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Increasing circulating ESM-1 and adhesion molecules are associated with earlystage atherosclerosis in OSA patients:A cross-sectional study

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ABSTRACT

Background: There are increasing evidences for a direct relationship between the vascular system and obstructive sleep apnea (OSA). The aim of this study was to investigate the relationship between circulating endothelial cell specific molecule-1 (ESM-1), adhesion molecules and subclinical atherosclerosis in patients with OSA.
Methods: This was a cross-sectional study in which 161 patients with OSA and 56 controls were recruited. Demographic data, biochemical and polysomnography parameters were collected. We used a powerful high-throughput Multiplex Immunobead Assay technique to simultaneously test plasm levels of ESM-1, P-selectin, E-selectin, L-selectin, inter-cellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (*VCAM-1*). Carotid intima-media thickness (CIMT) were measured as parameters of vascular endothelial dysfunction and early atherosclerosis.
Results: Increasing circulating levels of ESM-1, P-selectin, E-selectin, L-selectin, ICAM-1 and VCAM-1 were found increased in patients with OSA (all $P < 0.001$). Furthermore, OSA patients exhibited increased CIMT than controls ($P < 0.05$). Multivariate linear analysis indicated that elevated ESM-1, P-Selectin, E-selectin, and L-selectin levels were associated with AHI (all $P < 0.05$). Moreover, multivariate analysis showed that increasing ESM-1, VCAM-1, P-Selectin, and L-selectin were significantly associated with thick CIMT in OSA patients (all $P < 0.05$).
Conclusions: Increased circulating ESM-1 and adhesion molecules associated with thick CIMT in OSA, which is a marker of subclinical atherosclerosis. Strict attention to monitor circulating ESM-1 and adhesion molecules is necessary for early detection of subclinical atherosclerosis in OSA patients.

1. Introduction

Obstructive sleep apnoea (OSA) is the most common phenotype of the serious sleep-related breathing disorders which is characterized by repetitive narrowing or occlusion of the pharynx causing intermittent hypoxia, repetitive arousals, sleep deprivation, and excessive daytime sleepiness. Several studies have reported an association between OSA and an increased risk for cardiovascular disease, including hypertension, coronary artery disease, and atherosclerosis [1–5]. A number of studies suggest that OSA could directly impairs the endothelium cells, providing a possible link between OSA and the development of cardiovascular disease [6,7].

Although studies have revealed a direct relationship between the vascular system and OSA, the pathogenesis of this relationship has yet to be fully explained. There are increasing evidences that Carotid intima-media thickness (CIMT) is widely used as a marker for the detection of early endothelial dysfunction and subclinical

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atherosclerosis [8]. Recent studies suggested the presence of OSA is independently associated with increased CIMT [9], and CIMT is recognized as an ideal marker for subclinical atherosclerosis in OSA [9,10].

Cellular adhesion molecules are good indicators of endothelial dysfunction and vascular inflammation, OSA is also be associated with increased adhesion molecules [11]. The adhesion of circulating leukocytes to the endothelial cells is believed to be one of the initial steps in the pathogenesis of atherosclerosis. Selectin family of adhesion molecules includes 3 receptors expressed from various cells including endothelial cells (E-selectin), leukocytes (L-selectin) to platelets and endothelium cells (P-selectin). Vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) are synthesized by inflamed endothelium [12]. Adhesion molecules are known to be involved in vascular dysfunction and are considered as markers of atherosclerosis.

ESM-1 (previously known as endothelial cell specific molecule-1, Endocan), is a novel immunoinflammatory marker that may be linked to cardiovascular disease. ESM-1 has a significant effect on the regulation of cell adhesion and increases cellular adhesion moleculesthat reflect endothelial dysfunction [13–15]. In our previous study, we found Circulating ESM-1 levels are correlated with the presence of coronary artery disease in patients with OSA [16].

Several studies have reported associations between OSA and subclinical atherosclerosis [9,10]. However, few studies have investigated the role of combined adhesion molecules and ESM-1 in evaluating the early vascular injury in patients with OSA. Therefore, we sought to investigate the relationship between circulating ESM-1, adhesion molecules and subclinical atherosclerosis, which may play a vital role in estimating the risk of earlystage atherosclerosis in patients with OSA.

2. Materials and methods

2.1. Study population

This cross sectional study was performed in the sleep center and was approved by the ethics committee of Beijing Anzhen Hospital capital medical university (approval NO:2017005). All participants gave written informed consent.

All consecutive patients who underwent overnight full polysomnography study due to snoring, apnea, and excessive daytime sleepiness in the sleep center of Beijing Anzhen Hospital between July 2017 and March 2019 were included in this study. A total of 217 patients were eligible for the study. Subjects younger than 18 years, with central sleep apnea syndrome, coronary artery disease, heart failure, arrhythmias, stroke, chronic kidney disease, chronic obstructive pulmonary diseases, pulmonary hypertension, active infections, malignancy and on therapy for OSA were excluded from the study. Moreover, patients with significant carotid atherosclerosis (CIMT$\geq$1.5 mm) were excluded in our study. Blood pressure was measured at the nondominant arm in the morning of the procedure after a 5min resting interval. Hypertension was defined for the study purpose according to the 2007 European Society of Hypertension and of the European Society of Cardiology task force for the management of arterial hypertension guidelines [17].

Demographic characteristics including age, gender, smoking and drinking status were recorded for each study participant. Body mass index (BMI) was calculated by dividing weight in kilograms by squared height in meters (kg/m^2). Sleepiness was evaluated using the Epworth sleepiness scale (ESS) [18].

2.2. Sleep study

All participants underwent a standard overnight PSG (Siesta, Compumedics, Melbourne, Australia). All sleep studies were manually interpreted according to the standard criteria of the American Academy of Sleep Medicine Manual by experienced physicians [19]. An apnea event was defined as a drop in the peak thermal sensor excursion by $\geq$ 90% of the baseline for at least 10s. Hypopnea was defined as a decrease in the nasal pressure signal excursion by $\geq$ 30% for at least 10s, accompanied by desaturation of 4% or more from the pre-event baseline or an arousal from sleep [20]. Obstructive sleep apnea was defined as an apnea-hypopnea index (AHI) score of 5 or more events per hour, with the severity described as mild for an AHI score of 5 to less than 15, moderate for an AHI score of 15 to less than 30, and severe for an AHI score of more than 30. We recorded the AHI, Lowest oxygen saturation ($LSaO_2$), Mean oxygen saturation ($MSaO_2$), Oxygen desaturation index (ODI), Mean apnea-hypopnea duration (MAD), percentage of cumulative time with oxygen saturation below 90% (CT90) and Arousal index (ArI).

A final total of 217 participants were enrolled (Supplemental Fig. 1). According to the diagnostic standard, the participants were divided into two groups: OSA (n = 161) and non-OSA (n = 56).

2.3. Blood sample

All blood samples were collected after the participants had fasted overnight. Blood samples were then centrifuged for 10 min at 3000 rpm and 4 °C. Plasma samples were subsequently stored in a freezer at −80 °C before analysis. Serum triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) levels and fasting blood glucose (FBG), and other routine serum biochemical parameters were measured in a biochemical analyzer (Hitachi-7600, Tokyo, Japan) using blinded quality control specimens in the Department of the Biochemical Laboratory at Beijing An Zhen Hospital.

2.4. Carotid artery ultrasonographic measurements

All patients underwent Duplex Doppler ultrasonography of the common carotid artery (Epiq 5, Philips Medical Systems) by qualified researchers. Carotid arteries were scanned longitudinally to visualize the CIMT in the far wall of the artery. The best images of the far wall that could be obtained were used to determine the common carotid artery. Measurements were made on frozen images, magnified to standard size online. The CIMT value was defined as the mean of the CIMT of the common carotid artery, calculated from 10 measurements on each side, taken 10 mm proximal to the carotid bifurcation. A carotid plaque was defined as a focal thickening encroaching into the carotid lumen of more than 1.5 mm (as measured from the intima-lumen interface to the media-adventitia interface) or at least 50% of the surrounding CIMT. Therefore, participants were further categorized into three carotid artery profiles: normal carotid arteries (CIMT<1.0 mm), thick CIMT (1.0 mm $\leq$ CIMT<1.5 mm), significant carotid atherosclerosis (CIMT$\geq$1.5 mm) [21]. Patients with significant carotid atherosclerosis (CIMT$\geq$1.5 mm) were excluded in our study. The reproducibility of CIMT measurements between and within sonographers had been checked previously.

2.5. Quantification of plasm molecules

Circulating P-selectin, E-selectin, L-selectin, ICAM-1, VCAM-1 and ESM-1 levels were determined simultaneously by Human Magnetic Luminex Screening Assay (R&D Systems, MN, USA) in accordance with the manufacturer's instructions. In order to ensure the accuracy and validity of the results, we evaluated the Luminex multiplex assay system by the standard curve and intra-assay variability. Firstly, the standard curve was critical for quantitation

measurements. In our study, the intra-assay CV of standard was <4.0%. Each well absorbance was determined at 450 nm. The concentrations of each cytokine were determined using Bio-Rad Bio-Plex 200 system (BioRad, CA, USA). Data were analyzed by 5-parameter curve fitting using Bio-Plex Manager software, version 6.1.1 (Bio-Rad).

2.6. Statistical analysis

Continuous variables are expressed as mean ± standard deviation or **median (interquartile range)** and categorical variables as numerals (percentages). The independent Student's *t*-test for normal distribution and the Wilcoxon rank sum test for asymmetric distribution were used to analyze the differences in continuous variables. The Chi square test was used to analyze categorical variables. Pearson's analysis was performed to evaluate correlations among the measured parameters. Spearman correlation was used to determine the correlation between the circulating cytokines and CIMT. Multiple logistic regression analyses were used to investigate the potential effects of the interaction between the cytokines concentrations and thick CIMT (1.0 mm ≤ CIMT<1.5 mm). Multiple logistic regression analyses were performed to assess the influence of variables (age, sex, BMI and other variables with $P < 0.05$ in the univariate model analysis) analyzed on circulating cytokines levels and the incidence of thick CIMT. In addition, multiple linear regression analyses were also performed to examine the association of circulating ESM-1, adhesion molecules concentrations and the severity of OSA (assessed by AHI). Multiple linear regression analyses were performed to assess the influence of variables (age, sex, BMI and other variables with $P < 0.05$ in the univariate model analysis) analyzed on circulating cytokines and AHI. A P value of <0.05 was considered statistically significant. Statistical analysis was performed with SPSS 20.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Baseline clinical characteristics of the study population

The present study included 161 patients with OSA and 56 control subjects. Baseline demographic and laboratory data of the individuals were shown in Table 1. There were significant differences in BMI, SBP, DBP, TC, TG, LDL, FBG, and CIMT between the severe OSA and Control groups ($P < 0.05$, Table 1). The patients in the OSA group were more likely to experience excessive daytime sleepiness than those in the non-OSA group ($P < 0.05$, Table 1).

3.2. Elevated circulating ESM-1 and adhesion molecules in patients with OSA

Plasma ESM-1, ICAM-1, VCAM-1, P-Selectin, E-selectin and L-selectin concentrations were significantly increased in mild, moderate and severe OSA group than those in the control group ($P < 0.05$, Fig. 1). Meanwhile, we explore the levels of ESM-1, ICAM-1, VCAM-1, P-Selectin, E-selectin and L-selectin among three different OSA status. We found that the these inflammatory markers were the lowest in control subjects and increased in OSA group ($P < 0.05$, Table 1).

To investigate whether the levels of ESM-1 and adhesion molecules were correlated with clinical and biological parameters, we used Spearman's correlation to explore the associations. As shown in Table 2, ESM-1 levels in all individuals were closely correlated with AHI (rho = 0.501, $P < 0.001$), LSaO2 (rho = −0.454, $P < 0.001$), MSaO2 (rho = −0.469, $P < 0.001$), ArI (rho = 0.431, $P < 0.001$), CT90 (rho = 0.504, $P < 0.001$), ODI (rho = 0.413, $P < 0.001$, Table 2). Meanwhile circulating ESM-1 levels showed significantly positive correlation with plasma ICAM-1 (rho = 0.347, $P < 0.001$),VCAM-1 (rho = 0.424, $P < 0.001$),P-selectin (rho = 0.431, $P < 0.001$), E-selectin (rho = 0.476, $P < 0.001$) and L-selectin (rho = 0.482, $P < 0.001$, Table 2). CIMT was also closely correlated with circulating ESM-1, VCAM-1, P-selectin and L-selectin (all $P < 0.05$, Table 2).

Table 1
Baseline characteristics of non-OSA and OSA groups.

Variables	non-OSAn = 56	Mild OSA n = 29	Moderate OSA n = 33	Severe OSA n = 99	P value
Male, n (%)	38 (67.85%)	21 (72.41%)	29, (89.87%) *	85 (85.85%) *	0.053
Ages (years)	48.0 ± 16.0	47.5 ± 14.7	48.0 ± 14.7	45.0 ± 10.3	0.393
Smoke,n (%)	18 (32.14%)	12 (41.37%)	10 (30.30%)	32 (32.33%)	0.704
alcohol, n (%)	8 (14.28%)	10 (34.48%)*	8 (24.24%)*	35 (35.35%)*	0.018
BMI (kg/m^2)	23.67 ± 3.78	25.13 ± 2.89	26.67 ± 3.71*	29.03 ± 4.67*†§	<0.001
SBP (mmHg)	119.40 ± 12.73	126.04 ± 9.50	122.09 ± 29.36	128.72 ± 13.34*	<0.001
DBP (mmHg)	74.17 ± 10.52	79.26 ± 6.27	78.22 ± 20.02	83.03 ± 10.74*	<0.001
TC (mmol/l)	4.62 (0.96)	4.60 (1.36)	4.95 (2.19)*†	5.10 (2.89)*†§	0.003
TG (mmol/l)	1.14 (0.84)	1.32 (0.98)*	1.44 (1.00)*	1.73 (1.02)*†§	0.002
HDL (mmol/l)	1.23 (0.35)	1.01 (0.45)	1.14 (0.24)	1.16 (0.49)	0.506
LDL (mmol/l)	2.97 (1.16)	2.88 (1.13)	2.95 (1.25)	3.33 (1.49)*†§	0.004
FBG (mmol/l)	5.03 (0.56)	5.00 (0.95)	5.39 (1.67)	5.75 (2.22)*†	0.002
ALT (mmol/l)	21.12 ± 10.26	23.90 ± 16.70	26.16 ± 14.47	34.98 ± 30.76*†	0.013
AST (mmol/l)	20.55 ± 5.02	22.76 ± 12.56	22.04 ± 7.79	25.32 ± 13.18	0.045
creatinine (mmol/l)	63.22 ± 13.48	71.44 ± 41.64	74.59 ± 10.71*	71.09 ± 13.91*	0.006
uric acid (mmol/l)	329.55 ± 79.77	377.73 ± 122.56*	397.66 ± 70.22*	420.14 ± 98.36*†	<0.001
ESM-1 (pg/ml)	277.70 (389.03)	717.57 (311.21)*	805.80 (344.17)*†	924.60 (882.56)*†§	<0.001
ICAM-1 (ng/ml)	149.21 (344.86)	575.60 (523.93)*	496.02 (447.96)*	624.60 (482.56)*	<0.001
VCAM-1 (ng/ml)	268.48 (308.51)	600.16 (536.88)*	739.11 (463.68)*†	398.09 (188.56)*†	<0.001
P-selectin (ng/ml)	10.15 (11.03)	23.43 (8.73)*	26.50 (10.71)*	23.12 (10.38)*	<0.001
E-selectin (ng/ml)	7.06 (7.14)	23.60 (18.21)*	28.70 (15.77)*†	23.94 (14.60)*	<0.001
L-selectin (ng/ml)	187.65 (418.63)	618.22 (274.40)*	575.09 (234.48)*	549.10 (159.85)*	<0.001
CIMT (mm)	0.95 ± 0.21	0.93 ± 0.26	1.08 ± 0.32	1.17 ± 0.27*†	0.019
ESS score	7.00 (6.00)	8.00 (7.00)	11.00 (7.00)*†	12.00 (7.00)*†	0.001

Data are presented as mean values ± standard deviation, **median (interquartile range,IQR) or n (%).**
BMI, body Mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; FBG: fasting blood glucose; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ESM-1, endothelial cell specific molecule-1; ICAM-1, inter-cellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; CIMT, carotid intima media thickness; ESS, Epworth Sleepiness Scale.
* $P < 0.05$ versus Control.† $P < 0.05$ versus Mild OSA. § $P < 0.05$ versus Moderate OSA.

3.3. Baseline clinical characteristics of the OSA patients according to CIMT

Table 3 summarizes the basic characteristics of OSA patients stratified by CIMT (normal CIMT: CIMT<1.0 mm, n = 56; thick CIMT:1.0 mm ≤ CIMT<1.5 mm, n = 105). Patients in the thick CIMT group had dramatically higher levels of circulating ESM-1 [627.00 (488.16)vs.396.72 (475.77)pg/ml], ICAM-1 [403.89 (349.66) vs.217.35 (444.66) ng/ml], VCAM-1 [477.88 (452.66) vs. 351.36 (372.99)ng/ml], P-selectin [23.13 (11.80) vs 17.95 (15.51)ng/ml], and E-selectin [22.60 (19.25) vs. 20.20 (21.90)ng/ml] than patients in the normal CIMT group in this study (P < 0.05, Table 3). Significant differences showed in SBP, TC, TG, LDL between normal CIMT group and thick CIMT group (P < 0.05). Moreover, participants in the thick CIMT group had higher AHI, $MSaO_2$, CT90, ODI and ArI than normal CIMT group (P < 0.05, Table 3).

3.4. Association of circulating ESM-1 and adhesion molecules with CIMT

The association between the occurrence of CIMT thickness and circulating inflammatory factors were also investigated in different models of logistic regression. Patients who had higher ESM-1 levels had a higher OR (OR = 1.112/100 pg ESM-1, 95% CI = 1.024–1.027, P = 0.011) with the incidence of thick CIMT. After adjustment for BMI, age, sex, TC, LDL, FBG, creatinine and uric acid, we found that circulating ESM-1 levels is associated with the incidence of thick CIMT (OR = 1.181/100 pg increase, 95%CI = 1.035–1.256, P = 0.004, Table 4). We also found that circulating VCAM-1, P-selectin, L-selectin were associated with the increased incidence of thick CIMT (all P < 0.05, Table 4).

Association of circulating ESM-1 and adhesion molecules levels with the severity of OSA.

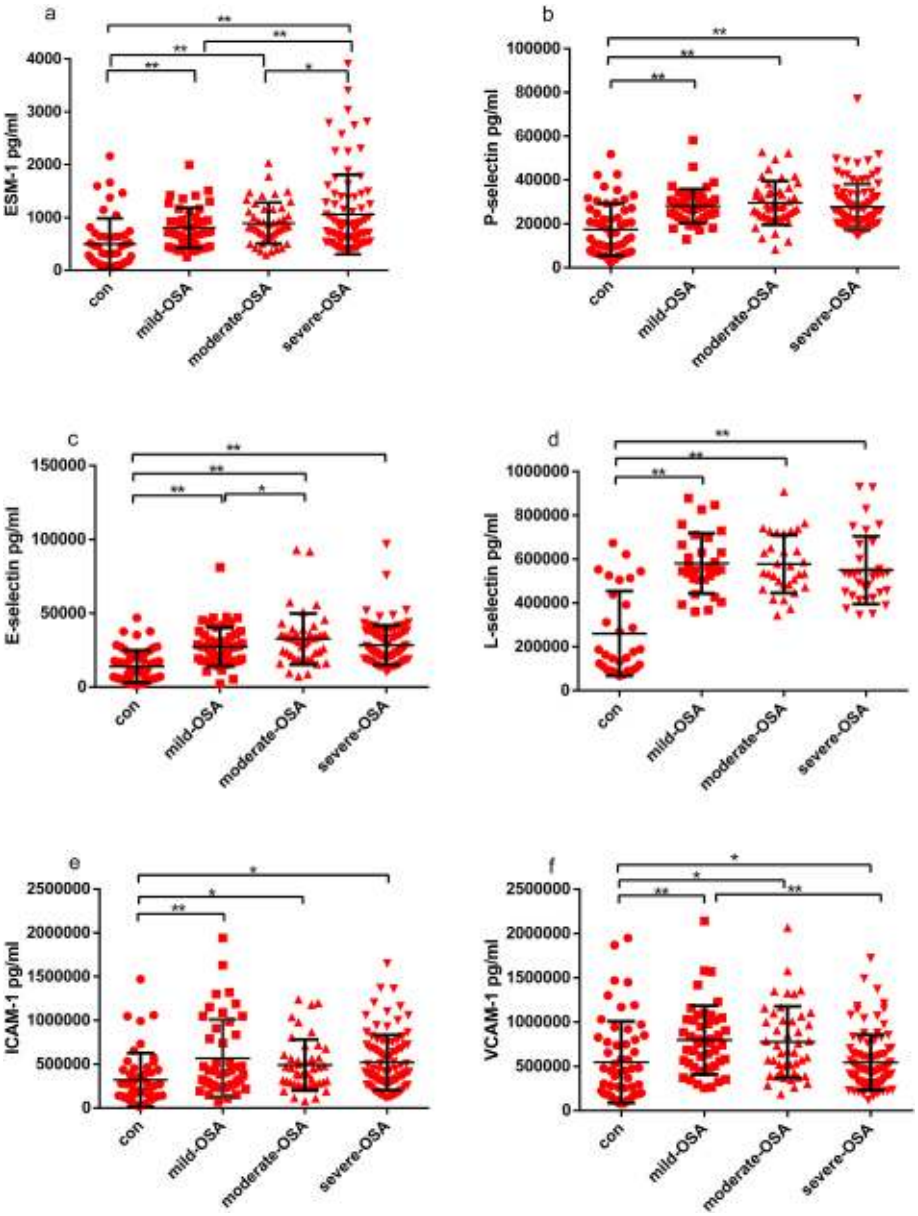

Fig. 1. Circulating P-selectin, E-selectin, L-selectin, ICAM-1, VCAM-1 and ESM-1 levels in Control, Mild OSA, Moderate OSA and Severe OSA groups, the dots represent each individual. ESM-1, Endothelial cell specific molecule 1; ICAM-1, inter-cellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1.
*P < 0.05, **P < 0.01, ***P < 0.001.

Table 2
Correlation between polysomnography parameters and inflammatory factors in OSA patients.

	ESM-1		ICAM-1		VCAM-1		P-selectin		E-selectin		L-selectin	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
AHI	0.501	<0.001**	0.259	0.001*	0.011	0.789	0.449	< 0.001**	0.513	< 0.001**	0.339	< 0.001**
$LSaO_2$	−0.454	<0.001**	0.238	0.003*	0.029	0.718	−0.439	< 0.001**	−0.482	< 0.001**	−0.310	< 0.001**
$MSaO_2$	−0.469	<0.001**	−0.200	<0.001**	−0.144	0.158	−0.346	<0.001**	−0.224	0.028	−0.178	0.095
MAD	0.099	0.345	0.100	0.352	0.132	0.209	0.161	0.125	0.070	0.509	0.117	0.290
CT90	0.504	<0.001**	0.166	0.136	0.148	0.172	0.314	0.003*	0.420	< 0.001**	0.280	0.013*
ODI	0.413	<0.001**	0.147	0.177	0.229	0.221	0.246	0.019*	0.378	< 0.001**	0.231	0.257
ArI	0.431	<0.001**	0.204	0.352	0.061	0.769	0.370	0.063	0.289	0.152	0.654	<0.001**
CIMT	0.248	0.035*	0.176	0.066	0.193	0.045**	0.301	0.002**	0.147	0.129	0.178	0.046*
ESM-1	___	___	0.347	< 0.001**	0.424	0.004	0.431	< 0.001**	0.476	< 0.001**	0.482	< 0.001**
ICAM-1	0.347	< 0.001**	___	___	0.233	0.004	0.374	< 0.001**	0.427	< 0.001**	0.287	0.001**
VCAM-1	0.424	<0.001**	0.233	< 0.001**	___	___	0.286	< 0.001**	0.359	< 0.001**	0.621	< 0.001**
P-selectin	0.431	<0.001**	0.286	< 0.001**	0.374	< 0.001**	___	___	0.816	< 0.001**	0.634	< 0.001**
E-selectin	0.476	<0.001**	0.359	< 0.001**	0.427	< 0.001**	0.816	< 0.001**	___	___	0.654	< 0.001**
L-selectin	0.482	<0.001**	0.287	< 0.001**	0.621	< 0.001**	0.634	< 0.001**	0.654	< 0.001**	___	___

ESM-1, endothelial cell specific molecule-1; ICAM-1, inter-cellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; AHI: apnea-hypopnea index; $LSaO_2$: lowest oxygen saturation; $MSaO_2$: mean oxygen saturation; ODI, Oxygen desaturation index; MAD: Mean apnea−hypopnea duration; ESS: Epworth Sleepiness Scale; CT90: percentage of cumulative time with oxygen saturation below 90%; CIMT, carotid intima media thickness; ESS, Epworth Sleepiness Scale.

Table 3
Baseline clinical characteristics of the study participants according to CIMT.

Variables	OSA		P value
	CIMT<1.0 mm n = 56	1.0 mm ≤ CIMT<1.0 mm n = 105	
Male, n (%)	41 (73.21%)	84 (80.00%)	0.319
Ages (years)	46.1 ± 12.9	48.5 ± 11.3	0.104
Current smokers,n (%)	37 (66.07%)	65 (61.90%)	0.502
Current drinkers,n (%)	47 (83.92%)	80 (76.19%)	0.773
BMI (kg/m^2)	26.12 ± 4.84	27.10 ± 4.55	0.195
SBP (mmHg)	122.29 ± 13.65	126.46 ± 12.35	0.038*
DBP (mmHg)	78.40 ± 10.68	79.42 ± 10.93	0.607
TC (mmol/l)	4.82 (1.18)	5.11 (2.09)	<0.001**
TG (mmol/l)	1.27 (1.03)	1.96 (0.97)	0.027*
HDL (mmol/l)	1.17 (0.65)	1.11 (0.55)	0.174
LDL (mmol/l)	2.62 (1.23)	3.23 (1.52)	0.001**
ESM-1 (pg/ml)	396.72 (475.77)	627.00 (488.16)	0.001**
ICAM-1 (ng/ml)	217.35 (444.66)	403.89 (349.66)	0.028*
VCAM-1 (ng/ml)	351.36 (372.99)	477.88 (452.66)	0.010**
P-selectin (ng/ml)	17.95 (15.51)	23.13 (11.80)	<0.001**
E-selectin (ng/ml)	20.20 (21.90)	22.60 (19.25)	0.002**
L-selectin (ng/ml)	486.92 (447.37)	516.12 (419.30)	0.114
AHI(events/h)	25.34 ± 28.95	35.24 ± 27.68	0.035*
$LaSo_2$(%)	82.73 ± 11.18	79.3 ± 14.50	0.125
$MSaO_2$ (%)	94.48 ± 3.17	91.40 ± 10.10	0.030*
MAD(s)	27.66 ± 7.15	30.59 ± 7.53	0.131
CT90 (%)	0.80 (0.20–6.25)	2.25 (0.25–8.25)	0.031*
ODI (events/h)	9.00 (2.25–24.23)	26.35 (9.18–40.48)	0.117
ArI(events/h)	12.50 (3.00–29.87)	7.00 (4.75–9.93)	0.006**
CIMT(mm)	0.89 ± 0.12	1.26 ± 0.20	<0.001**
ESS score	8.00 (7.00)	7.00 (8.00)	0.455

Data are presented as mean values ± standard deviation or as number. ESM-1, endothelial cell specific molecule-1; ICAM-1, inter-cellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; AHI: apnea-hypopnea index; $LSaO_2$: lowest oxygen saturation; $MSaO_2$: mean oxygen saturation; ODI, Oxygen desaturation index; MAD: Mean apnea−hypopnea duration; CT90: percentage of cumulative time with oxygen saturation below 90%; ArI, Arousal index; ESS: Epworth Sleepiness Scale; CIMT carotid intima media thickness.
*P < 0.05, **P < 0.001.

Multiple linear regression analyses were also performed to examine the association of circulating inflammatory factors levels and the AHI. After adjustment for demographics (age, sex, BMI, alcohol), biological parameters (TG, TC, LDL, FBG, creatinine and uric acid), circulating ESM-1 levels were positively associated with the AHI (Beta = 2.309, 95%CI = 1.534–3.084, P < 0.001, Table 5). We also found that circulating P-selectin, E-selectin, L-selectin levels were associated with AHI(P < 0.05, Table 5).

4. Discussion

In this study, some inflammatory parameters that indicate the activation of endothelium and platelets were evaluated in the circulating of adhesion molecules and ESM-1. Circulating levels of P-selectin, E-selectin, L-selectin, ICAM-1, VCAM-1 and ESM-1 levels were found to be significantly elevated compared to healthy individuals. **We showed that i**ncreasing circulating ESM-1 and adhesion molecules strongly and independently associated with CIMT, which is a marker of early vascular injury.

Endothelial dysfunction is an early indicator of vascular damage and may be related to increased cardiovascular risk in OSA patients [6]. Despite increased research regarding OSA, there are still unknowns regarding pathophysiology, treatment and outcome. New biomarkers showing OSA prognosis will be of value especially for

Table 4
Association between thick CIMT and variables in multiple logistic regression models.

	Unadjusted		Model 1		Model 2	P value
	OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)	
ESM-1 (per 100 pg increase)	1.112 (1.024–1.027)	0.011**	1.115 (1.035–1.213)	0.001**	1.181 (1.035–1.256)	0.004**
ICAM-1 (per ng increase)	1.050 (1.019–1.082)	0.199	1.001 (0.999–1.002)	0.378	1.000 (0.99–1.000)	0.395
VCAM-1 (per ng increase)	1.012 (1.001–1.021)	0.006**	1.002 (1.001–1.003)	0.067	1.062 (1.004–1.153)	0.014*
P-selectin (per ng increase)	1.147 (1.048–1.323)	0.002**	1.159 (1.019–1.303)	0.006**	1.153 (1.012–1.309)	0.013*
E-selectin (per ng increase)	1.002 (0.999–1.005)	0.144	1.002 (0.999–1.005)	0.166	1.002 (0.999–1.005)	0.256
L-selectin (per ng increase)	1.349 (1.112–1.649)	0.002**	1.309 (1.077–1.592)	0.007**	1.348 (1.098–1.655)	0.004**

Multiple logistic regression model includes age, sex, BMI, and other variables with $P < 0.05$ in univariate model analysis.
Note: **Dependent variable: thick cIMT(yes vs. no)**.
Model 1: adjusted for age, sex, BMI, SBP.
Model 2: adjusted for Model 1 +TC, LDL, FBG, creatinine and uric acid.
ESM-1, endothelial cell specific molecule-1; ICAM-1, inter-cellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; BMI, body mass index; SBP, systolic blood pressure; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; **FBG, fasting blood glucose; CIMT, carotid intima media thickness**.

Table 5
Association between levels of AHI and variables in multiple linear regression models.

	Unadjusted	P value	Model 1	P value	Model 2	P value
	Beta (95%CI)		Beta(95%CI)		Beta(95%CI)	
ESM-1 (per 100 pg increase)	2.152 (1.421–2.882)	<0.001**	2.109 (1.373–2.845)	<0.001**	2.309 (1.534–3.084)	<0.001**
ICAM-1 (per ng increase)	0.012 (−0.002-0.025)	0.086	0.011 (−0.001-0.022)	0.093	0.010 (−0.003-0.023)	0.119
VCAM-1 (per ng increase)	−0.012 (−0.025-0.002)	0.092	−0.003 (−0.016-0.010)	0.658	−0.001 (−0.014-0.013)	0.924
P-selectin (per ng increase)	0.954 (0.587–1.321)	<0.001**	0.549 (0.192–0.902)	0.003**	0.511 (0.096–0.927)	0.016*
E-selectin (per ng increase)	0.688 (0.420–0.926)	<0.001**	0.426 (0.163–0.690)	0.002**	0.398 (0.104–0.692)	0.008**
L-selectin (per ng increase)	3.948 (1.962–5.934)	<0.001**	2.645 (0.714–4.576)	0.008**	2.807 (0.832–4.782)	0.006**

Note: **Dependent variable: AHI**.
Model 1: adjusted for age, sex, BMI, alcohol.
Model 2: adjusted for Model 1 +TG, TC, LDL, FBG, creatinine and uric acid.
ESM-1, endothelial cell specific molecule-1; ICAM-1, inter-cellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; **FBG, fasting blood glucose**.

future cardiovascular risk. Cellular adhesion molecules are good indicators of endothelial dysfunction and vascular inflammation [11]. These adhesion molecules favor the pathogenesis of atherosclerosis by promoting the adhesion of circulating leukocytes to vascular endothelial cells, which then migrate beneath the endothelium and favor the formation of atherosclerotic lesions [22]. Elevation of adhesion molecules may be responsible for endothelial dysfunction and atherosclerosis [11]. Research into subclinical cardiovascular disease in OSA patients has identified associations between cellular adhesion molecules and the severity of atherosclerosis [11,23]. Many studies also shown that level of circulating adhesion molecules levels were all significantly increased in the OSA patients [11,15,23]. Another article also investigated the relationship between OSA and circulating ESM-1 levels, and found that intermittent hypoxia can up-regulate the level of ESM-1 via HIF-1α/VEGF pathway, which could also enhance adhesion molecules expression and promote adhesion between monocytes and endothelial cells [15]. All evidence indicated that increased adhesion molecules may be another link between ESM-1 and OSA. OSA induced intermittent hypoxia may play a vital role in elevating circulating ESM-1 levels in patients with OSA. However, future studies are needed to highlight underlying mechanisms and the predictive value of ESM-1 for outcome in patients with OSA.

Enhanced CIMT is also an established predictor for early impairment of the vascular endothelium and endothelial dysfunction [24]. CIMT thickening is the manifestation of early atherosclerosis, and the change of CIMT can be used as a marker of early vascular injury [25]. CIMT is recognized as an established biomarker of atherosclerosis and subclinical vascular damage for predict future clinical cardiovascular events. We found that circulating ESM-1 and adhesion molecules were both associated with early vascular injury in OSA patients. Elevated CIMT and diminished FMD are early subclinical indicators of atherosclerosis [26]. Similar to our observations, a recent study also reported a negative correlation between ESM-1 and flow-mediated dilation (FMD) [27]. Overall, more clinical data are required to investigate the effect of ESM-1 and adhesion molecules on early atherogenesis in OSA patients of different ages and with various complications.

ESM-1 was initially cloned from human umbilical vein endothelial cell (HUVEC) cDNA library in 1996 and a novel Proteoglycans secreted by vascular endothelium [28]. ESM-1 can be detected in the circulation and is an indicator of angiogenesis and endothelial cell activation [13,28]. ESM-1 can take part in molecular interactions with wide range of biologically active moieties, which are essential for regulation of biological processes such as cell adhesion, migration, proliferation, and neovascularization. Circulating ESM-1 levels are elevated in conditions such as chronic kidney disease [29], renal transplant rejection [30], tumor progression [31], coronary artery disease [32], and hypertension [33]. ESM-1 is a potential inflammatory marker and raised circulating levels may reflect endothelial dysfunction.

Circulating ESM-1 may be a surrogate endothelial dysfunction marker and may have a functional role in endothelium-dependent pathological disorders. Kose et al. evaluated the relationship between serum ESM-1 levels and acute coronary syndrome (ACS), and demonstrated that ESM-1 was an independent predictor of endothelial dysfunction, which was significantly increased in patients with ACS [32]. Balta et al. suggested that circulating ESM-1 levels may represent a new marker for essential hypertension. They found that in 18 patients with newly diagnosed hypertension, serum ESM-1 levels were correlated significantly with CIMT and high-sensitivity C-reactive protein compared with 23 normotensive controls [33].

ESM-1 plays a vital role in many biological actions including cell

adhesion, migration and proliferation and it can be a marker for endothelial dysfunction [13]. However, there are few studies on circulating ESM-1 levels related with the presence of OSA and mechanisms of OSA involving endothelial dysfunction. Kanbay et al. demonstrated that ESM-1 were significantly up-regulated in OSA patients [27], which is in accord and similar with our findings. We found that increased circulating ESM-1 and adhesion molecules correlated with CIMT, which suggests that there is a potential of treatment on the early cardiovascular risk. All evidence suggested that ESM-1 may be a potential predictor of vascular injury in patients with OSA.

However, some limitations of our study need to be mentioned. First, the study is a cross-sectional and the sample size is relatively small, so longitudinal study cause and effect relationship can not be suggested. Second, our study does not allow to elucidate the role of ESM-1 in OSA, nevertheless, a significant association with OSA and adhesion molecules suggest that ESM-1 can be considered as an early marker of vascular damage in patients with OSA. Third, the measurements were made only for once so temporal suggestions cannot be made. Fourth, we did not consider the effect of medication on circulating ESM-1 and adhesion molecules.

In conclusion, our study indicated that increasing circulating ESM-1 and adhesion molecules strongly associated with CIMT, which is a marker of early vascular injury. Therefore, strict attention to monitor circulating ESM-1 and adhesion molecules in patients with OSA is necessary for early detection of subclinical atherosclerosis, and avoiding adverse major adverse cardiovascular events.

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CRediT authorship contribution statement

Haili Sun: Methodology, Formal analysis, Investigation, Writing – original draft. **Yunhui Du:** Data curation, Visualization, Investigation. **Lichuan Zhang:** Data curation, Visualization, Investigation. **Huahui Yu:** Supervision. **Xiaolu Jiao:** Supervision. **Qianwen Lv:** Software. **Fan Li:** Validation. **Yu Wang:** Visualization. **Qiuju Sun:** Investigation. **Chaowei Hu:** Supervision. **Linyi Li:** Investigation. **Huina Zhang:** Data curation. **Zhiyong Du:** Supervision. **Yanwen Qin:** Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

All authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sleep.2022.06.015.

References

[1] Muxfeldt S, Margallo VS, Guimarães GM, et al. Prevalence and associated factors of obstructive sleep apnea in patients with resistant hypertension. Am J Hypertens 2014;27:1069–78.
[2] Peker Y, Carlson J, Hedner J. Increased incidence of coronary artery disease in sleep apnoea: a long-term follow-up. Eur Respir J 2006;28:596–602.
[3] Shah NA, Yaggi HK, Concato J, et al. Obstructive sleep apnea as a risk factor for coronary events or cardiovascular death. Sleep Breath 2010;14:131–6.
[4] Yaggi HK, Concato J, Kernan WN, et al. Obstructive sleep apnea as a risk factor for stroke and death. N Engl J Med 2005;353:2034–41.
[5] Arnaud C, Dematteis M, Pepin JL, et al. Obstructive sleep apnea, immunoinflammation, and atherosclerosis. Semin Immunopathol 2009;31:113–25.
[6] Atkeson A, Yeh SY, Malhotra A, et al. Endothelial function in obstructive sleep apnea. Prog Cardiovasc Dis 2009;51:351–62.
[7] Kent BD, Ryan S, Mcnicholas WT. Obstructive sleep apnea and inflammation: relationship to cardiovascular co-morbidity. Respir Physiol Neurobiol 2011;78:475–81.
[8] Mancini GB, Dahlöf B, Díez J. Surrogate markers for cardiovascular disease: structural markers. Circulation 2004;109(25 Suppl. 1). IV22–30.
[9] Gunnarsson SI, Peppard PE, Korcarz CE, et al. Obstructive sleep apnea is associated with future subclinical carotid artery disease: thirteen-year follow-up from the Wisconsin sleep cohort. Arterioscler Thromb Vasc Biol 2014;34:2338–42.
[10] Sarioglu N, Demirpolat G, Erel F, Kose M. Which is the ideal marker for early atherosclerosis in obstructive sleep apnea (OSA)-Carotid intima-media thickness or mean platelet volume? Medical Science Monitor International Medical Journal of Experimental & Clinical Research 2017;23:1674–81.
[11] Pak VM, Grandner MA, Pack AI. Circulating adhesion molecules in obstructive sleep apnea and cardiovascular disease. Sleep Med Rev 2014;18(1):25–34.
[12] Blankenberg S, Barbaux S, Tiret L. Adhesion molecules and atherosclerosis. Atherosclerosis 2013;170:191–203.
[13] Balta S, Mikhailidis DP, Demirkol S, et al. Endocan: a novel inflammatory indicator in cardiovascular disease? Atherosclerosis 2015;243:339–43.
[14] Kali A, Shetty KS. Endocan: a novel circulating proteoglycan. Indian J Pharmacol 2015;46:579–83.
[15] Sun HL, Zhang HN, Li K, et al. ESM-1 promotes adhesion between monocytes and endothelial cells under intermittent hypoxia. J Cell Physiol 2019;234:1512–21.
[16] Sun H, Fang F, Li K, Zhang H, Zhang M, Zhang L, Li J, Qin Y, Wei Y. Circulating ESM-1 levels are correlated with the presence of coronary artery disease in patients with obstructive sleep apnea. Respir Res 2019;20:188.
[17] Mancia G, De Backer G, Dominiczak A, et al. ESH-ESC practice guidelines for the management of arterial hypertension: ESHESC task force on the management of arterial hypertension. J Hypertens 2007;25:1751–62.
[18] Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540–5.
[19] Epstein LJ, Kristo Jr D, Sp, et al. Clinical guideline for the evaluation, management and long-term care of obstructive sleep apnea in adults[J]. J Clin Sleep Med 2009;5:263–76.
[20] Berry RB, Budhiraja R, Gottlieb DJ, et al. Rules for scoring respiratory events in sleep: update of the 2007 AASM manual for the scoring of sleep and associated events. deliberations of the sleep apnea definitions task force of the American academy of sleep medicine. J Clin Sleep Med 2012;8(5):597–619.
[21] Stein JH, Korcarz CE, Hurst RT, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008;21(2):93–111.
[22] Ramar K, Caples SM. Vascular changes, cardiovascular disease and obstructive sleep apnea. Future Cardiol 2011;7(2):241–9.
[23] Juradogamez B, Bujalance CC, Caballero BL, et al. Association of cellular adhesion molecules and oxidative stress with endothelial function in obstructive sleep apnea. Intern Med 2012;51:363–8.
[24] Sun CQ, Zhong CY, Sun WW, et al. Elevated type II secretory phospholipase A2 increases the risk of early atherosclerosis in patients with newly diagnosed metabolic syndrome. Sci Rep 2016;6:34929.
[25] Huang K, Zou CC, Yang XZ, et al. Carotid intima-media thickness and serum endothelial marker levels in obese children with metabolic syndrome. Arch Pediatr Adolesc Med 2010;164:846–51.
[26] Huang NK, Bůžková P, Matthan NR, et al. Serum non-esterified fatty acids, carotid artery intima-media thickness and flow-mediated dilation in older adults: the cardiovascular health study (CHS). Nutrients 2021;13 [undefined].
[27] Kanbay A, Ceylan E, Mustafa Ç, et al. Endocan: a novel predictor of endothelial dysfunction in obstructive sleep apnea syndrome. Clinical Respiratory Journal 2018;149:588.
[28] Kali A, Shetty KS. Endocan: a novel circulating proteoglycan. Indian J Pharmacol 2014;46:579–83.
[29] Lee HG, Choi HY, Bae JS. Endocan as a potential diagnostic or prognostic biomarker for chronic kidney disease. Kidney Int 2014;86:1079–81.
[30] Su YH, Shu KH, Hu CP, et al. Serum Endocan correlated with stage of chronic kidney disease and deterioration in renal transplant recipients. Transplant Proc 2014;46:323–7.
[31] Roudnicky F, Poyet C, Wild P, et al. Endocan is upregulated on tumor vessels in invasive bladder cancer where it mediates VEGF-A-induced angiogenesis. Cancer Res 2013;73:1097–106.
[32] Kose M, Emet S, Akpinar TS, Koccaga M, Cakmak R, et al. Serum endocan level and the severity of coronary artery disease: a pilot study. Angiology 2015;66:727–31.
[33] Balta S, Mikhailidis DP, Demirkol S, et al. Endocan-a novel inflammatory indicator in newly diagnosed patients with hypertension: a pilot study. Angiology 2014;65:773–7.