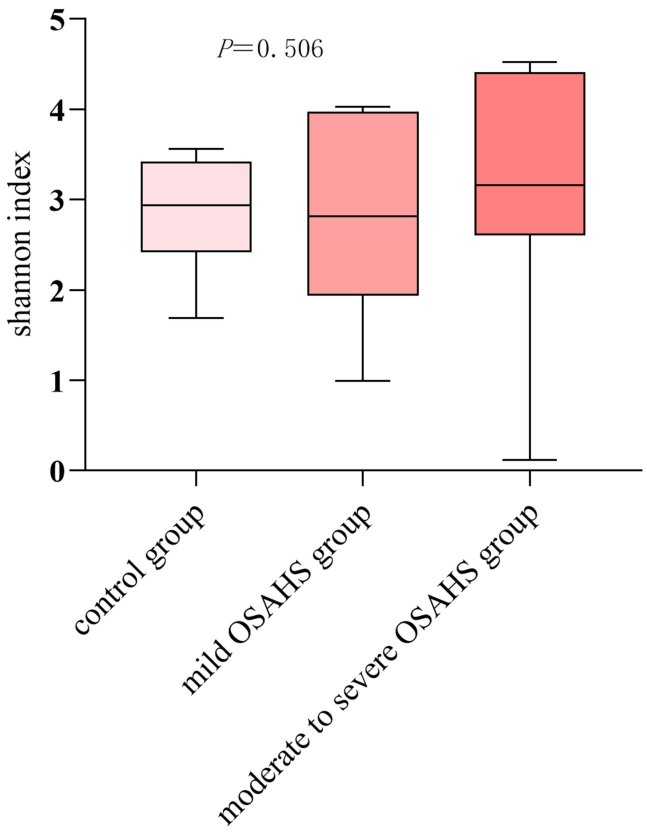

Figure 1 Comparison of Shannon Indexes Among the Three Groups.

In the overall comparison among the three groups, the differences in the relative abundance of *Bifidobacterium pseudocatenulatum, Bifidobacterium longum, Klebsiella pneumoniae*, and *Haemophilus parainfluenzae* were not statistically significant ($P > 0.05$, Table 2).

Comparison of Cytokine and CRP Levels among the Three Groups

The differences in IL-17a and TNF-α were statistically significant when compared among the three groups overall (with F values of 18.119 and 10.691, respectively, $P < 0.05$). IL-17A levels were significantly higher in both the mild OSAHS group and the moderate-to-severe OSAHS group when compared to the control group ($P < 0.001$). TNF-α levels were significantly higher in the moderate-severe OSAHS group than in the control group ($P < 0.05$). There were no significant differences in IL-10, IL-6, and CRP in the overall comparison among the three groups ($P > 0.05$, Table 3).

Table 2 Comparison of the Relative Abundance of Intestinal Microbiota Species at the Species Level Among the Three Groups

Indicator	Control Group (n=10)	Mild OSAHS Group (n=10)	Moderate-to-Severe OSAHS Group (n=17)	F(X^2)	*P* value
Faecalibacterium prausnitzii (‰)	1.99±1.61	0.76±1.00[a]	0.74±1.02[a]	3.955	0.029*
Bifidobacterium pseudocatenulatum (‰)[f]	0.12(0.00,0.42)	0.32(0.00,0.61)	0.02(0.00,0.17)	0.211	0.900
Bifidobacterium longum (‰)[f]	0.30(0.21,0.65)	0.03(0.00,0.26)	0.04(0.01,0.53)	2.693	0.260
Bifidobacterium adolescentis (‰)[f]	0.37(0.00,2.48)	0.01(0.00,0.67)	0.00(0.00,0.01)[a]	7.24	0.027*
Klebsiella pneumoniae (‰)[f]	0.01(0.00,0.51)	0.05(0.01,0.84)	0.07(0.01,0.61)	2.380	0.304
Haemophilus parainfluenzae (‰)[f]	0.02(0.00,0.29)	0.01(0.00,0.16)	0.01(0.00,0.07)	1.26	0.351

Notes: $F(X^2)$ denotes F-statistic or Chi-square value; * denotes overall comparison among the three groups ($P < 0.05$); [a] denotes comparison with the control group ($P < 0.05$); and [f] is expressed as M (P_{25}, P_{75}).

Table 3 Comparison of Cytokine and CRP Levels Among the Three Groups

Indicator	Control Group (n=10)	Mild OSAHS Group (n=10)	Moderate-to-Severe OSAHS Group (n=17)	$F(X^2)$	*P* value
IL-17a(pg/ml)	2.70±1.78	12.51±5.81[a]	10.41±3.42[a]	18.119	<0.001*
IL-10 (pg/ml)[f]	2.20(1.43,4.12)	1.90(0.00,3.57)	1.74(1.12,3.63)	0.875	0.646
TNF-a(pg/ml)[f]	1.38(0.93,1.84)	2.39(0.95,6.06)	10.40(4.26,17.82)[a]	10.691	0.005*
IL-6(pg/ml)[f]	2.38(1.15,4.54)	2.92(0.60,6.48)	5.79(0.75,21.99)	2.709	0.258
CRP(mg/L)[f]	4.77(1.25,8.16)	4.04(1.00,5.62)	3.67(2.75,12.58)	1.147	0.564

Note: $F(X^2)$ denotes F-statistic or Chi-square value; * denotes overall comparison among the three groups ($P < 0.05$); [a] denotes comparison with the control group ($P < 0.05$); and [f] is expressed as M (P_{25}, P_{75}).

Correlation Analysis of Species-Level Abundance of Intestinal Microbiota with Cytokines, CRP, and PSG Monitoring Indexes

Correlation Analysis Between the Species-Level Relative Abundance of Intestinal Microbiota and Each Variable

At the species level, the relative abundance of *F. prausnitzii* had no significant correlation with IL-10, TNF-α, ESS, SpO_2 mean, SpO_2min, or TAmax ($P > 0.05$) but had a significant negative correlation with IL-17a and AHI (r-values of −0.340 and −0.396, respectively) (Figure 2A and D) and a significant positive correlation with CRP and BMI (r-values of 0.368 and 0.672) (Figure 2B and C). There was no significant correlation between the relative abundance of *B. adolescentis* and IL-10, TNF-α, CRP, BMI, ESS, SpO_2mean, SpO_2min, or TAmax ($P > 0.05$), but it exhibited a significant negative correlation with IL-17a and AHI levels (with r values of −0.407 and −0.354, respectively) (Figure 3). The *P* values of the above tests were all < 0.05 (Table 4).

Multiple Linear Regression Analysis of the Species-Level Relative Abundance of Intestinal Microbiota and Related Variables

Using the relative abundance of *F. prausnitzii* as the dependent variable and IL-17a, CRP, BMI, and AHI as the independent variables, a multiple linear regression analysis was conducted. The results indicated a correlation between the relative abundance of *F. prausnitzii* and changes in BMI and AHI (with β values of 2.585 and −0.157, respectively, $P < 0.05$, Table 5).

Multiple linear regression analysis with the relative abundance of *B. adolescentis* as the dependent variable and IL-17a and AHI as the independent variables revealed a correlation between the relative abundance of *B. adolescentis* and changes in IL-17 (β-value of −0.161, $P < 0.05$, Table 6).

Discussion

CIH is the primary pathophysiological mechanism of OSAHS, a process that can result in systemic inflammation and trigger or worsen the development of OSAHS-related diseases and complications.[10] Intestinal microbiota plays a crucial role in the development of several chronic diseases, maintenance of intestinal immune function, and systemic dynamic equilibrium.[11] In recent years, studies have found intestinal microbiota disorders in patients with varying degrees of OSAHS, which are closely linked to the development of OSAHS. However, the specific mechanisms underlying this association remain unclear.

In this study, mNGS was used to analyze the intestinal microbiota of patients with varying degrees of OSAHS. In our study, there was no statistically significant difference in the comparison of the Shannon index of intestinal microbiota among the three groups: the normal control group, the mild OSAHS group, and the moderate-to-severe OSAHS group. This finding is consistent with the results reported by Wu et al.[6] In the study by Francesco et al,[12] children were divided into an OSAHS group and a healthy control group. The results indicated that the microbial diversity in the OSAHS group was significantly lower than that in the healthy group, as measured by observed species richness and the Chao1 index. It remains unclear whether there are significant changes in α-diversity of the gut microbiota in OSAHS. The differences in the results of these studies could be attributed to factors such as dietary habits, geographic location, and age of the study

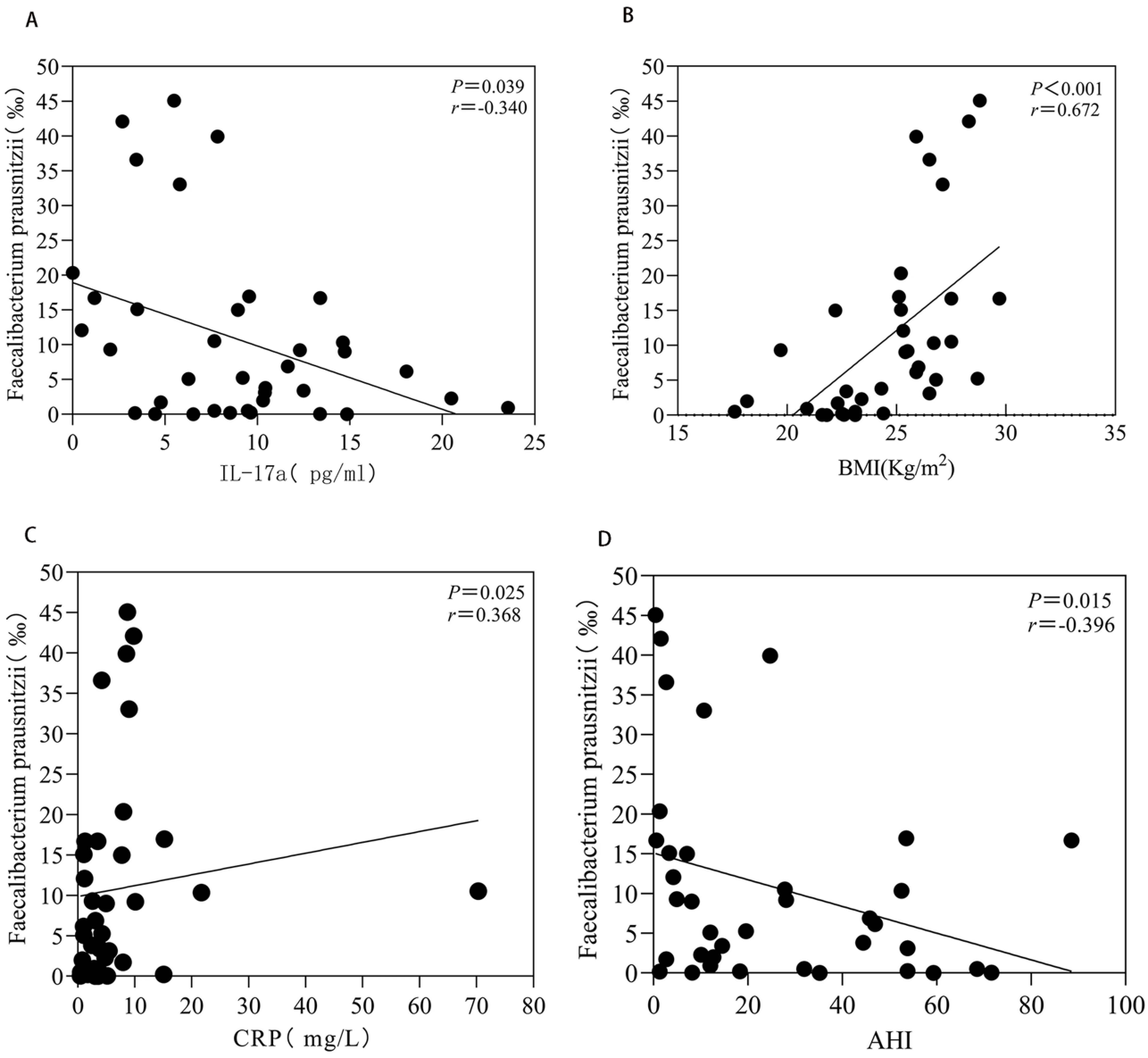

Figure 2 Relationships between *F. prausnitzii* and IL-17a levels, BMI, CRP, and AHI. (**A**): Relationships between *F. prausnitzii* and IL-17a levels; (**B**): Relationships between *F. prausnitzii* and BMI; (**C**): Relationships between *F. prausnitzii* and CRP; (**D**): Relationships between *F. prausnitzii* and AHI. *r* means correlation coefficient value; *P* means *P* value.

participants, as well as the rigor of controlling confounding factors in the animal model. β-diversity, on the other hand, is a measure of similarity in microbial composition between individuals. In our study, we observed differing levels of variation in the β-diversity of the intestinal microbiota among patients with OSAHS. The mammalian gut is mainly composed of specialized anaerobic organisms from the phylum Firmicutes and the phylum Bacteroidetes.[13] Moreno-Indias et al[4] found no significant difference between the intestinal microbiota of the normoxic and intermittent hypoxic groups when analyzed at the phylum level. However, they noted that at the genus level, the abundance of *Odoribacter, Bacteroides, Turicibacter*, and *Allobaculum* was significantly higher in the normoxic group, while *Paraprevotella* and *Prevotella* showed a significant increase in abundance in the intermittent hypoxia group. These results indicate that various degrees of intestinal microbiota disorders occur in patients with OSAHS, further confirming that intestinal microbiota disorders are closely associated with this syndrome.

In this study, a detailed analysis of the relative abundance of intestinal microbiota at the species level was conducted. The findings demonstrated a significantly lower relative abundance of *F. prausnitzii* and *B. adolescentis* was significantly

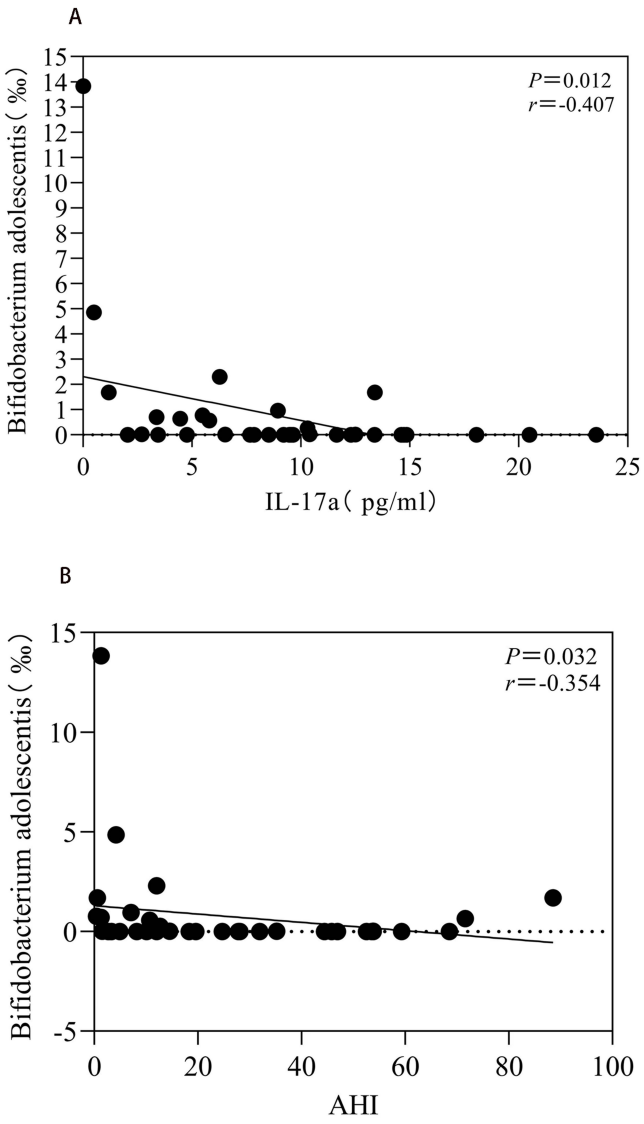

Figure 3 Relationships between *B. adolescentis* and both of IL-17a levels and AHI. (**A**): Relationships between *B. adolescentis* and IL-17a levels; (**B**): Relationships between *B. adolescentis* and AHI. *r* means correlation coefficient value; *P* means *P* value.

lower in the moderate-to-severe OSAHS group compared to the control group. Additionally, the abundance of *F. prausnitzii* was negatively correlated with AHI. One study using a CIH animal model found that cyclic decreases in oxygen partial pressure (PO_2) in the intestinal lumen during sleep induced by CIH led to changes in the relative abundance of aerobic bacteria and an increase in the relative abundance of both obligate and facultative anaerobes.[3] Wang et al[5] observed that the relative abundance of gut microbiota at the genus level significantly varied among patients with different severities of OSAHS, and this variation was notably associated with the severity of the OSAHS disease. These findings suggest that there were changes in the intestinal microbiota of patients with varying degrees of OSAHS, and the extent of disruption of the intestinal microbiota was closely linked to the severity of the disease in such patients. It may be related to the degree and time of chronic hypoxia of OSAHS. Significant variations in the composition, diversity, and relative abundance of the intestinal microbiota in patients with OSAHS were also observed. These differences may be associated with the specific survival environment—either aerobic or anaerobic—of each type of microbiota.

CIH is the main characteristic of OSAHS. Various bacterial microbiota exhibit varying levels of tolerance to hypoxia, and hypoxia can alter the pH of the intestine, inflammation levels, and other factors. Consequently, the impact of hypoxia

Table 4 Correlation Analysis Between the Species-Level Relative Abundance of Intestinal Microbiota and Each Variable

Indicator	Faecalibacterium prausnitzii		Bifidobacterium adolescentis	
	r	*p* value	r	*p* value
IL-17a	−0.340	0.039*	−0.407	0.012*
IL-10	0.021	0.900	0.089	0.601
TNF-α	−0.022	0.897	−0.282	0.091
CRP	0.368	0.025*	0.002	0.990
BMI	0.672	<0.001*	0.130	0.433
ESS	−0.280	0.093	−0.070	0.682
AHI	−0.396	0.015*	−0.354	0.032*
SpO_2mean	0.111	0.512	0.235	0.162
SpO_2min	0.112	0.511	0.123	0.408
TAmax	−0.164	0.331	−0.286	0.087

Notes: "r" means correlation coefficient value, * indicates $P < 0.05$.

Table 5 Multiple Linear Regression Analysis of Faecalibacterium Prausnitzii and Related Variables

Independent variable	β	Beta	t	*P* value
IL-17a	−0.481	−0.203	−1.498	0.144
CRP	−0.024	−0.022	0.169	0.867
BMI	2.585	0.581	4.385	<0.001*
AHI	−0.157	−0.294	−2.172	0.037*

Notes: β denotes Non-standardized regression coefficients; Beta denotes standardized regression coefficients; t denotes t-statistics; * indicates $P < 0.05$.

Table 6 Multiple Linear Regression Analysis of Bifidobacterium Adolescentis and Related Variables

Independent variable	β	Beta	t	*P* value
IL-17a	−0.161	−0.366	−2.178	0.036*
AHI	−0.008	0.080	−0.476	0.637

Notes: β denotes Non-standardized regression coefficients; Beta denotes standardized regression coefficients; t denotes t-statistics; * indicates $P < 0.05$.

on the bacterial microbiota, whether promoting or inhibiting, varies. Therefore, the diversity of intestinal microbiota differs among patients with OSAHS of varying severity, leading to variations in dominant bacterial microbiota.

An increasing number of studies have shown that OSAHS can activate the sympathetic nervous system in vivo, leading to the generation of significant amounts of oxidative reactive products and chronic inflammatory factors.[14] OSAHS leads to chronic inflammation involving a variety of immune cells, among which the activation of T lymphocytes is one of the key steps leading to the release of inflammatory mediators and adhesion molecules.[15] In recent years, studies have found that T helper cell subsets T regulatory (T reg) and Th17 have opposite effects on autoimmunity and inflammation, and secrete a variety of inflammatory factors to participate in systemic inflammatory response.[16] Numerous studies have revealed elevated levels of CRP, TNF-α, IL-6, and IL-17a in the serum of patients

with OSAHS, with a significant correlation to disease severity.[17,18] Notably, one study found that IL-10 levels were lower in patients with OSAHS than in normal subjects. Additionally, IL-1b, IL-6, and IL-8 decreased significantly in patients with OSAHS post-treatment compared to before treatment.[19] Another study demonstrated that patients with OSAHS who underwent uvulopalatopharyngoplasty (UPPP) exhibited significant improvements in the respiratory disturbance index, SpO_2min, and sleep quality following treatment. Additionally, post-treatment levels of serum IL-23 and IL-17 were found to be notably decreased compared to pre-treatment levels.[20] These findings underscore the existence of a systemic inflammatory response in patients with OSAHS, closely related to the severity of the disease. Consequently, it is postulated that cytokines may play a role in assessing the disease during its dynamic development in OSAHS patients. Therefore, we hypothesize that changes in the levels of inflammatory factors may serve as indicators for assessing the effectiveness of OSAHS treatment.

In the current study, significantly higher IL-17a levels were noted in both the mild OSAHS group and the moderate-to-severe OSAHS group compared to the control group. Additionally, TNF-α levels were found to be significantly elevated in the moderate-to-severe OSAHS group than in the control group. These findings are consistent with the results of studies both in China and internationally regarding IL-17a and TNF-α levels in patients with OSAHS. No significant difference was observed in IL-10 and IL-6 levels in the mild and moderate-to-severe OSAHS groups when compared to the control group.

We additionally found that the relative abundance of intestinal microbiota species at the species level in patients with OSAHS was correlated with cytokines, CRP, and PSG monitoring indexes. Subsequent multiple linear regression analysis revealed that AHI and BMI may be influencing factors for the relative abundance of *F. prausnitzii* in patients with OSAHS, while IL-17a may be an influencing factor for the relative abundance of *B. adolescentis*. The impact of BMI on the disruption of the intestinal microbiota may be associated with dietary habits and obesity. Mozes et al[21] observed that feeding young rats a high-fat diet led to obesity and disturbances in their intestinal microbiota. Similarly, Kheirandish-Gozal et al[22] discovered that changes in the intestinal microbiota of children in the OSAHS group could elevate lipopolysaccharide levels, increase circulating endotoxins, and worsen the systemic inflammatory response in patients with OSAHS.

The above findings suggest that disturbances in the intestinal microbiota of patients with OSAHS may exacerbate the inflammatory response and potentially play a role in the pathogenesis of OSAHS. Therefore, we hypothesize that intestinal microbiota disorders and chronic inflammation mutually influence one another in patients with OSAHS. The persistence of chronic inflammation in such patients may be a mechanism involved in intestinal microbiota disorders. This, in turn, could exacerbate the inflammatory response in patients with OSAHS by increasing harmful bacteria and toxins and enhancing intestinal permeability, with this cascade effect contributing to the progression of OSAHS and its associated complications.

At present, Continuous Positive Airway Pressure (CPAP) and surgery are still the main treatment methods for OSAHS. In the future, whether the intervention of intestinal flora can become a new treatment method for the occurrence and development of OSAHS may be a research hotspot. This study helps us understand the relationship between the pathogenesis of OSAHS and the intestinal microbiota. It offers novel insights for further exploring the pathophysiological mechanisms of intestinal microbiota disorders in OSAHS and offers a new theoretical basis for targeting the intestinal microbiota to intervene in OSAHS and its associated complications, thereby presenting an innovative therapeutic approach. The current experimental study has certain limitations, primarily characterized by a small sample size, which may potentially introduce biases and limit the generalizability of the findings. Individual differences may have an impact on the study results. We acknowledge that multicenter and large-sample studies can provide stronger evidentiary support to substantiate the observed trends. Our future endeavors will focus on collecting data to compare the levels of intestinal microbiota and cytokines both pre- and post-treatment for OSAHS.

Conclusion

The results of this study indicate a significant correlation between the relative abundance of gut microbiota and cytokine levels in OSAHS, suggesting a potential link between gut microbiota dysbiosis in OSAHS patients and systemic chronic inflammation as well as the progression of OSAHS.

Abbreviations

AHI, apnea hypopnea index; BMI, body mass index; CIH, chronic intermittent hypoxia; CPR, cardiopulmonary resuscitation; ESS, Epworth sleepiness scores; IL-6, interleukin-6; IL-10, interleukin-10; IL-17a, interleukin-17a; mNGS, metagenomic next-generation sequencing; OSAHS, obstructive sleep apnea hypopnea syndrome; PSG, polysomnography; SpO_2mean, mean oxygen saturation; SpO_2min, lowest oxygen saturation; TAmax, the longest time of sleep apnea; TNF-α, tumor necrosis factor.

Data Sharing Statement

The datasets used or analysed during the current study are available from the corresponding author Jie Li and Ni Ye on reasonable request.

Ethics Approval and Consent to Participate

This study was conducted with approval from the Ethics Committee of The First Affiliated Hospital of Soochow University [No.(2023)-005]. This study was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflict of interest regarding this work.

References

1. Lévy P, Kohler M, McNicholas WT, et al.. Obstructive sleep apnoea syndrome. *Nat Rev Dis Primers*. 2015;1:15015. PMID: 27188535. doi:10.1038/nrdp.2015.15
2. Pan YD, Wang DB, Han DM. A meta-analysis of the prevalence of obstructive sleep apnea hypopnea syndrome in Chinese adults. *J Med Inform*. 2019;32(07):73–77,81. doi:10.3969/j.issn.1006-1959
3. Albenberg L, Esipova TV, Judge CP, et al.. Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. *Gastroenterology*. 2014;147(5):1055–63.e8. Epub 2014 Jul 18. PMID: 25046162; PMCID: PMC4252572. doi:10.1053/j.gastro.2014.07.020
4. Moreno-Indias I, Torres M, Montserrat JM, et al.. Intermittent hypoxia alters gut microbiota diversity in a mouse model of sleep apnoea. *Eur Respir J*. 2015;45(4):1055–1065. Epub 2014 Dec 23. PMID: 25537565. doi:10.1183/09031936.00184314
5. Wang LJ, Yang C, Dou ZJ, Wang PP, Hu ZX, Wang B. Preliminary analysis of intestinal microflora in patients with different severity of OSAHS. *Chin J Tubercul Respirat Dis*. 2021;44(6):543–549. doi:10.3760/cma.j.cn112147-20201009-01027
6. Wu J, Lu Y, Cai X, Chen Y, Shen Z, Lyv Q. Gut microbiota dysbiosis in 4- to 6-year-old children with obstructive sleep apnea-hypopnea syndrome. *Pediatr Pulmonol*. 2022;57(9):2012–2022. doi:10.1002/ppul.25967
7. Orrù G, Storari M, Scano A, Piras V, Taibi R, Viscuso D. Obstructive sleep apnea, oxidative stress, inflammation and endothelial dysfunction–an overview of predictive laboratory biomarkers. *Eur Rev Med Pharmacol Sci*. 2020;24(12):6939–6948. doi:10.26355/eurrev_202006_21685
8. Liu X, Ma Y, Ouyang R, et al. The relationship between inflammation and neurocognitive dysfunction in obstructive sleep apnea syndrome. *J Neuroinflammation*. 2020;17(1):229. doi:10.1186/s12974-020-01905-2
9. Ko CY, Hu AK, Chou D, et al.. Analysis of oral microbiota in patients with obstructive sleep apnea-associated hypertension. *Hypertens Res*. 2019;42(11):1692–1700. doi:10.1038/s41440-019-0260-4

10. Faraut B, Boudjeltia KZ, Vanhamme L, Kerkhofs M. Immune, inflammatory and cardiovascular consequences of sleep restriction and recovery. *Sleep Med Rev*. 2012;16(2):137–149. Epub 2011 Aug 10. PMID: 21835655. doi:10.1016/j.smrv.2011.05.001
11. Singh RK, Chang HW, Yan D, et al.. Influence of diet on the gut microbiome and implications for human health. *J Transl Med*. 2017;15(1):73. PMID: 28388917; PMCID: PMC5385025. doi:10.1186/s12967-017-1175-y
12. Valentini F, Evangelisti M, Arpinelli M, et al.. Gut microbiota composition in children with obstructive sleep apnoea syndrome: a pilot study. *Sleep Med*. 2020;76:140–147. Epub 2020 Oct 17. PMID: 33181474. doi:10.1016/j.sleep.2020.10.017
13. Vinolo MA, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. *Nutrients*. 2011;3(10):858–876. Epub 2011 Oct 14. PMID: 22254083; PMCID: PMC3257741. doi:10.3390/nu3100858
14. Huang YS, Guilleminault C, Hwang FM, et al.. Inflammatory cytokines in pediatric obstructive sleep apnea. *Medicine*. 2016;95(41):e4944. PMID: 27741107; PMCID: PMC5072934. doi:10.1097/MD.0000000000004944
15. Freire AX, Kadaria D, Avecillas JF, Murillo LC, Yataco JC. Obstructive sleep apnea and immunity: relationship of lymphocyte count and apnea hypopnea index. *South Med J*. 2010;103(8):771–774. PMID: 20622723. doi:10.1097/SMJ.0b013e3181e6dabf
16. Ye J, Liu H, Zhang G, et al.. The treg/th17 imbalance in patients with obstructive sleep apnoea syndrome. *Mediators Inflamm*. 2012;2012:815308. Epub 2012 Dec 31. PMID: 23345934; PMCID: PMC3549368. doi:10.1155/2012/815308
17. Zhang NN, Li BN, Xu WM, Li YN. Expression and clinical significance of Th17 cell related cytokines in peripheral blood of patients with OSAHS. *Acta Medicinae Universitatis Scientiae et Technologiae Huazhong*. 2020;49(5):587–590. doi:10.3870/j.issn.1672-0741
18. Fleming WE, Holty JC, Bogan RK, et al.. Use of blood biomarkers to screen for obstructive sleep apnea. *Nat Sci Sleep*. 2018;10:159–167. PMID: 29942168; PMCID: PMC6005302. doi:10.2147/NSS.S164488
19. Yi M, Zhao W, Fei Q, et al.. Causal analysis between altered levels of interleukins and obstructive sleep apnea. *Front Immunol*. 2022;13:888644. PMID: 35967324; PMCID: PMC9363575. doi:10.3389/fimmu.2022.888644
20. Zhou Q, Ling YF. Changes and significance of IL-23 and IL-17 in peripheral blood of patients with OSAHS. *Acta Universitatis Medicinalis Anhui*. 2017;52(4):601–604. doi:10.19405/j.cnki.issn1000-1492
21. Mozes S, Bujnáková D, Sefcíková Z, Kmet V. Intestinal microflora and obesity in rats. *Folia Microbiol*. 2008;53(3):225–228. Epub 2008 Jul 27. PMID: 18661297. doi:10.1007/s12223-008-0031-0
22. Kheirandish-Gozal L, Peris E, Wang Y, et al.. Lipopolysaccharide-binding protein plasma levels in children: effects of obstructive sleep apnea and obesity. *J Clin Endocrinol Metab*. 2014;99(2):656–663. Epub 2013 Nov 25. PMID: 24276451; PMCID: PMC3913804. doi:10.1210/jc.2013-3327

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