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Oxidative stress does not contribute to the release of proinflammatory cytokines through activating the Nod-like receptor protein 3 inflammasome in patients with obstructive sleep apnoea

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Abstract

Purpose The study was conducted to test the hypothesis that oxidative stress leads to the release of proinflammatory cytokines by activating the Nod-like receptor protein (NLRP)3 inflammasome in patients with obstructive sleep apnoea (OSA).

Methods The study recruited 247 participants who were divided into cases and healthy control groups. OSA patients were subdivided into four subgroups according to sex, blood pressure, body mass index (BMI), and severity of disease. No significant differences were found between cases and controls with respect to age or sex. Peripheral blood samples were collected for analysis after examination, and the serum concentrations of oxidative stress (8-isoprostane), inflammation (interleukin (IL)-18, IL-1β, IL-6, tumour necrosis factor (TNF)-α), and NLRP3 inflammasome components (NLRP3, caspase-1, and ASC) were detected by enzyme-linked immunosorbent assay.

Results The serum concentrations of both oxidative stress and proinflammatory factors were higher in OSA patients than healthy controls. Subgroup analysis also revealed significant differences according to the apnoea–hypopnea index and BMI. Additionally, correlations were identified between 8-isoprostane and proinflammatory factors (IL-1β, IL-18, and TNF-α). Multiple regression analysis suggested that sleep parameters and BMI affected inflammation. However, no differences were observed in the serum level of NLRP3 inflammasome components between patients and controls. Furthermore, stratified analysis revealed no additional differences.

Conclusions The current study suggests that oxidative stress leads to inflammation by mechanisms other than activation of the NLRP3 inflammasome in OSA patients. Furthermore, both sleep apnoea and BMI influenced the serum concentration of inflammatory mediators.

Keywords Obstructive sleep apnoea · Oxidative stress · NLRP3 inflammasome · Inflammation · 8-Isoprostane

Introduction

Obstructive sleep apnoea (OSA) is a common disorder caused by partial or complete upper airway obstruction or airflow stopping, leading to chronic intermittent hypoxaemia, hypercapnia, and sleep fragmentation. There is also a high prevalence of respiratory disorders occurring during sleep, especially for cardiovascular or arrhythmic patients [1]. These events are closely associated with a series of adverse complications, including metabolic disturbance, acute cerebral accident, cardiovascular disorders, diabetes, and sudden cardiac death [2–5]. Because of increases in obesity and ageing, the global prevalence of OSA is also rising, and it is estimated that up to 9% of 30-year-olds and 17% of 70-year-olds have moderate to severe forms of OSA [6].

Increasing evidence suggests that inflammation plays an important part in the targeted organ damage caused by OSA, and a higher level of proinflammatory cytokines is observed in OSA patients than controls [7]. Moreover, Spießhöfer et al. [5] demonstrated that OSA independently contributes to inflammation. The systemic inflammation and adverse sequelae caused by OSA, such as heart failure and reduced ejection fraction, were suggested to be ameliorated by continuous positive airway pressure (CPAP) treatment [8]. Additionally, a meta-analysis conducted by Xie et al. [9] reported that inflammatory cytokines, including tumour necrosis factor (TNF)-α,

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interleukin (IL)-6, and IL-8, were elevated in OSA patients and partially alleviated after CPAP intervention.

However, the mechanisms that initiate the increased inflammatory burden are not well understood in OSA patients. The elevation of oxidative stress in OSA has been established by several studies [10]. Through partial or complete upper airway obstruction, the apnoeic and hypopneic events that define OSA lead to a repeated process of hypoxaemia–reoxygenation, similar to ischaemia–reperfusion injury, with subsequent reactive oxygen species (ROS) generation. A study conducted by Asker et al. [11] noted that oxidative stress was significantly upregulated in OSA patients. Sánchez-Armengol et al. [12] also found a higher level of serum markers of oxidative stress, which was positively associated with disease severity. Additionally, Carpagnano et al. [13] reported that both the serum and exhaled breath condensate concentrations of 8-isoprostane were elevated in OSA patients compared with healthy controls.

Focusing on the relationship between oxidative stress and inflammation not only helps understand the underlying pathogenesis of OSA, but also contributes to the development of diagnostic, prognostic, and therapeutic measures. Activation of the Nod-like receptor protein 3 (NLRP3) inflammasome, consisting of the NLRP3 protein, the adaptor protein ASC, and caspase-1, leads to maturation of the proinflammatory cytokines IL-1β and IL-18. The increased concentration of IL-1β and IL-18 is closely linked to inflammatory disorders. ROS is crucial to activate the NLRP3 inflammasome, as shown by the fact that ROS reduction inhibits NLRP3 inflammasome activation [14, 15]. Several studies have reported the association between ROS elevation and activation of the NLRP3 inflammasome in other inflammatory diseases [16–18]. However, none have investigated whether oxidative stress leads to the release of inflammatory factors by activating the NLRP3 inflammasome in OSA patients.

In view of previous research findings, the present case-controlled study was performed to examine the hypothesis that oxidative stress causes NLRP3 inflammasome activation and the release of inflammatory factors in patients with OSA.

Methods

Study design and population

A total of 120 OSA patients and 127 healthy controls were consecutively recruited into the study between January 2014 and October 2016 from the sleep disorder centre of Zhejiang Hospital, China. Baseline clinical characteristics were collected as previously described [7]. Information on the history of arterial hypertension and hyperlipidaemia were obtained from medical records and the enrolment interview. The research protocol was conducted according to the World Medical Association Declaration of Helsinki of 1975, as revised in 1983, and was approved by the Ethics Committee of Zhejiang Hospital. All subjects provided their written informed consent.

Polysomnography and exclusion criteria

All subjects received an overnight polysomnography (PSG) test according to standardised criteria prior to joining the study [19]. Two sleep specialists (Liang Gu and Jianzong Du) reviewed the PSG results independently. Patients with a reduction of airflow ≥ 90% for ≥ 10 s were diagnosed with apnoea. Hypopnea was defined as a decrement in nasal pressure flow amplitude ≥ 30% lasting ≥ 10 s with oxygen desaturation of ≥ 3% associated with sleep arousal. The number of respiratory events per hour of sleep was calculated to acquire the apnoea–hypopnea index (AHI). An obstructive event was defined as oronasal airflow cessation and chest and abdominal respiratory movement presence during sleep. The arousal index and oxygen desaturation index (ODI) were defined according to the American Academy of Sleep Medicine Scoring Manual [20]. Patients with an AHI 5–14.9, 15–30, and > 30 events/h were divided into mild, moderate, and severe groups, respectively; those with an AHI < 5 events/h were classed as controls. Exclusion criteria were as follows: (1) chronic hypoxaemia caused by other respiratory diseases, such as obstructive pulmonary disease, interstitial pneumonia, and pulmonary embolism; (2) drug or alcohol abuse or depression; (3) maxillofacial deformities affecting ventilation; and (4) diseases affecting activation of the NLRP3 inflammasome, including rheumatic diseases, diabetes, tumours, and other inflammatory disorders.

Blood collection and analysis

Venous blood samples were collected from each patient on the morning following PSG examination. All samples were centrifuged within 30 min of collection at 3000 rpm for 15 min, and serum was stored at − 70 °C until analysis. Enzyme-linked immunosorbent assay kits for NLRP3, caspase-1, ASC, IL-18, IL-1β, IL-6, TNF-α, and 8-isoprostane (R&D Systems, Minneapolis, MN) were used to quantify serum protein levels according to the manufacturer's instructions. Serum levels of triglyceride, total cholesterol, low-density lipoprotein, and high-density lipoprotein were detected by an automated biochemical analyser (UniCel DxC 800 Synchron, Beckman Coulter, Inc., Indianapolis, IN).

Statistical analysis

SPSS software version 19.0.0 (IBM Corporation, Somers, NY) was used for statistical analysis. Continuous variables are expressed as the mean ± standard deviation. Differences

within groups were analysed using *t* tests for continuous variables and χ^2 tests for categorical variables. The potential association between 8-isoprostane and inflammation was evaluated by Spearman's correlation analysis. Multiple regression analysis was used to assess the role of confounding factors on inflammation. $p < 0.05$ was considered statistically significant.

Results

Patient characteristics

Clinical, polysomnographic, and biochemical characteristics of the study participants are outlined in Table 1. Of the 120 OSA patients, nine were classed as mild, 33 moderate, and 78 severe. Average ages were 48.88 ± 9.76 years for OSA patients and 47.37 ± 9.12 years for controls. A significant difference was identified between cases and controls in terms of body mass index (BMI), blood pressure, and parameters of PSG ($p < 0.05$).

Table 1 Clinical characteristics of study subjectives

Variables	Patients	Controls	*p* value
Demographics			
Gender			0.862
Male	96	100	
Female	24	27	
Age (years)	48.88 ± 9.76	47.37 ± 9.12	0.421
BMI	26.86 ± 3.12	22.46 ± 3.29	0.000
Normal weight	11 (10.77%)	27 (32.35%)	
Overweight	40 (89.23%)	50 (67.65%)	
SBP	133.60 ± 15.79	121.27 ± 17.79	0.001
DBP	80.90 ± 13.48	74.79 ± 12.08	0.019
AHI	39.00 ± 18.38	3.31 ± 1.09	< 0.001
$LSaO_2$	74.39 ± 12.22	95.95 ± 4.65	< 0.001
$mSaO_2$	93.55 ± 2.22	96.35 ± 3.87	< 0.001
ODI	43.18 ± 25.6	3.56 ± 1.12	< 0.001
Arousal index	24.59 ± 16.0	4.27 ± 2.16	< 0.001
TG	3.10 ± 2.84	1.83 ± 1.10	0.002
TC	5.01 ± 1.08	4.75 ± 0.95	0.224
HDL	1.07 ± 0.24	1.19 ± 0.27	0.036
LDL	2.93 ± 1.16	3.03 ± 0.89	0.633
NLRP3	365.96 ± 68.69	377.74 ± 56.07	0.102
ASC	176.31 ± 31.17	173.44 ± 41.2	0.703
Caspase-1	20.04 ± 3.42	20.99 ± 2.93	0.129
IL-18	94.13 ± 7.98	89.72 ± 12.71	0.028
IL-1β	23.00 ± 2.87	20.86 ± 2.56	0.007
IL-6	63.60 ± 8.29	59.16 ± 12.27	0.026
TNF-α	336.34 ± 40.16	321.90 ± 27.18	0.047
8-Isoprostane	143.48 ± 16.77	131.25 ± 20.75	0.001

AHI apnoea-hypopnea index, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, $LSaO_2$ lowest saturation oxygen, $mSaO_2$ mean saturation oxygen, *ODI* oxygen desaturation index, *TG* triglyceride, *TC* total cholesterol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein

Comparison of 8-isoprostane and inflammation in cases and controls

Serum levels of 8-isoprostane and inflammatory factors IL-18, IL-1β, IL-6, and TNF-α showed significant differences between cases and controls (all $p < 0.05$; Table 1). Subgroup analysis of differences according to severity of disease (AHI), sex, blood pressure, and BMI revealed significant differences in the mild vs. moderate (IL-18, $p = 0.013$; IL-1β, $p = 0.040$; TNF-α, $p = 0.045$; 8-isoprostane, $p = 0.001$), mild vs. severe (IL-18, $p = 0.003$; IL-1β, $p = 0.008$; IL-6, $p = 0.007$; TNF-α, $p = 0.000$; 8-isoprostane, $p = 0.001$), and moderate vs. severe (IL-18, $p = 0.036$; IL-1β, $p = 0.022$; IL-6, $p = 0.028$; TNF-α, $p = 0.045$; 8-isoprostane, $p = 0.030$) groups, except for IL-6 in the mild vs. moderate patients with OSA ($p = 0.127$). Furthermore, marked differences in proinflammatory factors were observed between healthy weight and overweight groups (IL-18, $p = 0.025$; IL-1β, $p = 0.011$; IL-6, $p = 0.032$; TNF-α, $p = 0.022$; 8-isoprostane, $p = 0.019$). No other significant differences were determined between subgroups ($p > 0.05$; Tables 2 and 3).

Comparison of the NLRP3 inflammasome between OSA patients and healthy controls

No differences were observed in the serum levels of NLRP3, caspase-1, or ASC between patients and controls. Furthermore, stratified analysis according to blood pressure, sex, BMI, and AHI revealed no additional differences.

Correlations between 8-isoprostane and proinflammatory factors

Correlation analyses between 8-isoprostane and proinflammatory cytokines revealed significant positive correlations between 8-isoprostane and IL-1β ($r = 0.395$, $p = 0.012$), IL-18 ($r = 0.255$, $p = 0.035$), and TNF-α ($r = 0.217$, $p = 0.039$), but not IL-6 ($r = 0.152$, $p = 0.076$) (Fig. 1).

Effect of confounding factors on the concentration of inflammatory mediators

To further identify possible factors affecting serum levels of inflammation, we conducted multiple regression analysis. This indicated that PSG parameters (AHI, ODI, arousal index, and mean saturation oxygen) and BMI significantly affected the concentrations of IL-1β, IL-18, IL-6, and TNF-α,

Table 2 Comparison of various indexes in OSA subgroups according to blood pressure, gender, and BMI

Variables	HBP vs. normal BP		*p* value	Male vs. female		*p* value	Normal weight vs. overweight		*p* value
	HBP (75)	Normal BP (45)		Male (96)	Female (24)		Normal weight (15)	Overweight (105)	
Demographics									
Age	49.84 ± 10.11	47.27 ± 9.26	0.213	48.81 ± 9.76	49.13 ± 10.44	0.937	51.20 ± 12.91	48.54 ± 9.42	0.576
BMI	27.83 ± 3.11	27.03 ± 3.09	0.218	27.98 ± 3.24	25.74 ± 1.44	0.007	23.40 ± 0.38	28.12 ± 2.84	0.000
SBP	138.92 ± 16.25	122.73 ± 15.6	0.050	134.24 ± 17.65	132.86 ± 18.45	0.861	125.20 ± 20.03	135.39 ± 17.09	0.233
DBP	85.00 ± 15.63	78.45 ± 11.24	0.110	83.97 ± 15.36	79.00 ± 10.88	0.427	75.60 ± 11.76	84.19 ± 14.82	0.227
TG	3.75 ± 3.31	2.26 ± 1.18	0.076	3.48 ± 3.02	2.02 ± 0.68	0.029	3.85 ± 4.96	3.04 ± 2.41	0.767
TC	5.24 ± 1.09	4.93 ± 1.08	0.429	5.18 ± 1.11	4.88 ± 0.99	0.500	4.38 ± 0.89	5.21 ± 1.08	0.151
HDL	1.02 ± 0.22	1.05 ± 0.28	0.721	1.03 ± 0.25	1.05 ± 0.24	0.836	1.03 ± 0.41	1.04 ± 0.22	0.959
LDL	3.10 ± 1.19	3.00 ± 1.17	0.800	2.99 ± 1.25	3.30 ± 0.88	0.520	2.35 ± 0.63	3.16 ± 1.19	0.198
AHI	40.41 ± 19.03	36.64 ± 17.63	0.537	39.13 ± 16.10	38.46 ± 27.09	0.948	33.30 ± 9.81	39.81 ± 19.25	0.466
$LSaO_2$	72.04 ± 13.36	78.43 ± 9.07	0.122	73.32 ± 12.61	79.14 ± 9.74	0.261	77.25 ± 10.44	74.06 ± 12.52	0.628
$mSaO_2$	93.67 ± 2.01	93.36 ± 2.61	0.684	93.47 ± 2.32	93.93 ± 1.84	0.627	93.50 ± 2.65	93.56 ± 2.21	0.961
ODI	47.14 ± 26.02	36.40 ± 24.28	0.217	45.17 ± 24.61	34.39 ± 30.06	0.321	31.70 ± 18.30	44.54 ± 26.21	0.350
Arousal index	26.02 ± 17.00	21.35 ± 13.67	0.428	25.51 ± 16.73	20.77 ± 12.92	0.490	15.82 ± 9.04	26.00 ± 16.52	0.191
NLRP3	369.47 ± 72.77	360.12 ± 63.32	0.683	373.34 ± 66.5	346.46 ± 73.56	0.178	383.87 ± 95.89	360.55 ± 63.95	0.098
ASC	178.42 ± 34.2	172.79 ± 26.09	0.586	176.33 ± 30.35	176.22 ± 36.53	0.993	187.65 ± 20.87	174.69 ± 32.28	0.392
Caspase-1	19.92 ± 3.01	20.26 ± 4.10	0.770	20.31 ± 3.45	19.92 ± 3.31	0.347	20.53 ± 3.32	19.98 ± 3.48	0.739
IL-18	95.65 ± 13.04	92.65 ± 17.11	0.304	95.65 ± 13.69	92.28 ± 18.83	0.458	92.21 ± 12.29	97.75 ± 18.6	0.025
IL-1β	24.29 ± 3.28	22.65 ± 3.08	0.740	21.22 ± 3.09	23.27 ± 3.58	0.410	20.10 ± 3.51	25.47 ± 3.18	0.011
IL-6	65.85 ± 13.26	61.46 ± 10.46	0.404	66.37 ± 12.21	62.4 ± 12.72	0.420	60.41 ± 13.75	66.88 ± 12.09	0.032
TNF-α	338.91 ± 48.7	330.39 ± 39.3	0.274	339.95 ± 48.35	334.63 ± 35.11	0.970	312.00 ± 55.6	344.1 ± 44.87	0.022
8-Isoprostane	146.36 ± 27.73	137.41 ± 32.43	0.914	147.92 ± 29.87	138.11 ± 27.55	0.621	121.64 ± 20.04	148.77 ± 17.67	0.019

AHI apnoea-hypopnea index, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, $LSaO_2$ lowest saturation oxygen, $mSaO_2$ mean saturation oxygen, *ODI* oxygen desaturation index, *TG* triglyceride, *TC* total cholesterol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein

Table 3 Comparison of various indexes in subgroups according to severity of disease

Variables	OSA			Mild vs. moderate		Mild vs. severe		Moderate vs. severe	
	Mild (9)	Moderate (33)	Severe (78)	*t*	*p* value	*t*	*p* value	*t*	*p* value
Demographics									
Age	50.33 ± 10.26	48.82 ± 8.58	48.73 ± 10.51	0.262	0.798	0.251	0.804	0.024	0.981
BMI	25.76 ± 1.45	27.71 ± 3.34	27.66 ± 3.14	− 0.965	0.354	− 1.022	0.316	0.460	0.964
SBP	146.67 ± 13.32	129.70 ± 19.10	134.17 ± 17.14	1.417	0.184	1.207	0.239	− 0.666	0.510
DBP	91.67 ± 9.61	82.00 ± 17.34	82.30 ± 14.00	0.905	0.385	1.114	0.276	− 0.053	0.958
AHI	10.63 ± 4.00	23.59 ± 3.77	48.78 ± 14.81	− 5.227	0.000	− 4.376	0.000	− 8.077	0.000
$LSaO_2$	87.00 ± 1.00	79.45 ± 9.13	70.50 ± 12.47	1.389	0.190	6.322	0.000	2.128	0.041
$mSaO_2$	95.67 ± 1.16	94.21 ± 2.09	92.99 ± 2.19	1.140	0.277	2.057	0.500	1.153	0.130
ODI	13.17 ± 7.21	24.18 ± 15.83	55.65 ± 22.11	− 1.147	0.274	− 3.256	0.003	− 4.233	0.000
Arousal index	15.97 ± 2.94	16.37 ± 8.41	29.29 ± 17.71	− 0.800	0.938	− 3.277	0.003	− 2.838	0.008
TG	2.17 ± 0.90	2.60 ± 1.28	3.49 ± 3.25	− 0.541	0.601	− 0.689	0.498	− 0.784	0.439
TC	5.28 ± 1.05	4.72 ± 1.05	5.25 ± 1.10	0.804	0.440	0.037	0.970	− 1.264	0.223
HDL	1.11 ± 0.23	1.00 ± 0.23	1.04 ± 0.26	0.714	0.492	0.450	0.657	− 0.366	0.717
LDL	3.59 ± 1.12	2.73 ± 1.21	3.13 ± 1.17	1.090	0.301	0.640	0.529	− 0.860	0.397
NLRP3	359.42 ± 79.33	360.25 ± 66.13	369.13 ± 71.26	− 0.019	0.985	− 0.222	0.826	− 0.354	0.726
ASC	176.39 ± 29.07	185.78 ± 21.56	172.30 ± 34.73	− 0.627	0.542	0.195	0.847	1.189	0.243
Caspase-1	19.63 ± 4.03	20.43 ± 3.63	19.93 ± 3.41	− 0.332	0.745	− 0.142	0.888	0.401	0.691
IL-18	81.80 ± 2.68	91.24 ± 5.34	96.77 ± 7.63	− 2.901	0.013	− 3.326	0.003	2.182	0.036
IL-1β	20.10 ± 1.48	22.89 ± 1.92	24.92 ± 2.86	− 2.039	0.040	− 2.873	0.008	2.166	0.022
IL-6	52.86 ± 5.47	60.16 ± 7.08	66.29 ± 7.62	− 1.638	0.127	− 2.944	0.007	2.285	0.028
TNF-α	264.64 ± 14.74	324.21 ± 44.26	349.74 ± 29.09	− 2.239	0.045	− 4.936	0.000	2.081	0.045
8-Isoprostane	116.12 ± 4.68	137.37 ± 11.37	149.24 ± 15.65	− 4.869	0.001	− 3.592	0.001	2.264	0.030

AHI apnoea-hypopnea index, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, $LSaO_2$ lowest saturation oxygen, $mSaO_2$ mean saturation oxygen, *ODI* oxygen desaturation index, *TG* triglyceride, *TC* total cholesterol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein

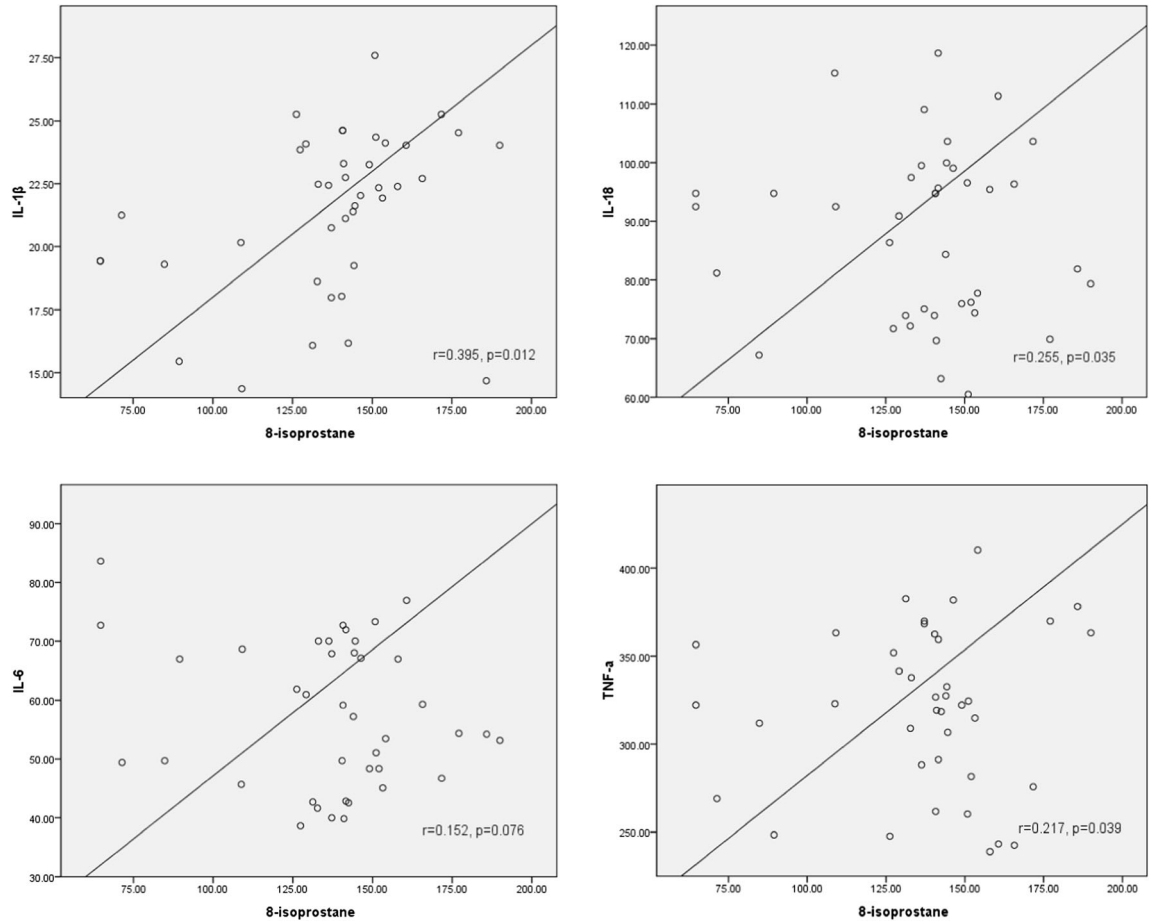

Fig. 1 The scatter plots of the correlation of 8-isoprostanes vs. IL-1β, IL-18, IL-6, and TNF-α

suggesting that sleep apnoea and obesity influence serum levels of inflammatory mediators. No associations with other variables were determined ($p > 0.05$) (Table 4).

Discussion

The current research showed that OSA patients had elevated levels of 8-isoprostane and inflammatory cytokines, including IL-18, IL-1β, IL-6, and TNF-α, compared with control groups. Further subgroup analysis revealed higher serum 8-isoprostane and inflammation in OSA patients with a higher BMI and AHI. Additionally, correlation analysis showed that 8-isoprostane was positively associated with inflammation (IL-18, IL-1β, and TNF-α). However, serum concentrations of NLRP3 inflammasome were similar between patients and controls. Multiple regression analysis suggested that sleep apnoea and obesity strongly influenced the secretion of inflammatory factors. Therefore, our current findings did not support our hypothesis that oxidative stress activates the NLRP3 inflammasome in OSA patients leading to the release of proinflammatory cytokines.

Patients with OSA are known to have increased levels of inflammation, which may contribute to adverse complications. The current study showed that this inflammation was positively associated with the severity of disease and obesity, which is in accordance with previous research. For instance, a study by Huang et al. [21] reported significantly higher levels of IL-17 and IL-23 in OSA patients compared with controls. Furthermore, Ifergane et al. [22] revealed elevated TNF-α, IL-6, and plasminogen activator inhibitor-1 in OSA patients with an AHI ≥ 15. Taken together, these findings indicated that proinflammatory cytokines are upregulated in OSA patients.

Several studies have previously confirmed that oxidative stress plays an important role in the pathogenesis of OSA [13]. 8-Isoprostanes represent a unique group of arachidonic acid derivatives produced by the free radical-catalysed peroxidation of arachidonic acid in vivo that may also be useful markers of oxidative stress [23]. They are present in the plasma of healthy individuals and are elevated by oxidative stress. Higher 8-isoprostane levels were also observed in patients with inflammatory airway diseases compared with healthy controls [24].

The present study observed higher serum levels of factors associated with oxidative stress in OSA patients with AHI and

Table 4 The effect of confounding factor on concentration of inflammation in OSA patients

Variables	IL-1β			IL-18			IL-6			TNF-α		
	β	95% CI	*p* value	*β*	95% CI	*p* value	*β*	95% CI	*p* value	*β*	95% CI	*p* value
AHI	− 0.123	(− 0.285, 0.040)	0.039	− 13.323	(− 29.838, 3.638)	0.043	6.052	(− 2.466, 14.569)	0.034	− 11.261	(− 18.887, 3.365)	0.036
ODI	0.109	(0.004, 0.215)	0.042	6.451	(− 0.302, 13.204)	0.060	8.072	(− 0.0404, 16.548)	0.049	− 6.294	(− 13.788, 1.200)	0.039
Arousal index	− 0.094	(− 0.182, − 0.007)	0.035	− 0.152	(− 0.730, 0.426)	0.582	− 0.338	(− 0.744, 0.067)	0.035	0.551	(− 0.723, 1.824)	0.369
$LSaO_2$	0.086 (− 0.068, 0.241)	0.257	0.179	(− 0.842, 1.200)	0.013	− 0.175	(− 0.891, 0.541)	0.609	− 1.391	(− 3.640, 0.858)	0.206	
$mSaO_2$	0.322	(0.064, 0.580)	0.017	2.512	(− 2.117, 7.141)	0.264	0.376	(− 2.872, 3.624)	0.808	− 0.531	(− 10.729, 9.688)	0.913
BMI	0.153	(− 0.250, 0.557)	0.037	14.047	(− 6.153, 34.247)	0.025	5.174	(− 2.072, 12.419)	0.017	2.755	(− 7.295, 12.805)	0.020
SBP	0.028	(− 0.093, 0.149)	0.635	− 0.654	(− 1.541, 0.234)	0.136	− 0.340	(− 0.962, 0.283)	0.261	− 0.251	(− 2.206, 1.704)	0.787
DBP	− 0.031	(− 0.179, 0.118)	0.669	0.687	(− 0.429, 1.804)	0.208	0.351	(− 0.433, 1.134)	0.353	0.167	(− 2.293, 2.627)	0.866
TG	0.483	(− 0.458, 1.424)	0.297	1.388	(− 4.290, 1.067)	0.024	4.269	(− 0.469, 9.007)	0.074	− 7.090	(− 21.969, 7.788)	0.324
TC	− 1.878	(− 4.846, 1.089)	0.202	− 21.132	(− 43.254, 0.991)	0.060	− 13.795	(− 29.317, 1.726)	0.138	− 2.460	(− 51.201, 46.282)	0.915
HDL	1.447	(− 7.090, 9.984)	0.727	52.088	(− 5.678, 109.854)	0.074	27.612	(− 12.918, 68.142)	0.166	− 84.255	(− 211.526, 43.015)	0.178
LDL	1.884	(− 0.775, 4.542)	0.155	10.884	(− 8.133, 29.901)	0.153	5.947	(− 7.396, 19.290)	0.355	− 6.652	(− 48.551, 35.246)	0.739

AHI apnoea-hypopnea index, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, $LSaO_2$ lowest saturation oxygen, $mSaO_2$ mean saturation oxygen, *ODI* oxygen desaturation index, *TG* triglyceride, *TC* total cholesterol, *HDL* high-density lipoprotein, LDL low-density lipoprotein

obesity that were present at disease severity-dependent levels. This was in accordance with previous publications. A study by Dinc et al. [25] demonstrated increased oxidative stress in OSA patients, which was significantly positively correlated with disease severity. Another study by Villa et al. [26] reported elevated 8-isoprostane levels in the patient group with AHI ≥ 5 events/h, compared with the control group with AHI < 5 events/h. These studies suggested that oxidative stress is upregulated in OSA patients.

Elevated oxidative stress resulting from repeated hypoxia and reoxygenation in patients with OSA is thought to trigger systemic inflammation via activation of the proinflammatory transcription factor nuclear factor kappa B [27]. It has also been reported that ROS are the strongest activator of the NLRP3 inflammasome, and an association of serum NLRP3 levels with oxidative stress was investigated in other inflammatory diseases. For example, Carta et al. [16] reported a link with cryopyrin-associated periodic syndromes, Ding et al. [17] with renal injury, and Dashdorj et al. [18] with inflammatory bowel disease. However, no studies have investigated whether the NLRP3 inflammasome is activated in OSA patients. We observed no differences in the serum level of NLRP3 inflammasome components (NLRP3, caspase-1, and ASC) between patients and controls, and this was confirmed by subgroup analysis. These results suggest that although oxidative stress leads to inflammation in OSA patients, it does not occur through activation of the NLRP3 inflammasome.

Excess weight or obesity increasing the mechanical burden of the upper airway and leading to a repetitive partial or complete collapse is thought to be an important risk factor for OSA. Obesity was also reported to be a contributing factor of inflammation in OSA patients [28]. Multivariate regression analysis in the current study suggested that both sleep apnoea and obesity had a marked effect on the level of inflammation in OSA. Taken together with other findings, this indicates that sleep apnoea in association with other factors such as obesity contribute to inflammation in OSA.

The present study has a number of limitations. The first is the relatively small sample size of 120 OSA patients. Second, a higher proportion of OSA patients had high blood pressure and were overweight compared with controls, meaning that findings were more likely to be inconclusive. Future studies should include larger sample sizes and take into account these risk factors.

In summary, the present study found that OSA patients with a higher BMI and AHI had higher serum concentrations of 8-isoprostane and inflammation, which was correlated with oxidative stress. Furthermore, sleep apnoea and obesity affected the concentration of inflammatory mediators. However, no differences in the serum level of NLRP3 inflammasome components were identified between cases and controls. We therefore speculate that oxidative stress

leads to inflammation by mechanisms other than activation of the NLRP3 inflammasome in OSA patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Research involving human participants All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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