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ORIGINAL RESEARCH

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Association of matrix metalloproteinase-9 and nitric oxide with hypertension in obstructive sleep apnea

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Abstract

Background: Patients with obstructive sleep apnea (OSA) are more likely to suffer from hypertension. At the same time, the serum levels of matrix metalloproteinase-9 (MMP-9) and nitric oxide (NO) in patients with OSA are also changed in OSA patients. We investigated the correlation between serum levels of MMP-9, NO in patients with OSA and their association with hypertension in those patients, and the effects of continuous positive airway pressure therapy (CPAP) on these serum biomarkers and blood pressure.

Methods: Serum MMP-9 and NO levels and blood pressure of 57 patients with newly diagnosed OSA and 30 controls were measured; among them, 30 patients with moderate to severe OSA underwent 3-month CPAP treatment.

Results: In comparison to the control group, the MMP-9 serum levels were higher (232.8 ± 103.2 ng/ml versus 161.6 ± 56.5 ng/ml, $p < .001$*), there was no statistical significance difference among serum NO (26.7 ± 9.1 IU/ml versus 31.0 ± 11.7 IU/ml, $p = .06$), and MMP-9 was negatively correlated to NO, especially in patients with hypertension ($r = -.644$, $p = .02$*). MMP-9, NO, and blood pressure were significantly recovered in the patients with OSA after CPAP treatment for 3 months ($p < .05$*).

Conclusion: The MMP-9 level and the NO level were altered in OSA patients. The relationship between the two especially in patients with hypertension suggests the potential mechanism of OSA-induced hypertension.

KEYWORDS
blood pressure, CPAP, hypertension, MMP-9, NO, OSA

1 | INTRODUCTION

Obstructive sleep apnea (OSA) is a disease of apnea and hypopnea caused by upper airway collapse and obstruction during sleep, often accompanied by acute autonomic and hemodynamic disruptions. With these features, the OSA patients gradually develop obvious arterial hypercapnia and hypoxemia.[1] Currently, OSA is considered to be a public disease with high morbidity in both adults and children.[2]

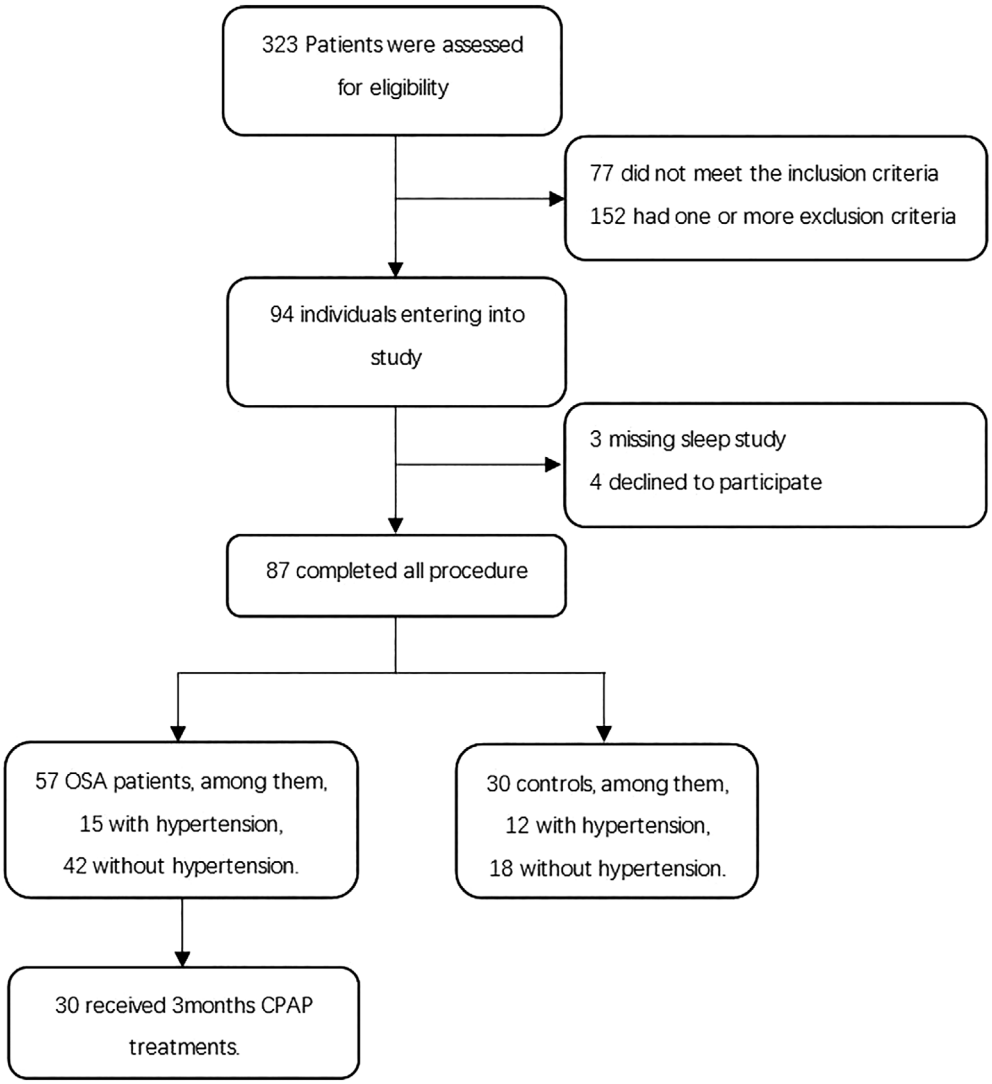

FIGURE 1 Subject enrollment and study design

Researchers believe that OSA is a risk factor for many cardiovascular diseases, including hypertension.[3,4] The epidemiologic association between OSA and cardiovascular diseases could be attributed to many factors, like nocturnal intermittent hypoxemia, hypercapnia, highly active sympathetic nervous system, change of intrathoracic pressure, endothelial dysfunction, metabolic anomalies, and systemic inflammation.[3,5,6] It is reported that the blood pressure of patients with OSA can be significantly improved after continuous positive airway pressure ventilation (CPAP).[7] The specific pathophysiological mechanism between OSA and hypertension is unclear. The importance of oxidative stress and inflammation in OSA has been stressed by tons of studies[8] and the changes of cytokines or their combinations in OSA patients have been getting increasing attention.[9] Finding out which inflammatory factors are changed in OSA and whether alterations of these inflammatory factors are inter-correlated could be clinically significant.

Matrix metalloproteinase-9 (MMP-9) is a member of the zinc-metalloproteinase family. It is involved in the degradation of proteins in extracellular matrix (ECM) in many normal physiological processes and disease processes.[10] It is reported that the level of activity of MMP-9 was changed in cardiovascular diseases and other pathological backgrounds.[11] Patients with hypertension express higher serum levels of MMP-9.[12] In addition, studies have shown that the serum level and activity of MMP-9 were increased in patients with OSA.[13,14] Our previous study found that level of MMP-9 was positively correlated with the severity of OSA and the degree and duration of hypoxia.[15] Cell experiments showed that the inhibitor of nitric oxide (NO) and nitric oxide synthase (NOS) could enhance the expression of MMP-9.[16,17] NO is a physiologically related gasotransmitter that can regulate blood pressure and vascular function.[18] The effect of NO on the heart could be a universal defense against ischemia–reperfusion.[19] Decreased plasma concentration of NO and NO metabolites has been thought to be associated with cardiovascular events.[20,21] And the plasma levels of nitric oxide derivative[22] and endothelial nitric oxide synthetase (eNOS)[23] were downregulated in OSA patients. Although the two cytokines are altered in OSA patients and were separately reported to be associated with hypertension, the correlation between the four has not been studied, which is also the purpose of this article.

2 | MATERIALS AND METHODS

2.1 | Participants

The study recruited 87 participants who complained of snoring, with ages ranging from 19 to 64 years old (see Figure 1). The inclusion criteria were no CPAP therapy or surgery therapy for OSA. Exclusion criteria for all subjects included a history of smoking or alcoholism and previous or current treatment for cardiovascular disease, stroke, severe renal insufficiency, severe lung disease, acute infection, trauma, taking medications for hypertension, diabetes mellitus, or dyslipidemia or surgeries 1 month before the study. Among the 87 patients, 57 patients with apnea–hypopnea index (AHI) ≥ 5 were defined as the OSA group, 30 patients with AHI <5 were defined as the control group. The study was approved by the ethics committee of the Second Xiangya Hospital (No.2019001), Central South University in China, and all patients gave written informed consent.

2.2 | Polysomnography

All participants received standard nighttime polysomnography (EMBLA; Flagra high frequency. Medical Devices, Reykjavik, Iceland). OSA is defined as the AHI≥5 events per hour, with the severity

TABLE 1 Baseline characteristics in patients with OSA and control subjects

	Patients with OSA ($n = 57$)	Healthy subjects ($n = 30$)	p
Age (years)	42.3 ± 10.6	44.1 ± 10.5	.60
BMI (kg/m^2)	27.5 ± 3.8	27.0 ± 3.8	.56
AHI (events/h)	44.0 ± 20.1	2.9 ± 1.2	<.001*
Lower nadir SaO_2 (%)	67.5 ± 11.0	85.8 ± 3.5	<.001*
CT90 (%)	31.9 ± 24.1	1.0 ± 1.2	<.001*
Systolic pressure (mmHg)	117.7 ± 24.1	123.9 ± 25.2	.30
Diastolic pressure (mmHg)	75.4 ± 14.7	81.7 ± 16.5	.09
ESS	12.7 ± 4.5	5.4 ± 2.8	<.001*
NO (IU/ml)	26.7 ± 9.1	31.0 ± 11.7	.06
MMP-9 (ng/ml)	232.8 ± 103.2	161.6 ± 56.5	<.001*
Male/female (n)	52/5	26/4	.77

Note: *$p < .05$.
Abbreviations: AHI, apnea–hypopnea index; BMI, body mass index; CT90, a greater percentage of sleep time when blood oxygen saturation is less than 90%; ESS, Epworth Sleepiness Scale; $LSaO_2$, lowest saturation oxygen; MMP-9, matrix metalloproteinase-9; NO, nitric oxide synthetase; SaO_2,saturation oxygen.

TABLE 2 Parameters in patients with mild to moderate OSA compared with those with severe OSA

	Mild to moderate ($n = 18$)	Severe ($n = 39$)	p
Age (years)	34.1 ± 10.1	45.7 ± 8.7	<.001*
BMI (kg/m^2)	26.2 ± 3.4	28.2 ± 3.9	.07
AHI (events/h)	20.3 ± 7.8	54.9 ± 13.5	<.001*
Lower nadir SaO_2 (%)	77.7 ± 5.9	62.8 ± 9.5	<.001*
CT90 (%)	9.6 ± 8.0	42.3 ± 22.0	<.001*
Systolic pressure (mmHg)	109.3 ± 17.5	121.6 ± 25.9	.04*
Diastolic pressure (mmHg)	70.7 ± 9.8	77.6 ± 16.2	.19
ESS	12.3 ± 4.4	12.8 ± 4.6	.68
NO (IU/ml)	31.5 ± 7.3	24.4 ± 9.1	.006*
MMP-9 (ng/ml)	199.8 ± 89.2	248.0 ± 106.7	.10
Male/female (n)	16/2	36/3	.65

Note: *$p < .05$.
Abbreviations: AHI, apnea–hypopnea index; BMI, body mass index; CT90, a greater percentage of sleep time when blood oxygen saturation is less than 90%; ESS, Epworth Sleepiness Scale; $LSaO_2$, lowest saturation oxygen; MMP-9, matrix metalloproteinase-9; NO, nitric oxide synthetase; SaO_2,saturation oxygen.

described as mild for an AHI score of 5 to less than 15(5 ≤ AHI < 15), moderate for an AHI score of 15 to 30 (15 ≤ AHI < 30), and severe for an AHI score of more than 30 (AHI ≥ 30).[24]

2.3 | Blood pressure measurement

The blood pressure (BP) of all participants was measured by periodically calibrated mercury sphygmomanometer the next morning after the PSG test. A cuff of appropriate size was applied on the nondominant arm. A trained nurse measured the BP and heart rate of participants three times at 5-min intervals after 15 min of resting in a seated position. The average of three measurements was adopted.

TABLE 3 Correlation coefficients between levels of NO or MMP-9 and clinical variables, and PSG parameters in patients with OSA

	NO		MMP-9	
	r	*p*	*r*	*p*
Age	−.219	.10	.392	.003*
BMI	−.137	.31	.151	.26
AHI	−.365	.005*	.108	.42
Lower nadir SaO_2	.422	.001*	−.291	.03*
CT90	−.558	<.001*	.316	.02*
Systolic pressure	−.006	.96	.343	.009*
Diastolic pressure	−.009	.95	.290	.029*
ESS	.042	.76	.007	.96
NO	-	-	−.269	.04*
MMP-9	−.269	.04*	-	-

Note: *p < .05.
Abbreviations: AHI, apnea–hypopnea index; BMI, body mass index; CT90, a greater percentage of sleep time when blood oxygen saturation is less than 90%; ESS, Epworth Sleepiness Scale; $LSaO_2$, lowest saturation oxygen; MMP-9, matrix metalloproteinase-9; NO, nitric oxide synthetase; SaO_2, saturation oxygen.

2.4 | Medication history in the "past" 1 year

Through the questionnaire, we collected some general information including medical history, family history, smoking or drinking habits, operation history, and medication history in recent 1 year. Excessive daytime somnolence was assessed by the Epworth Sleepiness Scale (ESS).

2.5 | Measurement of NO and MMP-9

Blood samples were collected the morning following the PSG test, immediately centrifuged to collect plasma/serum, frozen at −80°C, and finally analyzed by enzyme-linked immunosorbent assay (ELISA). The ELISA Kit for MMP-9 (ELISA, R&D Systems, Inc.) was used to quantify MMP-9 levels in the serum of OSA patients. NO concentrations were determined using (R&D Systems, Catalog #KGE001, Inc.). This kit determines NO concentrations based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. After the reaction, the nitrite as the product of azo dye in Griess reaction was detected by colorimetry.

2.6 | CPAP treatment

After diagnostic studies, patients with moderate to severe OSA (AHI > 15 events/h) received a treatment of CPAP during the next

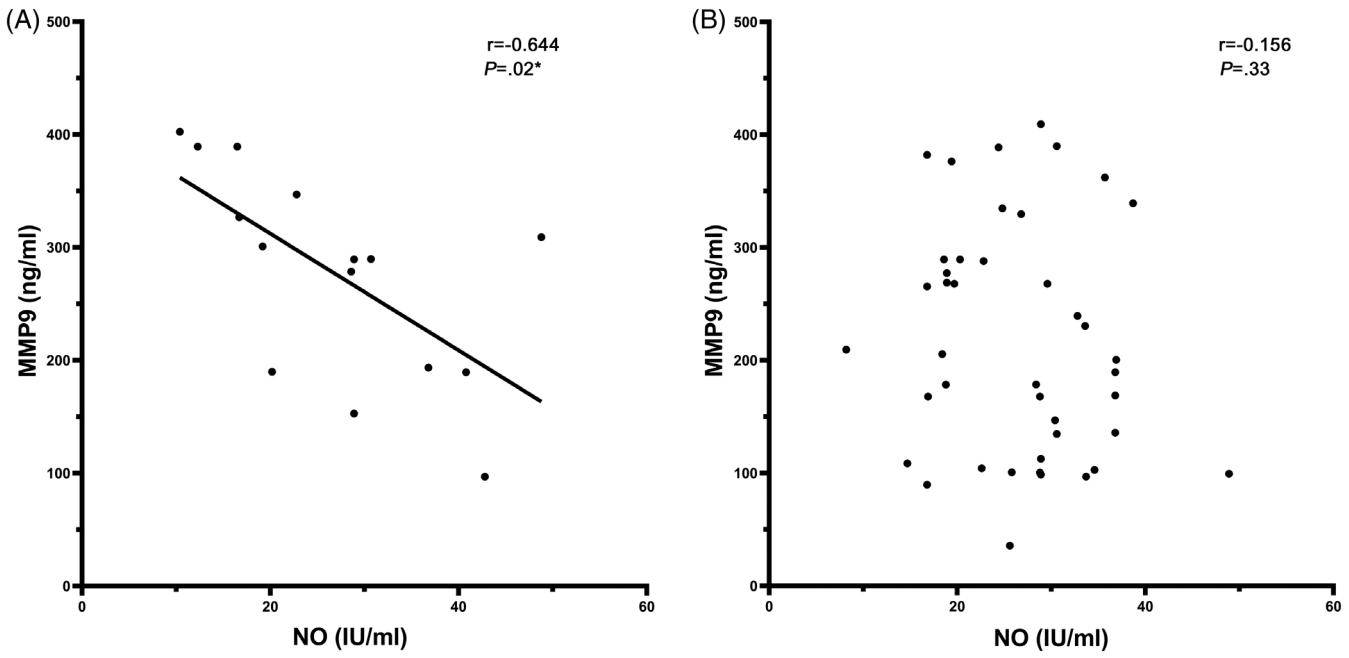

FIGURE 2 Correlation between serum NO and MMP-9 in OSA patients with hypertension (A) and without hypertension (B)

TABLE 4 Parameter changes of OSA patients before and after treatment

	preCPAP OSA ($n = 30$)	postCPAP ($n = 30$)	Difference value	p
Systolic pressure (mmHg)	127.6 ± 26.0	118.5 ± 19.5	8.8 ± 17.3	.007*
Diastolic pressure (mmHg)	81.6 ± 16.1	72.4 ± 12.0	8.1 ± 10.9	<.001*
ESS	13.3 ± 4.5	7.1 ± 2.5	6.2 ± 4.6	<.001*
NO (IU/ml)	25.6 ± 9.9	33.3 ± 9.0	−7.8 ± 7.9	<.001*
MMP-9 (ng/ml)	251.3.1 ± 106.8	160.8 ± 96.5	87.4 ± 91.2	<.001*

Note: *$p < .05$.
Abbreviations: ESS, Epworth Sleepiness Scale; MMP-9, matrix metalloproteinase-9; NO, nitric oxide.

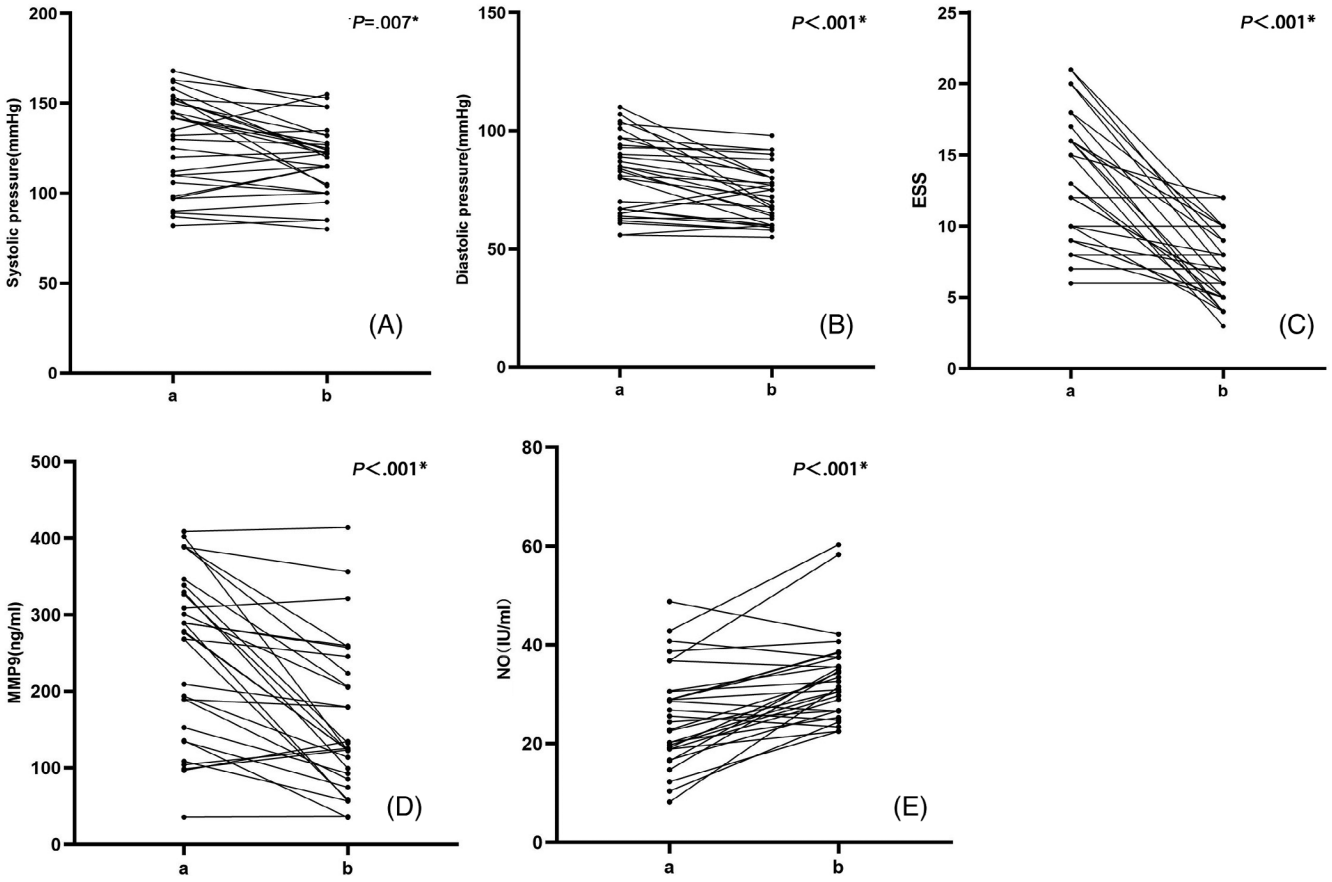

FIGURE 3 The changes of systolic pressure (A), diastolic pressure (B), ESS (C), MMP-9 (D), and NO (E) before and after treatment

3 months. Nasal CPAP titration using a commercial CPAP device (Philips, Respironics), which was performed to abolish apnea and snoring completely and to maintain SaO_2 >90% during sleep, and the optimal CPAP level was determined.

2.7 | Statistical analysis

IBM SPSS software 23 (https://www.ibm.com/products/spss-statisticsRRID:SCR_019096) was used for data processing. Measurement data or classified data are respectively represented by mean ± standard deviation (SD), number, or percentage. The normal distribution was verified by Kolmogorov–Smirnov test. Student's t test, Mann–Whitney test, and $\chi 2$ test were used to compare the variables. Differences of values before and after CPAP were compared using a two-tailed paired Wilcoxon test or Wilcoxon signed-rank test. The correlation of variables was tested by Spearman's rank. With regard to the relative strength of association of serum levels of MMP-9 and NO, stepwise multiple regression analysis was applied to patients with OSA as a single group. When $p < .05$, the difference was considered to be statistically significant.

3 | RESULTS

3.1 | Subject characteristics

The basic characteristics of all participants are shown in Table 1. Age, body mass index (BMI), blood pressure, and gender composition between two groups is of no statistical significance. Compared to the participants in the control group, the OSA patients have a higher AHI, lower nadir Arterial Oxygen Saturation (SaO_2), a greater percentage of sleep time when blood oxygen saturation is less than 90% (CT90), and higher ESS. Most importantly, OSA patients have a higher serum concentration of MMP-9 (232.8 ± 103.2 ng/ml versus 161.6 ± 56.5 ng/ml, $p < .001$*), but there was no statistical difference in concentration of serum NO (26.7 ± 9.1 IU/ml vs. 31.0 ± 11.7 IU/ml $p = .06$).

3.2 | Effect of the severity of OSA on NO and MMP-9 expression

Based on AHI, the OSA group was further divided into mild to moderate group (5 < AHI < 30) and severe group (AHI ≥ 30), and the results are shown in Table 2, the values of AHI in the two groups were 20.3 ± 7.8 events/h and 54.9 ± 13.5 events/h, respectively. There were no significant differences in gender composition, BMI, diastolic blood pressure, and ESS between the two groups. Patients with severe OSA are older than mild to moderate patients, which may be related to the development of OSA. And there was a tendency of increased overweight severity ($p = .07$) in the severe group, these results can be explained as follows obesity is a risk factor for OSA, and severe OSA patients had a higher systolic pressure ($p = .04$*). Patients with severe OSA had a significantly lower serum level of NO ($p = .006$*); however, there was no significant difference in the serum level of MMP-9 ($p = .10$).

3.3 | Correlation between NO and MMP-9 and clinical parameters and their relationship in OSA

Spearman's rank correlation coefficients between serum levels of NO or MMP-9 and various parameters in patients with OSA ($n = 57$) are shown in Table 3. We can see that NO is related to AHI, and MMP-9 is correlated with age and blood pressure, both of them are related to the lowest blood oxygen and CT90. There is also a correlation between MMP-9 and NO.

According to whether the OSA patients complained of hypertension, we divided them into the following two groups: OSA with hypertension ($n = 15$), OSA without hypertension ($n = 42$). Interestingly, we analyzed the correlation of MMP-9 and NO between the two in OSA with hypertension and OSA without hypertension, OSA patients with hypertension show a relatively strong correlation, although there is no significant correlation between them in OSA patients without hypertension (Figure 2).

3.4 | The effects of CPAP treatment on OSA patients

In 57 OSA patients, 30 patients were treated with CPAP. The data comparison before and after treatment is shown in Table 4. The MMP-9, NO, blood pressure, ESS in OSA patients were statistically significantly changed after treatment, reflecting that CPAP can alleviate or even reverse these parameters. Changes in data are illustrated in Figure 3.

4 | DISCUSSION

OSA has been getting increasing awareness among the public in recent years, and it is recognized that OSA could cause cardiovascular diseases such as hypertension.[3] Meanwhile, 30%–83% of hypertension patients have got OSA.[25] Therefore, it is of great clinical significance for the prevention and treatment of OSA and hypertension to understand the underlying mechanism of OSA. Our study has found that severe OSA patients suffered elevated blood pressure ، CPAP therapy can partially reverse hypertension in OSA patients, and this is consistent with the previous results.[7] However, the specific mechanism of OSA leading to cardiovascular diseases such as hypertension is not clear. Regarding MMP-9 and NO in OSA patients, our study showed that they have a certain correlation in OSA patients especially patients with hypertension. To the best of our knowledge, this is the first study to evaluate the relationship between MMP-9 and NO levels and hypertension in OSA patients.

Our study showed a higher serum MMP-9 level in OSA patients, and the MMP-9 is correlated with blood pressure. MMPs contain more than 20 subtypes, of which MMP-2 and MMP-9 have been mentioned in OSA-related studies.[26] Our previous study[15] and meta-analysis have shown that the peripheral level of MMP-9 was increased in OSA patients, which was related to the severity of OSA.[27] MMPs were up-regulated by inflammatory cytokines.[28] OSA provides such an inflammatory and hypoxia environment.[29] Our previous studies also found that the serum level of MMP-9 in OSA patients was increased and associated with hypertension and left ventricular hypertrophy.[15] Furthermore, it has been proved that activation of MMP-9 preceded left ventricular remodeling in hypertensive heart failure in a rat model.[30] Studies had found that the increase of MMP-9 could contribute to the change of cardiac structure and function, and may be involved in atherosclerosis,[31] which is associated with elevated blood pressure. Stiffening and extracellular matrix remodeling of arteries is associated with an increased cardiovascular risk.[32] MMP-9 has been reported to degrade elastin and collegan.[33] Elastin provides the main elastic element of arteries.[34] Therefore MMP-9 is thought to be involved in destruction of the arterial media and various heart diseases. Moreover, targeted deletion of the MMP-9 attenuates collagen accumulation suggesting that MMP-9 plays a prominent role in extracellular matrix remodeling.[35] Over time, collagen deposition in cardiovascular diseases also leads to the increase of MMP-9 level.[33] In OSA-related hypertension, the increase of MMP-9 level may be a vicious cycle. Although our study demonstrated that serum MMP-9

levels in patients with OSA were higher than those in control, there was no significant difference between patients with severe OSA and patients with mild to moderate OSA. This result may be related to population imbalance, and more patients with mild to moderate OSA should be included in the study.

In our study, although there is not a significant difference in serum NO between OSA and control participants, the NO level was lower in patients with severe OSA. NO is synthesized under the action of NO synthase (NOS), which occur in three isoforms: endothelial (eNOS), inducible (iNOS), and neuronal(nNOS). eNOS is the enzyme responsible for NO production in vascular.[36] And chronic intermittent hypoxia (CIH) led to eNOS uncoupling in mice model and OSA patients,[37,38] and inhibit eNOS at RNA level.[39] So, the decreasing of eNOS in OSA patients especially in severe OSA may lead the lower serum NO level. Numerous clinic papers have explored the NO levels in OSA patients. However, the results are controversial. Some researchers found that circulating NO was reduced in OSA patients,[40,41] whereas another study did not find difference of plasma stable NO products nitrite (NOx) between OSA subjects and controls.[42] In our research, we only found lower serum NO level in patients with severe OSA. For these controversial results, we considered that serum NO level might not be regulated by OSA only, not also obesity, systemic inflammation, which were confirmed in some studies.[43,44] Lavie[45] proposed that oxidative stress and inflammation are important mechanisms of endothelial dysfunction in OSA and CVD. The survival and ventricular enlargement trial showed that captopril reduced mortality by 19% in patients with acute myocardial infarction complicated with asymptomatic left ventricular dysfunction compared with placebo.[46] The common physiological mechanism of NO and captopril is vasodilation, which reminds us of its importance in cardiovascular disease.

Our research has found that there is a negative correlation between serum MMP-9 and NO, this relationship is more prominent in OSA patients with hypertension. It had been found that NO plays an important role in regulating the expression of MMPs at protein and gene levels.[47,48] And the inhibition of NOS could enhance the expression of MMP-9.[17] NO and MMP-9 are found to be reciprocally modulated in inflammation states and cardiovascular diseases.[49] Understanding the interactions may prove crucial in designing new therapeutics on hypertension in OSA. What is more, in one mouse model, effect of NO on the activity of MMP-9 is dose-dependent and biphasic.[50] So the relationship between them may be a process of mutual deterioration.

After 3 months of CPAP treatment, the blood pressure, serum NO, and MMP-9 levels of these patients were recovered to some extent. These results which are similar with previous studies confirmed the effect of serum NO and MMP-9 levels on blood pressure in OSA patients.[20,51] To be sure, from our research results, short-term ventilator treatment is conducive to the recovery of blood pressure and serum indicators, but the long-term efficacy of CPAP needs to be verified by more research.

In our study, NO and MMP-9 were simultaneously studied in OSA patients for the first time, and the relationship between their interaction and hypertension was discussed in OSA patients first. Our results might be conducive to the prevention and treatment of obstructive sleep apnea syndrome and its related hypertension. There are also some limitations in our research. First, relatively few patients were included in the study. Second, the dynamic changes of NO and MMP-9 were not observed during the follow-up period. We only observed the correlation between MMP-9 and NO in this clinical observational study, the causality between them needs to be verified by subsequent in vitro mechanism studies and relevant animal experiments. Finally, the process of OSA-induced hypertension is a very complex pathophysiological process, which many other serum biomarkers may also involve. Further analysis of other biomarkers would help to understand its pathogenesis.

5 | CONCLUSION

In summary, we have shown that the levels of MMP-9 are elevated in OSA patients and may have an effect on lowering NO levels, particularly in patients with hypertension. These changes can be reversed by CPAP therapy. The correlation between MMP-9 and NO in OSA patients especially in patients with hypertension suggests a potential underlying mechanism for the association between hypertension and OSA.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Shiying Zeng: conception, data collection, analysis, interpretation, drafting. **Shisheng Li**: conception, analysis, interpretation, revision, approval. **Qinglai Tang:** conception, revision, approval. **Ayinuer Tuerdi**: conception, revision. **Xinying Tong**: analysis, interpretation, drafting. **Xiaojun Tang**: data collection, analysis, drafting. **Danhui Yin**: data collection, analysis; **Mengmeng Li**: data collection, analysis. **Qian Yang**: data collection, analysis.

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