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Original Article

Altered amyloid-β and tau proteins in neural-derived plasma exosomes in obstructive sleep apnea

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ABSTRACT

Objective: The purpose of our study was to investigate the correlation between neural-derived plasma exosomal amyloid-β (Aβ)42, total tau (T-tau) and tau phosphorylated at threonine 181 (P-T181-tau) protein levels and cognitive impairment in patients with obstructive sleep apnea (OSA).
Methods: There were 122 subjects without dementia included in the study: 27 patients with OSA and mild cognitive impairment (MCI), 52 OSA patients without MCI, and 43 subjects diagnosed with simple snoring but not MCI as the control group. Neuronal-derived exosomal proteins were measured by ELISA kits for Aβ42, T-tau and P-T181-tau. The cognitive function was evaluated by a Chinese version of the Montreal Cognitive Assessment (MoCA) questionnaire, and a normal cognitive score was ≥26.
Results: The exosomal Aβ42, T-tau and P-T181-tau levels in the OSA with MCI group were higher than those in the OSA group. The Aβ42, T-tau, and P-T181-tau levels in the plasma neuronal-derived exosomes were associated with an increased risk of cognitive impairment in OSA patients after additional adjustment for age, gender, education, vascular risk factors, apnea–hypopnea index (AHI) or oxygen reduction index (ODI). Furthermore, there were also significant associations between Aβ42, T-tau, and P-T181-tau in neural-derived plasma exosomes and Epworth Sleepiness Scale, AHI, and ODI in OSA patients. After 1 year of continuous positive airway pressure (CPAP) intervention, the neuronal-derived exosome levels of Aβ42, T-tau, and P-T181-tau were significantly lower than those at baseline ($P = 0.001$, $P = 0.012$, and $P = 0.034$).
Conclusions: These findings indicate that peripheral blood levels of neuronal-derived exosomal Aβ and tau proteins were increased in OSA patients with cognitive impairment. CPAP interventions could possibly improve cognitive function and be associated with decreased levels of exosomal Aβ and tau proteins.

1. Introduction

Obstructive sleep apnea (OSA) is typified by repeated episodes of upper airway obstruction during sleep, resulting in recurrent intermittent hypoxia and repetitive arousal from sleep, thus disturbing the normal sleep pattern. Hypoxemia and the accompanying sleep structural disturbances result in an increased risk for cognitive deficits, even in young patients [1,2]. Some studies have demonstrated that OSA increases the risk of mild cognitive impairment (MCI) and Alzheimer's disease (AD) [3–5], suggesting that OSA may promote AD pathogenesis. As is known to all, amyloid-β (Aβ) and tau has been suggested to play a pivotal role in the pathogenesis of AD [6]. Of all

Abbreviations: AASM, American Academy of Sleep Medicine; AD, Alzheimer's disease; AHI, apnea–hypopnea index; Aβ, Amyloid-β; BSA, bovine serum albumin; CI, confidence interval; CPAP, continuous positive airway pressure; CSF, cerebrospinal fluid; DPBS, Dulbecco's phosphate-buffered saline; ELISAs, enzyme-linked immunosorbent assays; ESS, Epworth Sleepiness Scale; L1CAM, L1 cell adhesion molecule; MCI, mild cognitive impairment; MoCA, Montreal Cognitive Assessment; NIA-AA, National Institute on Aging-Alzheimer's Association; ODI, oxygen reduction index; OR, odds ratio; OSA, obstructive sleep apnea; SaO_2, oxyhemoglobin saturation; PSG, Polysomnography; P-T181-tau, tau phosphorylated at threonine 181; T-tau, total tau.

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Aβ isoforms, Aβ40 and Aβ42 are considered to be the most important ones. In vitro and in vivo experiments demonstrate that acute or sustained exposure to hypoxia increases β-secretase activity, causing the overproduction of Aβ and decreased clearance by reducing neprilysin [7–9]. Sleep fragmentation, which is integral to OSA and associated with increased neuronal firing, is likely to promote Aβ generation. Sleep disruption due to repetitive cortical arousals in OSA may impair sleep-dependent clearance of Aβ in interstitial fluid [10,11]. Moreover, sleep disruption results in a significant increase in tau pathology [12,13]. OSA upregulates Aβ, tau hyperphosphorylation, and synaptic dysfunction. Recently, some researchers found that in the cerebrospinal fluid (CSF), lower Aβ42 and higher total tau (T-tau)/Aβ42 levels were associated with OSA and cognitive impairments, and treatment with continuous positive airway pressure (CPAP) led to its normalization [14,15].

Exosomes are one class of endosome-derived membrane vesicles shed by most cell types that contain various molecular constituents, including proteins of their cellular origin [16]. Neurally derived exosomes are released into not only the CSF but also the blood under physiological and pathological conditions [17,18], and blood-neuronal-derived exosomes are considered as an ideal biomarker vector for AD screening. Jia et al. [19] reported that Aβ42, T-tau, and tau phosphorylated at threonine 181 (P-T181-tau) in blood-neuronal-derived exosomes can differentiate patients with AD from controls, the levels of these biomarkers were highly correlated with their levels in CSF, and can potentially be alternatives to CSF for the diagnosis of AD and MCI.

The suggested association between OSA and cognitive impairment needs further study. To the best of our knowledge, there have been few studies linking Aβ and tau protein with cognitive impairment in patients with OSA. Given that the levels of exosomal biomarkers reflect pathological brain changes, in this study, we hypothesized that neural-derived plasma exosomal Aβ and tau protein levels are associated with cognitive impairment in patients with OSA.

2. Materials and methods

2.1. Participants

Subjects with snoring and suspected OSA were prospectively recruited from the sleep center of the Weihai Municipal Hospital between September 2018 and September 2019. The inclusion criteria were as follows: aged 35–65 years, ≥9 years of education, and urban residence. The exclusion criteria were as follows: (1) other sleep disorders, such as insomnia, obesity hypoventilation syndrome, and central sleep apnea; (2) a concomitant neurological disorder that could potentially affect cognitive function or a family history of dementia; (3) >14 alcoholic drinks/week or neuroactive medications; and (4) other serious heart, lung, liver, kidney, or brain diseases that affect the quality of life. There were 122 subjects without dementia included in the study: 27 patients with OSA and mild cognitive impairment, 52 OSA patients without MCI, and 43 subjects diagnosed with simple snoring but not OSA as the control group. All groups were matched for age, male:female ratio, and education. Daytime sleepiness was measured by the Epworth Sleepiness Scale (ESS) [20]. The study was conducted in accordance with the principles of the Declaration of Helsinki [21] and approved by the Institutional Review Board of Weihai Municipal Hospital. In addition, written informed consent was obtained from every participant.

2.2. Evaluation of the risk factors for cerebrovascular disease and cognitive function

Hypertension was defined as ≥140/90 mmHg at three different times or as the use of antihypertensive medications. The diagnosis of diabetes mellitus was based on the use of antidiabetic treatments or on repeated pathological blood tests indicating fasting values ≥7 mmol/l (126 mg/dl); value loads ≥11.1 mmol/l (200 mg/dl) 2 h after an oral glucose challenge or hemoglobin A1c ≥6.5%. Hyperlipidemia was diagnosed when the total cholesterol was ≥200 mg/dl or when low-density lipoprotein cholesterol was ≥130 mg/dl. A history of smoking was coded if the subject had smoked in the last 3 months. Alcohol was accepted as a risk factor if the current consumption reached 300 g/week.

Mild cognitive impairment was diagnosed based on the criteria established by the National Institute on Aging-Alzheimer's Association (NIA-AA) work groups [22]. The criteria included (1) concern regarding a change in cognition (self/informant/clinician report); (2) objective impairment in one or more cognitive domains, which in this study were assessed by the Montreal Cognitive Assessment (MoCA); (3) the preservation of independence in functional abilities, as measured by the basic and instrumental activities of daily living questionnaires; and (4) not experiencing dementia (based on the DSM-V criteria). The cognitive function of all subjects was evaluated by a Chinese version of the MoCA, and a normal MoCA score was ≥26 [23]. The cognitive scale was administered in a quiet room by the same trained clinician blinded to the other information in the morning.

2.3. Polysomnography (PSG) recording

All subjects underwent overnight PSG in a temperature- and light-controlled, sound-attenuated room. The procedure began at 9:00–10:00 p.m. and ended at 6:00–7:00 a.m. the following day. Subjects were allowed to follow their habitual sleep time, with moderate adjustments, if necessary. All PSG data were collected and stored via an E-Series digital system (Compumedics, Abbotsford, Australia) used with the standard montage recommended by the American Academy of Sleep Medicine (AASM) [24], including electroencephalogram, electrooculogram, chin electromyogram, electrocardiogram, naso-oral thermistor, nasal pressure transducer, microphone, body position, thoracic and abdominal respiratory movement registration, pulse oximetry sensor and anterior tibial electromyography. All sleep stages (W, N1, N2, N3 and R) and parameters were manually interpreted according to the AASM criteria [24] by the sleep center's full-time PSG technicians, who were blind to any diagnosis. Hypopnea is defined as the peak signal excursions drop by ≥30% using nasal pressure, and the duration is ≥10 seconds, along with a ≥3% oxygen desaturation from the pre-event baseline or associated with an arousal. Apnea is defined as a drop in the peak signal excursion by ≥90% using a thermal sensor, and the duration is ≥10 seconds. The apnea–hypopnea index (AHI) was calculated as the average number of apneas and hypopneas per hour of sleep. The diagnosis of OSA was based on an AHI of ≥5 events/h. Subjects with an AHI of <5 events/h of sleep were considered controls. OSA was graded as mild (AHI ≥5 events/h and <15 events/h), moderate (AHI ≥15 events/h and <30 events/h), and severe (AHI ≥30 events/h) [24].

2.4. Collection of neuronal-derived exosomes from blood, the detection of exosomes, and the quantification of exosomal proteins

Fasting blood was sampled between 06:00–07:00 a.m and stored in a polypropylene tube containing EDTA. After drawing, the blood samples were centrifuged at 4000×*g* for 10 min to obtain the plasma. Specific neuronal-derived exosomes were immediately separated for consistency according to a published protocol [25]. Then, 0.5 ml of plasma was incubated with 0.15 ml of thromboplastin-D (Thermo Fisher Scientific, Waltham, MA, USA) at room temperature for 60 min, and 0.35 ml calcium- and

magnesium-free Dulbecco's phosphate-buffered saline (DPBS) (Thermo Fisher Scientific, Waltham, MA, USA) with protease inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA, USA) added. After centrifugation at 3000×*g* for 20 min at 4 °C, supernatants were incubated with ExoQuick exosome precipitation solution (SEXOQ; System Biosciences, CA) and incubated at 4 °C for 1 h. After centrifugation at 1500×*g* for 30 min at 4 °C, each pellet was resuspended in 250 μl DPBS. Each exosome suspension received 100 μl of 3% bovine serum albumin (BSA) (Thermo Fisher Scientific, Waltham, MA, USA) and was incubated for 1 h at 4 °C each with 3 μl of rabbit anti-L1 cell adhesion molecule (L1CAM) antibody (Abcam, Cambridge-UK). Then, 25 μl of streptavidin-agarose resin (Thermo Fisher Scientific, Waltham, MA, USA) containing 50 μl of 3% BSA was added. After centrifugation at 400×*g* for 10 min at 4 °C and the removal of the supernatant, each pellet was suspended in 50 μl of 0.05 M glycine-HCl (PH 3.0) by vortexing for 10 seconds. Each suspension then received 0.4 ml M-PER mammalian protein extraction reagent (Thermo Fisher Scientific, Waltham, MA, USA) that had been adjusted to pH 8.0 with 1 M Tris-HCl (PH 8.6). These suspensions were incubated at 37 °C for 10 min and vortexed for 15 seconds before storage at −80 °C until use in enzyme-linked immunosorbent assays (ELISAs).

Western blot and transmission electron microscopy were performed to confirm the success of exosomal collection according to our previous protocols [26].

Neuronal-derived exosomal proteins were measured by ELISA kits for human Aβ42 (Thermo Fisher Scientific kit), T-tau (Abcam kit), and P-T181-tau (Abcam kit). The amount of CD81 protein was measured to normalize the exosomal content. The mean value for all determinations of CD81 in each assay group was set at 1.00, and the relative values for each sample were used to normalize their recovery [19]. Exosomal protein assays were performed by investigators blinded to clinical and sleep data.

2.5. Intervention

Among 79 patients with OSA, only 16 patients with severe OSA agreed to be treated by CPAP. Three specific inclusion criteria in the longitudinal analyses: good compliance with CPAP therapy, with the use of the CPAP device for more than 4 h a night and for more than five nights per week documented by the CPAP device software [27]; the efficacy of the continuous positive airway pressure treatment, documented by the CPAP device software (AHI < 5 per hour); and at least one year of CPAP therapy. During the CPAP therapy period, 4 patients who did not meet the three specific inclusion criteria were excluded, and 12 patients were identified for analysis.

2.6. Statistical analysis

IBM SPSS Statistics 22.0 was used for statistical analysis. Categorical variables were analyzed using the chi-squared test. Tests on the homogeneity of variances were performed. Numerical data, such as concentrations of Aβ and tau proteins in exosomes and group differences, were analyzed by using Welch's t-test or ANOVA with Bonferroni's post hoc test. A binary logistic regression analysis was used to investigate the exosomal Aβ42 and Tau proteins influencing MCI in patients with OSA by calculating the odds ratio (OR) and 95% confidence interval (CI). Correlative analysis was performed using a linear regression model. Differences in concentrations of amyloid-β and tau proteins in exosomes before and after CPAP intervention were detected using paired-sample t-tests. All tests were two-tailed, and the threshold for statistical significance was $P < 0.05$.

3. Results

3.1. Clinical and demographic characteristics of enrolled participants

Table 1 shows the clinical and demographic characteristics of the enrolled controls and OSA patients with and without MCI. There were significant differences in ESS scores, AHI, oxygen reduction index (*ODI*), mean oxyhemoglobin saturation (SaO_2), lowest SaO_2, and MoCA scores among the three groups (all $P < 0.001$). There were no significant differences in age, sex, years of education, BMI, diabetes mellitus, hypertension, hyperlipidemia, or the rate of current drinking and smoking (all $P > 0.05$) between the three groups. The OSA and OSA with MCI groups were similar with regard to the AHI, *ODI*, mean SaO_2, and lowest SaO_2 ($P > 0.05$). Compared with the control and OSA groups, the OSA with MCI group had the highest ESS scores and MoCA scores ($P < 0.05$).

3.2. Levels of Aβ42, T-tau, and P-T181-tau in neural-derived plasma exosomes

Cross-sectional comparisons of 52 OSA patients, 27 OSA with MCI patients and 43 matched controls revealed that, compared to

Table 1
Demographic and clinical characteristics.

	Control (n = 43)	OSA (n = 52)	OSA with MCI (n = 27)	*P* value
Female/Male	13/30	14/38	10/17	0.650
Age (years)	54.61 ± 7.28	53.81 ± 7.04	55.59 ± 6.83	0.565
Education (years)	11.47 ± 2.36	11.79 ± 2.99	10.93 ± 2.15	0.406
BMI (kg/m^2)	26.23 ± 4.16	27.59 ± 4.58	28.83 ± 4.38	0.053
Diabetes mellitus, n (%)	4 (9.3)	7 (13.5)	4 (14.8)	0.748
Hypertension, n (%)	11 (25.6)	19 (36.5)	11 (40.7)	0.357
Hyperlipidemia, n (%)	14 (32.6)	23 (44.2)	13 (48.3)	0.357
Current smoker, n (%)	3 (7.0)	5 (9.6)	2 (7.4)	0.884
Current drinker, n (%)	6 (14.0)	9 (17.3)	5 (18.5)	0.858
Epworth Sleepiness Scale	8.58 ± 3.12	12.35 ± 3.49[a]	14.46 ± 3.06[a,b]	<0.001
AHI, events/h	2.81 ± 1.18	34.80 ± 20.45[a]	40.87 ± 21.54[a]	<0.001
ODI	2.19 ± 1.22	29.45 ± 16.99[a]	34.95 ± 20.80[a]	<0.001
Mean SaO_2, %	95.40 ± 1.43	92.73 ± 2.13[a]	91.96 ± 2.78[a]	<0.001
Lowest SaO_2, %	92.05 ± 2.10	80.29 ± 10.88[a]	77.85 ± 11.81[a]	<0.001
MoCA score	27.81 ± 1.26	27.54 ± 1.16	23.15 ± 1.54[a,b]	<0.001

Abbreviations: BMI, body mass index; AHI, apnea–hypopnea index; ODI, oxygen reduction index; SaO_2, oxyhemoglobin saturation; MOCA, Montreal Cognitive Assessment.
[a]Significant at $P < 0.05$ vs control.
[b]Significant at $P < 0.05$ vs OSA.

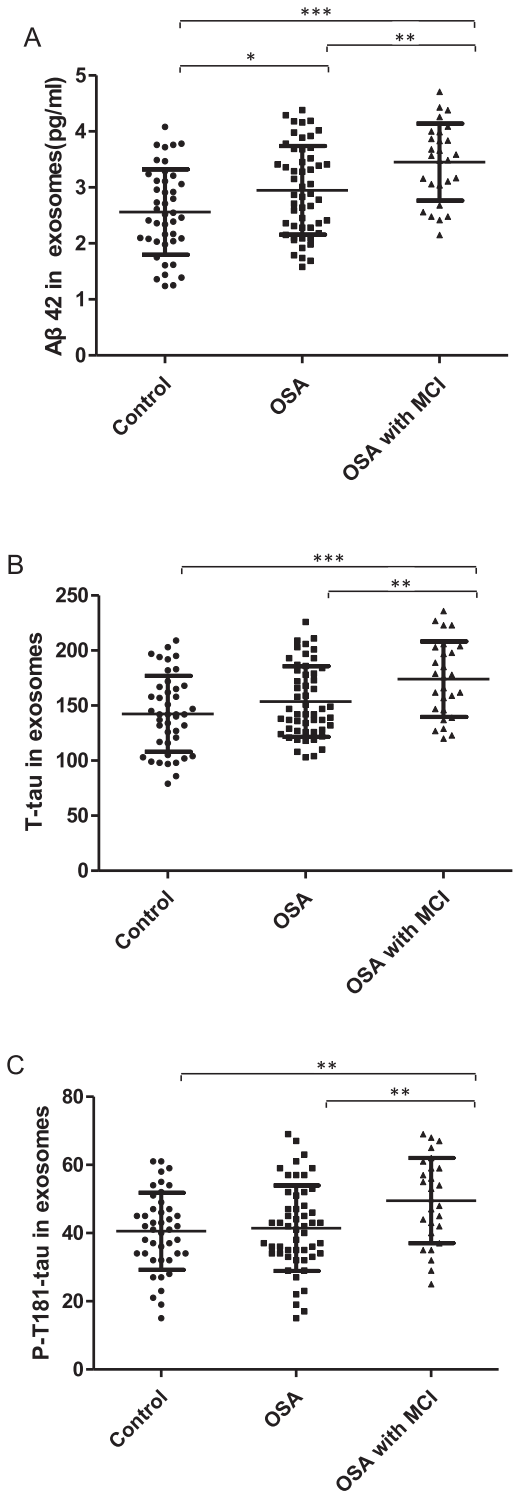

Fig. 1. Plasma neuronal-derived exosomes levels of amyloid-β and tau proteins in cross-sectional control, OSA, and OSA with MCI groups. **(A)** The Aβ42 levels in the plasma neuronal-derived exosomes from OSA and OSA with MCI patients were higher than those in control subjects; the exosomal Aβ42 levels in OSA with MCI were higher than those in OSA group. **(B)** The exosomal concentration of T-tau in OSA with MCI group was higher than those in the OSA and control group; there was no significant differences in the levels of T-tau between OSA and control group. **(C)** The exosomal concentration of P-T181-tau in OSA with MCI group was higher than those in the OSA and control group; there was no significant differences in the levels of P-T181-tau between OSA and control group. *P < 0.05, **P < 0.01, ***P < 0.001.

the controls (2.56 ± 0.76 pg/ml), the exosomal concentrations of Aβ42 in the OSA (2.95 ± 0.97 pg/ml, $P = 0.017$) and OSA with MCI groups (3.44 ± 0.69 pg/ml, $P < 0.001$) were significantly higher (Fig. 1A). Furthermore, the exosomal Aβ42 levels in the OSA with MCI group (3.44 ± 0.69 pg/ml) were higher than those in the OSA group (2.95 ± 0.97 pg/ml, $P = 0.005$) (Fig. 2A). The exosomal concentrations of T-tau and P-T181-tau in the OSA with MCI group (174.00 ± 34.22 and 49.52 ± 12.48) were higher than those in the OSA (153.65 ± 32.08, $P = 0.014$ and 41.42 ± 12.53, $P = 0.009$) and control group (142.47 ± 12.53, $P < 0.001$ and 40.53 ± 11.28, $P = 0.004$) (Fig. 1B and C). There were no significant differences in the levels of T-tau and P-T181-tau between the OSA (153.65 ± 32.08 and 41.42 ± 12.53) and control groups (142.47 ± 12.53, $P > 0.05$ and 40.53 ± 11.28, $P > 0.05$) (Fig. 1B and C).

3.3. Adjusted relationship between the exosomal Aβ42 and tau proteins and MCI in patients with OSA

A binary logistic regression analysis revealed that the levels of Aβ42 (OR: 3.223, 95% CIs: 1.182–8.794, $P = 0.022$), T-tau (OR: 1.019, 95% CIs: 1.001–1.038, $P = 0.043$), and P-T181-tau (OR: 1.050, 95% CIs: 1.005–1.097, $P = 0.029$) in neural-derived plasma exosomes contributed significantly to cognitive impairment in patients with OSA when adjusting for age, gender, education, vascular risk factors (eg, hypertension, diabetes mellitus, hyperlipidemia, current smoker, current drinker), and AHI (Table 2). In addition, the exosomal Aβ42 (OR: 2.739, 95% CIs: 1.074–6.990, $P = 0.035$) and P-T181-tau (OR: 1.048, 95% CIs: 1.003–1.095, $P = 0.038$) proteins were associated with an increased risk of cognitive impairment after adjustment for age, gender, education, vascular risk factors (eg, hypertension, diabetes mellitus, hyperlipidemia, current smoker, current drinker), and ODI (Table 2). There were no significant associations between T-tau (OR: 1.018, 95% CIs: 0.999–1.038, $P = 0.061$) in neural-derived plasma exosomes and cognitive impairment in OSA patients adjustment for age, gender, education, vascular risk factors, and ODI (Table 2).

3.4. Relative independent factors affect on the exosomal Aβ42 and tau proteins in patients with OSA

Furtherly, there were also significant associations between Aβ42, T-tau, and P-T181-tau in neural-derived plasma exosomes and ESS scores (Aβ42: β: 0.571; SE: 0.021; $P < 0.001$, T-tau: β: 0.432; SE: 1.010; $P < 0.001$, P-T181-tau: β: 0.407; SE: 0.405; $P < 0.001$, respectively), AHI (Aβ42: β: 0.622; SE: 0.003; $P < 0.001$, T-tau: β: 0.438; SE: 0.171; $P < 0.001$, P-T181-tau: β: 0.375; SE: 0.070; $P = 0.001$, respectively), ODI (Aβ42: β: 0.621; SE: 0.004; $P < 0.001$, T-tau: β: 0.479; SE: 0.191; $P < 0.001$, P-T181-tau: β: 0.395; SE: 0.079; $P = 0.001$, respectively) in OSA patients adjusted for age, gender, education, vascular risk factors (eg, hypertension, diabetes mellitus, hyperlipidemia, current smoker, current drinker) (Table 3). The exosomal Aβ42 and T-tau were associated with lowest SaO_2 (β: −0.379; SE: 0.008; $P = 0.001$, β: −0.283; SE: 0.347; $P = 0.016$) in patients with OSA adjustment for age, gender, education, vascular risk factors (Table 3).

3.5. Correlation between the exosomal Aβ42 and tau proteins and cognitive performance in patients with OSA

We performed correlation analysis and found that the levels of Aβ42, T-tau, and P-T181-tau in neuronal-derived exosomes were correlated with MoCA scores ($R^2 = 0.28$, $P < 0.001$, Fig. 2A; $R^2 = 0.19$, $P < 0.001$, Fig. 2B; $R^2 = 0.16$, $P < 0.001$, Fig. 2C).

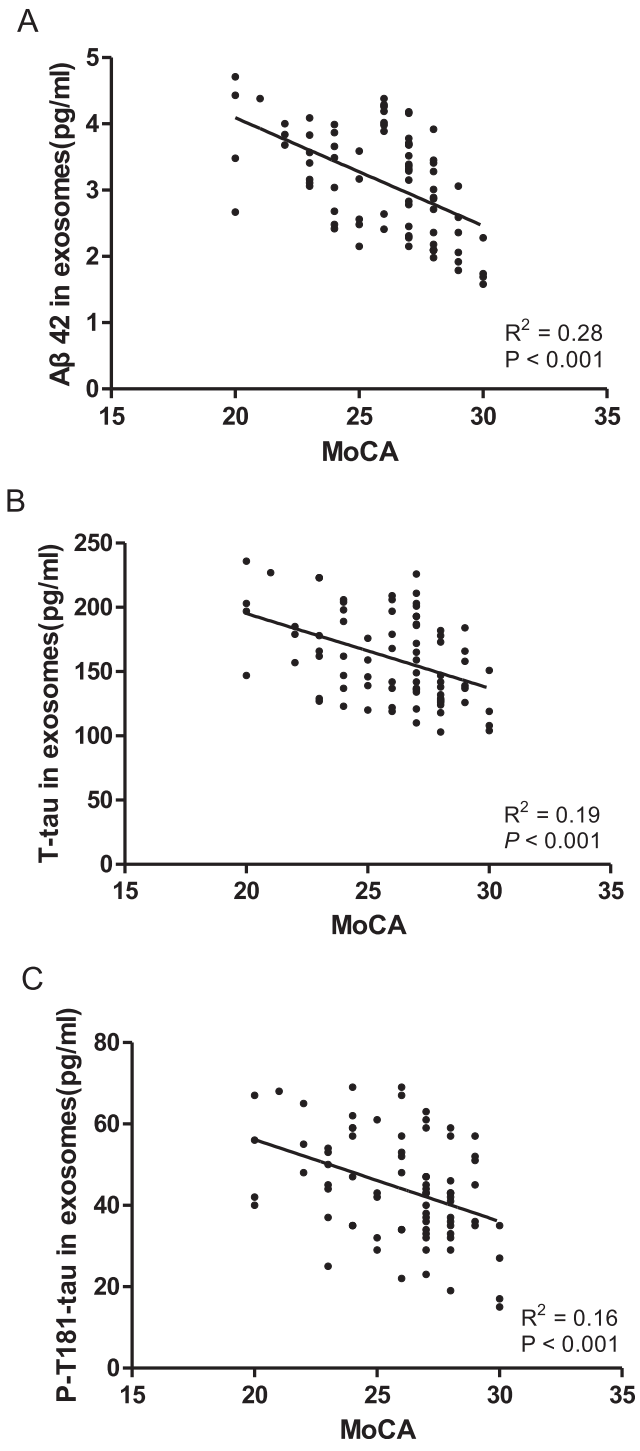

Fig. 2. Association between the levels of Aβ42, T-tau, and P-T181-tau and MoCA in patients with OSA. The levels of Aβ42 in neuronal-derived exosomes were correlated with MoCA scores (**A**). Plasma neuronal-derived exosomes levels of T-tau (**B**) and P-T181-tau (**C**) were correlated with MoCA scores.

3.6. Changes in Aβ42, T-tau, and P-T181-tau levels after CPAP intervention

Among 12 severe OSA patients who were treated by CPAP and identified for the longitudinal analysis, 9 patients had a clinical diagnosis of MCI. Compared with baseline, ESS scores decreased significantly (14.58 ± 3.34 vs 9.25 ± 2.30, $P < 0.001$) and MoCA scores increased significantly (23.75 ± 2.56 vs 26.17 ± 1.47, $P = 0.01$) after 1 year of CPAP treatment, indicating improvement in daytime excessive sleepiness and cognitive function after CPAP treatment. With regard to the neuronal-derived exosome biomarkers, the levels of Aβ42 (2.83 ± 0.31), T-tau (143.33 ± 22.62), and P-T181-tau (40.83 ± 9.06) after 1 year of CPAP intervention were significantly lower than those at baseline (3.54 ± 0.52, $P = 0.001$, Fig. 3A; 179.58 ± 38.51, $P = 0.012$, Fig. 3B; 51.33 ± 13.13, $P = 0.034$, Fig. 3C).

4. Discussion

In the present study, we provide additional evidence that OSA is associated with markers of altered pathological proteins in AD. OSA may represent a reversible risk factor for amyloid-β or tau pathology. The exosomal Aβ42, T-tau, and P-T181-tau levels in the OSA with MCI group were higher than those in the OSA group. The Aβ42, T-tau, and P-T181-tau levels in the plasma neuronal-derived exosomes were associated with an increased risk of cognitive impairment in OSA patients. The neuronal-derived exosome levels of Aβ42, T-tau, and P-T181-tau after 1 year of CPAP intervention were significantly lower than those at baseline.

To our knowledge, this is the first study to examine changes in amyloid-β and tau protein levels in neural-derived plasma exosomes in patients with OSA. AD-associated proteins, such as Aβ and tau protein, are secreted in exosomes during their formation in the brain [28,29]. Exosomes can cross the blood–brain barrier and be detected in the peripheral blood [30]. The blood exosomal Aβ42 levels have been confirmed by Jia et al. [19] to be negatively correlate with CSF levels of Aβ42, and the levels of neuronal-derived exosomal T-tau and P-T181-tau were positively correlated with their levels in CSF. These exosome findings suggest an accumulation of amyloid plaques and tau protein in the brain. Intermittent hypoxia and sleep fragmentation have been implicated as possible mechanistic links in a causal pathway between obstructive sleep apnea and the accumulation of Aβ. There is compelling evidence indicating that hypoxia significantly decreases the activity of α-secretase activity and increases β- and γ-secretase activity, consequently increasing Aβ production in the brain [31]. In addition to increased Aβ generation, hypoxia affects peptidases that degrade Aβ peptides, thus reducing Aβ clearance [32]. A recent study showed that lower CSF Aβ42 in patients with OSA than in healthy controls [14]. In that study, OSA patients treated with CPAP had normal Aβ42 levels. In our study, compared to the control group, the exosomal concentrations of Aβ42 in the OSA group and the OSA with MCI group were significantly higher in line with previous studies. After 1 year of CPAP treatment, the exosomal levels of Aβ42 significantly decreased compared with baseline. Furthermore, our study also revealed that the levels of Aβ42 in neuronal-derived exosomes affected by AHI, ODI and ESS scores were correlated with MoCA scores in patients with OSA.

Excessive daytime sleepiness related to sleep deprivation and sleep fragmentation are commonly observed in patients with OSA, and possibly contributes to the domain and extent of cognitive impairments in OSA [33]. Recently, some studies found that sleep deprivation or excessive daytime sleepiness was associated with increased Aβ accumulation in the brains of elderly individuals without dementia [34,35]. Evidence suggests that sleep affects tau, the second pathological hallmark of AD, in a similar manner as Aβ. Previous sleep-tau studies on animals were conducted by evoking acute sleep deprivation on animals with seeded tau in their brains [36]. Barthélemy et al. found that sleep deprivation increases the concentration of tau and affects tau phosphorylation in human cerebrospinal fluid [37]. Specifically, one study showed that informant-reported apneas during sleep (witnessed apneas)

Table 2
Logistic regression analysis between the exosomal Aβ42 and Tau proteins and MCI in patients with OSA.

	Model 1		Model 2		Model 3	
	OR (95% CIs)	*P* value	OR (95% CIs)	*P* value	OR (95% CIs)	*P* value
Aβ42	2.654 (1.283–5.490)	0.008	3.223 (1.182–8.794)	0.022	2.739 (1.074–6.990)	0.035
Age	1.071 (0.988–1.161)	0.098	1.072 (0.987–1.163)	0.098	1.070 (0.986–1.161)	0.104
Gender	0.603 (0.197–1.841)	0.374	0.574 (0.182–1.811)	0.344	0.580 (0.184–1.826)	0.352
Education	0.856 (0.706–1.037)	0.112	0.866 (0.705–1.065)	0.172	0.861 (0.700–1.059)	0.157
Hypertension	–	–	0.987 (0.331–2.940)	0.981	1.013 (0.333–3.085)	0.982
Diabetes mellitus	–	–	1.271 (0.270–5.976)	0.762	1.134 (0.248–5.194)	0.871
Hyperlipidemia	–	–	1.153 (0.381–3.490)	0.801	1.085 (0.362–3.245)	0.885
Current smoker	–	–	0.865 (0.132–5.679)	0.880	0.917 (0.141–5.953)	0.927
Current drinker	–	–	1.160 (0.286–4.706)	0.835	1.215 (0.299–4.937)	0.786
AHI	–	–	0.990 (0.956–1.025)	0.555	–	–
ODI	–	–	–	–	0.998 (0.962–1.035)	0.900
T-tau	1.019 (1.003–1.035)	0.017	1.019 (1.001–1.038)	0.043	1.018 (0.999–1.038)	0.061
Age	1.055 (0.978–1.139)	0.166	1.058 (0.978–1.144)	0.160	1.059 (0.979–1.146)	0.152
Gender	0.461 (0.152–1.396)	0.171	0.464 (0.148–1.455)	0.188	0.469 (0.149–1.472)	0.194
Education	0.915 (0.756–1.107)	0.359	0.900 (0.728–1.111)	0.325	0.894 (0.722–1.107)	0.305
Hypertension	–	–	1.251 (0.436–3.593)	0.678	1.280 (0.442–3.709)	0.649
Diabetes mellitus	–	–	1.341 (0.283–6.362)	0.711	1.305 (0.280–6.088)	0.735
Hyperlipidemia	–	–	0.882 (0.302–2.575)	0.818	0.886 (0.304–2.583)	0.825
Current smoker	–	–	1.319 (0.191–9.124)	0.779	1.323 (0.193–9.068)	0.776
Current drinker	–	–	1.239 (0.323–4.757)	0.755	1.280 (0.329–4.976)	0.722
AHI	–	–	1.003 (0.975–1.032)	0.847	–	–
ODI	–	–	–	–	1.007 (0.973–1.041)	0.696
P-T181-tau	1.051 (1.009–1.095)	0.016	1.050 (1.005–1.097)	0.029	1.048 (1.003–1.095)	0.038
Age	1.039 (0.965–1.119)	0.313	1.041 (0.964–1.124)	0.308	1.044 (0.966–1.129)	0.275
Gender	0.573 (0.190–1.725)	0.322	0.543 (0.172–1.708)	0.296	0.546 (0.173–1.724)	0.302
Education	0.875 (0.723–1.059)	0.170	0.856 (0.696–1.053)	0.142	0.851 (0.691–1.048)	0.129
Hypertension	–	–	1.375 (0.465–4.066)	0.565	1.421 (0.479–4.217)	0.527
Diabetes mellitus	–	–	0.878 (0.195–3.954)	0.865	0.885 (0.199–3.936)	0.872
Hyperlipidemia	–	–	0.947 (0.325–2.761)	0.921	0.937 (0.322–2.731)	0.906
Current smoker	–	–	0.955 (0.143–6.391)	0.962	0.971 (0.145–6.494)	0.975
Current drinker	–	–	1.652 (0.390–6.991)	0.495	1.719 (0.406–7.270)	0.462
AHI	–	–	1.007 (0.981–1.033)	0.619	–	–
ODI	–	–	–	–	1.012 (0.981–1.043)	0.447

Model 1: adjusted for age, gender, and education.
Model 2: adjusted for age, gender, education, vascular risk factors (eg, hypertension, diabetes mellitus, hyperlipidemia, current smoker, current drinker), and AHI.
Model 3: adjusted for age, gender, education, vascular risk factors (eg, hypertension, diabetes mellitus, hyperlipidemia, current smoker, current drinker), and ODI.

Table 3
Multivariable linear regression analys between the exosomal Aβ42 and Tau proteins and various indicators in patients with OSA.

	Aβ42		T-tau		P-T181-tau	
	β (SE)	*P* value	β (SE)	*P* value	β (SE)	*P* value
Epworth Sleepiness Scale	0.571 (0.021)	<0.001	0.432 (1.010)	<0.001	0.407 (0.405)	<0.001
AHI, events/h	0.622 (0.003)	<0.001	0.438 (0.171)	<0.001	0.375 (0.070)	0.001
ODI	0.621 (0.004)	<0.001	0.479 (0.191)	<0.001	0.395 (0.079)	0.001
Mean SaO_2, %	−0.169 (0.038)	0.144	−0.129 (1.645)	0.266	−0.123 (0.648)	0.305
Lowest SaO_2, %	−0.379 (0.008)	0.001	−0.283 (0.347)	0.016	−0.200 (0.140)	0.100

SE, standard error; AHI, apnea–hypopnea index; ODI, oxygen reduction index; SaO_2, oxyhemoglobin saturation.
Adjusted for age, gender, education, vascular risk factors (eg, hypertension, diabetes mellitus, hyperlipidemia, current smoker, current drinker).

in cognitively unimpaired elderly persons (≥65 years of age) are associated with elevated tau PET levels in tau-susceptible brain regions [38]. We found that there were no significant differences in the levels of T-tau and P-T181-tau in neuronal-derived exosomes between the OSA group and the control group in young and middle-aged persons (35–65 years of age). However, the exosomal concentrations of T-tau and P-T181-tau in OSA with MCI subjects were higher than those in the OSA group and the control group. We also found that degree of cognitive impairment in OSA patients measured by MoCA were correlated with the levels of T-tau and P-T181-tau in neuronal-derived exosomes, which were affected by AHI, ODI, and excessive daytime sleepiness measured by the ESS scores. This provides further supportive evidence that increased processing and release during sleep disturbances is the mechanism for the rise in tau protein levels that our study showed.

Recent studies have shown that the treatment of severe OSA with CPAP could lead to the improvement of cognitive function in patients with MCI and AD, which may be due to the correction of intermittent hypoxia [4,39]. Our results were also in line with these studies that showed that excessive daytime sleepiness and cognitive function were significantly improved after 1 year of CPAP treatment in severe OSA patients and were associated with decreases in exosomal Aβ and tau levels.

Our results have some limitations. First, the results were drawn from a small-scale hospital-based study, and future investigations are necessary to replicate and validate our findings in a large population of patients. Second, the present

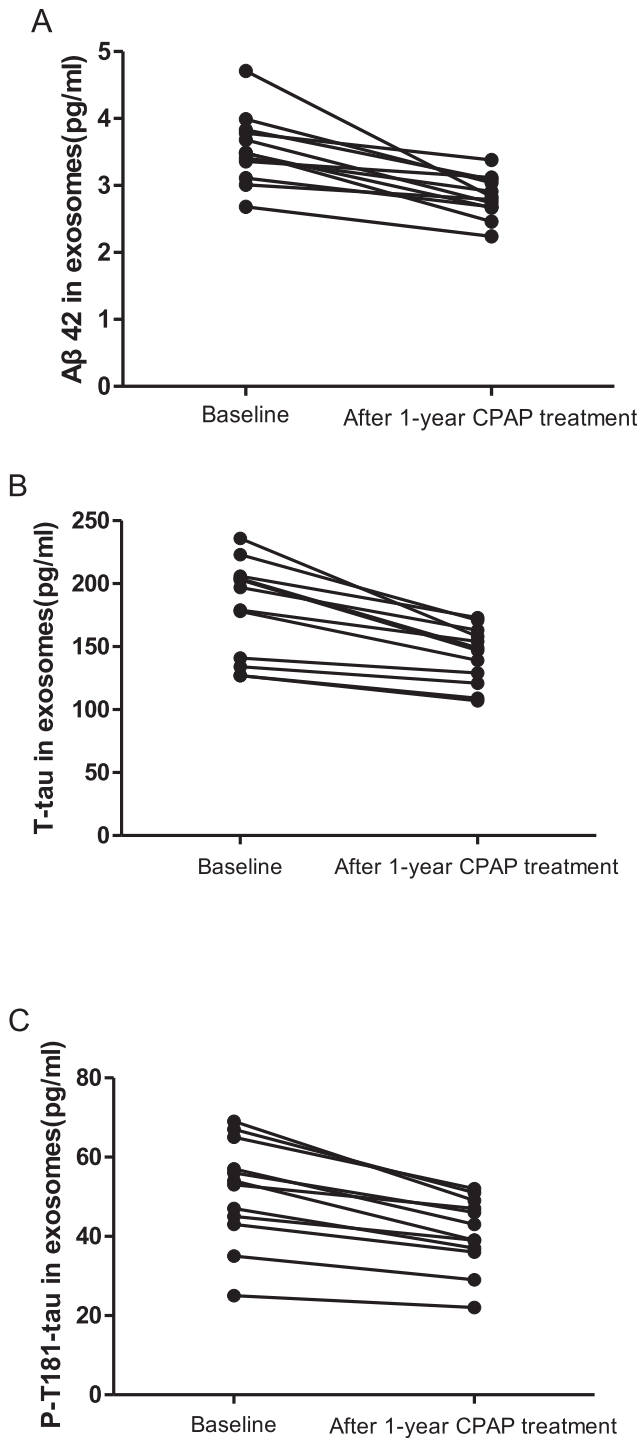

Fig. 3. Plasma neuronal-derived exosomes levels of Aβ42, T-tau, and P-T181-tau at baseline and after 1-year CPAP treatment. The neuronal-derived exosomes levels of Aβ42 **(A)**, T-tau **(B)**, and P-T181-tau **(C)** after 1-year CPAP intervention were significantly lower compared with the baseline.

investigation was mainly a cross-sectional study, and more longitudinal study data are needed to investigate the relationship between the levels of exosomal Aβ and tau and the decline in cognitive functions of OSA patients. Third, additional information, such as pathology or cerebrospinal fluid data, was not available to confirm the results.

5. Conclusions

We demonstrated that peripheral blood levels of neuronal-derived exosomal Aβ42, T-tau, and P-T181-tau were increased in OSA or OSA with MCI patients. Intermittent hypoxia is likely candidate mechanisms. Clinical interventions aimed at the treatment of OSA, such as CPAP, could possibly improve cognitive function and be associated with reductions in exosomal Aβ and tau levels.

Credit author statement

Jinbiao Zhang: Conceptualization, Methodology. **Hairong Sun, Yanling Gao**: Data curation, Writing- original draft. **Shukun Zhang**: Supervision, Validation. **Tengqun Shen**: Formal analysis, Visualization. **Xiuli Shang, Xiaoling Yuan**: Investigation. **Jinbiao Zhang, Mengfan Li**: Methodology, Writing-review and editing. **Zhenguang Li**: Project administration.

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Conflict of interest

The authors do not have any conflict of interest to disclose.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: https://doi.org/10.1016/j.sleep.2022.03.021.

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