

The Impact of Obstructive Sleep Apnea and Tobacco Smoking on Endothelial Function

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Key Words

Smoking · Obstructive sleep apnea · Biomarkers · Endothelial function

Abstract

Background: Endothelial dysfunction has been recognized to occur in the context of obstructive sleep apnea (OSA) or tobacco smoking. However, the deleterious effect on vascular function with concurrence of both conditions is largely unknown. **Objective:** To investigate whether the concurrence of OSA and smoking poses an additive detriment to endothelial dysfunction. **Methods:** Chinese men without a history of chronic medical illness were invited to complete a questionnaire including smoking pack-year exposure, polysomnography and peripheral arterial tonometry (PAT) for endothelial function. Serum 8-isoprostane, advanced oxidation protein products (AOPP) and monocyte chemo-attractant protein-1 (MCP-1) were measured. **Results:** 114 men were successfully enrolled. PAT ratio, adjusted for age and body mass index, correlated inversely with overall severity of OSA: apnea-hypopnea index (AHI), $r = -0.160$ ($p = 0.092$); oxygen desaturation index, $r = -0.214$ ($p = 0.024$); duration of oxygen saturation $<90\%$, $r = -0.219$ ($p = 0.020$); and mini-

num oxygen saturation, $r = 0.250$ ($p = 0.008$). The PAT ratio decreased with increasing pack-year group ($p = 0.018$). It was lower with concurrent smoking history and moderate-severe OSA (AHI $\geq 15/h$) compared to having one or neither factor ($p = 0.011$). Serum levels of 8-isoprostane and AOPP were positively related to severity of OSA, while MCP-1 correlated with smoking quantity. Multiple linear regression analyses showed that severity of intermittent hypoxia, MCP-1 and pack-year exposure were independent predictors of PAT ratio. **Conclusion:** While OSA, in particular intermittent hypoxemia, and tobacco smoking were independent risk factors, the concurrence of moderate-severe OSA and smoking was associated with the most severe impairment in endothelial function.

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Introduction

Both obstructive sleep apnea (OSA) and tobacco smoking are important conditions worldwide associated with increasing global health burden. While tobacco smoking is a traditional risk factor for cardiovascular morbidities, OSA is also increasingly recognized to be in-

dependently associated with adverse cardiovascular outcomes [1], with accumulating evidence supporting its causal role in endothelial dysfunction and atherosclerosis [2–4]. The prevalence of OSA, currently reported to range from 7 to 28% [5], is believed to be rising in parallel with the swapping epidemic of obesity [6]. Aside from obesity, tobacco smoking is also associated with more severe OSA and more prolonged nocturnal desaturation [7]. It is highly plausible that the concomitant presence of both OSA and tobacco smoking contributes additively to more cardiovascular complications.

Endothelial dysfunction is a precursor of atherosclerosis and cardiovascular morbidity [8]. A variety of pathologic mechanisms are believed to be linked to the endothelial dysfunction occurring in OSA, such as heightened sympathetic activity, systemic inflammation, oxidative stress, imbalanced coagulation cascade and reduction in endothelial progenitor cell activity [1, 9–12]. On the other hand, cigarette smoking is known to be proatherogenic and could thus exaggerate vascular inflammation, atheroma formation and lipid disorders in the setting of sleep apnea [13, 14]. Biomarker measurement is believed to be a potentially useful tool for cardiovascular risk assessment and stratification, and alterations in their levels shed light on the pathogenesis of cardiovascular sequelae in OSA [15]. Monocyte chemo-attractant protein-1 (MCP-1), a relatively novel biomarker mediating monocyte infiltration in atherosclerotic plaque, was found to be overly expressed in patients with severe OSA in a recent study [16]. Advanced oxidation protein products (AOPP) and 8-isoprostane, both biomarkers of oxidative stress, are being increasingly explored in various cardiometabolic disorders, including OSA [17, 18].

We hypothesize that concurrent tobacco smoking and OSA could aggravate the overall burden of vasculopathy and vascular endothelial dysfunction, as measured by digital peripheral arterial tonometry (PAT). In addition, we measured serum levels of MCP-1, 8-isoprostane and AOPP to explore whether these serum biomarkers could inform further regarding the potential pathologic mechanisms involved in endothelial dysfunction.

Methods

Subjects and Study Protocol

Consecutive Chinese men (age ≥ 18 years) undergoing overnight polysomnography (PSG) at the Ho Ting Sik Sleep Disorders Center, Queen Mary Hospital, Hong Kong were prospectively recruited from June 2008 to December 2011 in a cohort, with the aim of investigating the pathogenesis of vascular complications in OSA.

Exclusion Criteria

Exclusion criteria were history of hypertension, diabetes mellitus, hyperlipidemia on medication, atherosclerotic vascular diseases and other chronic medical diseases, including chronic obstructive pulmonary disease (COPD). Subjects on any regular medications, with a history of acute illness or hospitalization in the preceding 4 weeks or with inability to give written informed consent were also excluded. Only male subjects were included in the current study, as the PAT ratio is significantly affected by gender [19].

All enrolled subjects completed a standardized questionnaire including the Epworth Sleepiness Scale, demographic data, smoking status and relevant clinical history. Details of individual tobacco smoking history were recorded in number of pack-years and subsequently checked by research staff. Subjects were invited post hoc for lung function tests to exclude COPD.

PSG

All subjects underwent an overnight attended 16-channel PSG (Alice 5 Diagnostics System; Respironics, Murrysville, Pa., USA) in the sleep laboratory as previously described [20]. All recordings were then manually scored by a single qualified technologist according to international standard criteria by the American Academy of Sleep Medicine (AASM) [21]. Apnea was defined as cessation of air flow for 10 s or more, while hypopnea was defined as at least 30% reduction in air flow with at least 4% drop in oxygen saturation. Oxygen desaturation index (ODI) was defined as the number of episodes with at least 4% decrease in oxygen saturation per hour. The sleep study dataset was also scored using the updated AASM 2012 criteria [21]; the results are presented in the online supplementary materials (for all online suppl. material, see www.karger.com/doi/10.1159/000443527).

Anthropometric and Biochemical Measurements

Standardized anthropometric measurements were recorded as previously described [20]. Fasting venous blood was sampled at 8 a.m. the morning after PSG. Plasma glucose and lipid levels were measured as described previously [20]. Venous blood samples were centrifuged for serum and stored at -80°C before subsequent batch assays of MCP-1, AOPP and 8-isoprostane.

Measurement of MCP-1, AOPP and 8-Isoprostane

Serum MCP-1 concentrations were measured using the ELISA Development kit (eBioscience, San Diego, Calif., USA). Serum AOPP levels were measured in duplicate using a microplate reader by spectrophotometric detection method. Concentrations of serum 8-isoprostane were measured using a commercially available Enzyme Immunosorbent Assay kit (Cayman Chemical, Ann Arbor, Mich., USA). Details of the assays are available in the supplementary materials.

Reactive Hyperemia-PAT (RH-PAT)

Following overnight PSG, PAT (Endo-PAT 2000; Itamar Medical Ltd., Caesarea, Israel) was performed in a quiet, temperature-regulated room within the sleep laboratory by trained technicians according to the standard protocol. Fasting measurements were taken between 9 and 11 a.m., with at least 8 h prior abstinence from cigarette smoking, alcohol, caffeine and heavy exercise. A blood pressure cuff was placed above the elbow of the non-dominant arm for reactive hyperemia testing, while the dominant arm

Table 1. Demographic and clinical characteristics of the study subjects, divided into four groups according to smoking and OSA status (AHI <15/h or AHI ≥15/h)

	Non-OSA, non-smokers (n = 43)	Non-OSA, ever-smokers (n = 19)	OSA, non-smokers (n = 24)	OSA, ever-smokers (n = 28)
Age, years	42 (35–48)	44 (37–56)	47 (43–55)	45 (37–53)
BMI	25.8 (23.0–27.8)	24.6 (21.5–26.0)	26.0 (24.3–30.9)	27.9 (24.9–29.3)
Waist circumference, cm	88 (83–92)	84 (77–91)	91 (88–102)	95 (88–101)
Epworth Sleepiness Scale score	10 (7–13)	12 (10–16)	8 (5–12)	10 (8–13)
Mean systolic blood pressure, mm Hg	115 (108–122)	119 (106–128)	121 (116–131)	128 (121–136)
Mean diastolic blood pressure, mm Hg	69 (67–75)	75 (68–82)	78 (69–82)	77 (72–84)
Triglyceride, mmol/l	1.4 (1.1–1.9)	1.4 (1.2–1.9)	1.8 (1.2–2.7)	1.7 (1.4–2.6)
High-density lipoprotein, mmol/l	1.1 (0.9–1.3)	1.3 (0.9–1.4)	1.0 (0.9–1.3)	1.0 (0.9–1.1)
Low-density lipoprotein, mmol/l	2.9 (2.7–3.7)	3.0 (2.6–3.8)	3.0 (2.6–3.5)	2.8 (2.3–3.5)
Fasting glucose, mmol/l	5.0 (4.8–5.4)	5.0 (4.7–6.0)	5.2 (4.8–5.6)	5.3 (4.9–5.9)
AHI, events/h	4.3 (1.7–6.8)	2.3 (1.1–7.5)	32.2 (20.3–60.8)	30.6 (23.6–64.2)
Minimum oxygen saturation, %	88 (81–91)	88 (84–91)	78 (66–84)	79 (64–84)
Duration of oxygen saturation <90%, min	0.2 (0–5.1)	0.5 (0–1.6)	25.8 (4.6–101.1)	31 (4.5–81.5)
ODI, events/h	2.3 (1.0–4.7)	2.0 (0.2–5.0)	21.2 (13.2–53.9)	26.7 (18.4–59.6)
Arousal index, events/h	11.2 (7.4–15.7)	8.9 (6.6–16.1)	29.0 (22.2–44.6)	26.8 (22.1–44.8)
Sleep efficiency, %	81 (69–85)	75 (61–84)	79 (70–85)	83 (71–88)
FVC% predicted, %*	111 (103–122)	106 (100–116)	105 (97–111)	108 (99–119)
FEV ₁ % predicted, %*	109 (99–118)	106 (100–113)	101 (89–112)	107 (100–112)
FEV ₁ /FVC, %*	80 (76–83)	76 (75–83)	81 (74–85)	79 (76–84)
PAT ratio	0.70±0.31	0.60±0.28	0.57±0.30	0.41±0.40

Values represent mean ± standard deviation or median (interquartile range) depending on the data distribution.

* Spirometry was available in 37, 40 and 25 subjects, respectively.

served as the control. PAT probes were placed on the tip of each index finger. The test consisted of 5 min of baseline recording, followed by 5 min of brachial artery occlusion by cuff inflation to at least 60 mm Hg above systolic blood pressure (and not less than 200 mm Hg), and then at least 5 min of post-occlusion recording of the induced reactive hyperemia response. The PAT probes provided measurement of pulse volume changes in the index fingers of the occluded arm compared to the control arm. PAT ratios (Framingham reactive hyperemia index) were analyzed by standard automated algorithms (Itamar Medical Ltd.) as previously described [19, 22, 23] and used as the indicator of digital vasomotor response. The natural logarithm of the ratio of pulse amplitude during the 90–120 s post-deflation to that at baseline pulse in the hyperemic finger was calculated and then divided by the same ratio in the control finger. A lower PAT ratio reflects worse endothelial function as the vascular response to hyperemia relative to baseline is attenuated.

Data Analysis

Subjects with less than 1 pack-year equivalent were classed as non-smokers. Subjects who had ≥1 pack-year exposure were classified as ever-smokers (including ex-smokers if they had quit at the time of the study or active smokers otherwise). Tobacco smoking exposure was quantified in terms of pack-years and stratified into three groups (<1 pack-year, 1–10 pack-years and >10 pack-years) for purposes of stratified statistical analysis. Raw

data that did not conform to normal distribution were log- or logit-transformed. We performed t test to compare the means between two groups for continuous variables and χ^2 test to explore for any association between categorical variables. The linear relationships between PAT ratio and various parameters were examined by Pearson's correlation and partial correlation adjusted for potential confounders such as age and body mass index (BMI).

Subjects were divided into four groups according to their OSA and smoking status (non-smokers or ever-smokers): apnea-hypopnea index (AHI) <15/h non-smokers (group 1), AHI <15/h ever-smokers (group 2), AHI ≥15/h non-smokers (group 3) and AHI ≥15/h ever-smokers (group 4). General linear model analysis with adjustment for age and BMI was used to assess the difference between PAT ratios between the four groups and among different pack-year groups. A closed test procedure was used to account for the multiplicity in post hoc comparisons. Stepwise multiple regression analyses with PAT ratio as the dependent variable were performed. Independent variables that were considered included age, BMI, waist circumference, sleep indices (AHI, ODI, duration of oxygen saturation <90% and minimum oxygen saturation), pack-year group, AOPP, MCP-1 and 8-iso-prostane levels. Analyses were conducted using SPSS version 16.0. A two-sided p value <0.05 was considered to be statistically significant.

Table 2. Relationship between PAT ratio and sleep parameters (according to the AASM 2007 scoring criteria)

	PAT ratio		
	crude	adjusted for age, BMI	adjusted for age, waist
<i>AHI (events/h)*</i>			
r	-0.208	-0.160	-0.122
p value	0.026	0.092	0.202
<i>Duration of oxygen saturation <90% (min)*</i>			
r	-0.263	-0.219	-0.177
p value	0.005	0.020	0.062
<i>ODI (events/h)*</i>			
r	-0.259	-0.214	-0.165
p value	0.005	0.024	0.082
<i>Arousal index (events/h)*</i>			
r	-0.047	-0.006	0.022
p value	0.624	0.948	0.821
<i>Minimum oxygen saturation (%)#</i>			
r	0.293	0.250	0.209
p value	0.002	0.008	0.027

Data were * log-transformed and # logit-transformed before Pearson correlation (r) analysis.

Results

A total of 114 Chinese men were enrolled. 47 subjects (41.2%) were classed as ever-smokers; 52 subjects (45.6%) had an AHI $\geq 15/h$. A summary of the baseline demographics and clinical characteristics is given in table 1. 67 subjects (58.8%) were non-smokers, 22 (19.3%) ex-smokers and 25 (21.9%) active smokers. Smoking exposure, including active or past smoking, was associated with the presence of moderate-severe OSA (35.8% of non-smokers had an AHI $\geq 15/h$, as compared to 54.5 and 64.0% in ex-smokers and active smokers respectively, $p = 0.035$ by χ^2 test). Among the study cohort, the PAT ratio was significantly lower in those with AHI $\geq 15/h$ ($n = 52$) compared to those with AHI $< 15/h$ ($n = 62$) (0.48 ± 0.36 vs. 0.67 ± 0.30 , $p = 0.004$), and worse PAT ratio was associated with indices of OSA severity as continuous variables (table 2).

The PAT ratio was lower in ever-smokers ($n = 47$) compared with non-smokers ($n = 67$) (0.49 ± 0.36 vs. 0.65 ± 0.31 , $p = 0.011$) by t test, and increasing pack-year exposure was associated with a worse PAT ratio (0.65 ± 0.31 in the < 1 pack-year group, 0.54 ± 0.40 in the 1–10

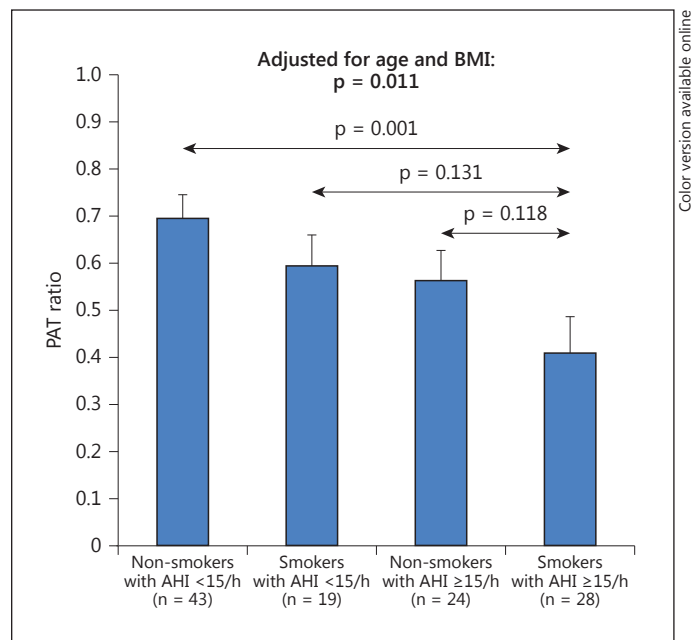


Fig. 1. Comparison of PAT ratios between four groups with different smoking exposure and OSA status by general linear model (with reference to group 4: smokers with an AHI $\geq 15/h$).

pack-years group and 0.42 ± 0.031 in the > 10 pack-years group, $p = 0.018$).

Differences in PAT ratios were statistically significant among the four groups as categorized by history of smoking and OSA status with adjustment for the effect of age and BMI ($p = 0.011$) (fig. 1). The group with both smoking exposure and moderate-severe OSA had a significantly lower PAT ratio compared to the group of non-smokers with AHI $< 15/h$ ($p = 0.001$) (fig. 1). A multiple linear regression model with PAT ratio as the dependent variable found that AHI $\geq 15/h$ and history of smoking were both independent predictors of PAT ratio ($p = 0.036$ and $p = 0.039$, respectively), and the interaction term of AHI and smoking was statistically insignificant.

Neither 8-isoprostane nor AOPP showed any association with smoking history (non-smokers versus ever-smokers). Nevertheless, the levels of 8-isoprostane and AOPP were associated with the severity of OSA (table 3), and the relationships remained significant even after adjustment for age and BMI or waist circumference for 8-isoprostane, but not for AOPP. The results remained consistent when re-analyzed using the AASM 2012 scoring criteria (see online suppl. materials). The MCP-1 lev-

Table 3. Relationship between biomarkers and sleep parameters (according to the AASM 2007 scoring criteria)

	8-isoprostane*			AOPP*			MCP-1*		
	crude	adjusted for age, BMI	adjusted for age, waist	crude	adjusted for age, BMI	adjusted for age, waist	crude	adjusted for age, BMI	adjusted for age, waist
<i>AHI (events/h)*</i>									
r	0.300	0.280	0.303	0.194	0.063	0.037	-0.059	0.049	-0.013
p value	0.001	0.003	0.001	0.040	0.513	0.690	0.536	0.611	0.891
<i>Duration of oxygen saturation <90% (min)*</i>									
r	0.252	0.224	0.250	0.195	0.044	0.020	0.004	0.140	0.065
p value	0.007	0.019	0.009	0.039	0.647	0.838	0.965	0.146	0.503
<i>ODI (events/h)*</i>									
r	0.232	0.200	0.230	0.226	0.074	0.037	-0.035	0.102	0.026
p value	0.014	0.036	0.016	0.017	0.440	0.700	0.719	0.290	0.786
<i>Arousal index (events/h)*</i>									
r	0.217	0.195	0.208	0.207	0.127	0.108	-0.072	-0.002	-0.038
p value	0.022	0.042	0.030	0.029	0.190	0.265	0.455	0.984	0.696
<i>Minimum oxygen saturation (%)[#]</i>									
r	-0.236	-0.201	-0.225	-0.140	0.039	0.050	0.061	-0.065	0.018
p value	0.012	0.036	0.018	0.142	0.684	0.600	0.527	0.504	0.850

Biomarker levels (n = 114): 8-isoprostane: 874 (638–1,137) pg/ml; AOPP: 196 (137–338) μ m; MCP-1: 286 (139–521) pg/ml. Data were *log-transformed and [#]logit-transformed before Pearson correlation (r) analysis.

el was higher in ever-smoking subjects compared to non-smoking counterparts [414 (216, 599) vs. 245 (79, 509), $p = 0.004$], but showed no association with the severity of OSA (table 3). The PAT ratio, adjusted for age and BMI, correlated significantly with MCP-1 ($r = -0.285$, $p = 0.003$), but not with AOPP or 8-isoprostane.

On multiple stepwise linear regression analysis, significant independent predictors of PAT ratio were AHI (as well as duration of oxygen saturation <90%, ODI and minimum oxygen saturation), MCP-1 level and pack-year group (for the AHI model, adjusted $R^2 = 13.5\%$) (table 4). Multiple regression analyses were further performed according to the AASM 2012 scoring criteria and yielded similar results (see online suppl. materials).

Discussion

To our knowledge, this is the first study to assess the relative impact of smoking and OSA on endothelial function in healthy subjects free of any cardiovascular comorbidities. Subjects with both moderate-severe OSA and a positive smoking history demonstrated an aggravated im-

pairment of digital endothelial function compared to those having only one or neither of the two conditions. The severity of OSA further correlated with increased oxidative stress as reflected by serum 8-isoprostane and AOPP levels, whereas the quantity of smoking exposure was associated with heightened inflammation as reflected by MCP-1. The respective associations with these biomarkers suggest potential mechanistic links to vascular complications.

PAT was used in the current study as an indicator of endothelial function, as it is non-invasive and less operator-dependent than previous methods such as Doppler ultrasonography of the brachial artery [24]. Digital pulse volume amplitude variation induced by post-occlusive reactive hyperemia (RH-PAT) reflects nitric oxide-mediated vasomotor response to ischemic challenge at the level of the small digital arterioles. Digital RH-PAT has been shown to be closely correlated with coronary blood flow response to acetylcholine infusion in subjects with no structural coronary lesions, with high sensitivity and specificity [25]. In the Framingham study, PAT ratio was observed to be inversely and significantly correlated with multiple cardiovascular risk factors including male sex, BMI, diabetes and tobacco smoking [19].

Table 4. Five multiple linear regression models of endothelial function (according to the AASM 2007 scoring criteria) with the PAT ratio as the dependent variable (n = 114)

	Estimate (SEM)	p value
<i>Model 1: AHI, adjusted R² = 13.5%</i>		
AHI*	-0.062 (0.026)	0.018
MCP-1*	-0.061 (0.028)	0.029
Pack-year group	-0.087 (0.040)	0.034
<i>Model 2: Duration of SaO₂ <90%, adjusted R² = 15.4%</i>		
Duration of SaO ₂ <90%	-0.052 (0.018)	0.005
MCP-1*	-0.058 (0.027)	0.037
Pack-year group	-0.084 (0.040)	0.038
<i>Model 3: Arousal index, adjusted R² = 5.9%</i>		
Pack-year group	-0.114 (0.041)	0.006
<i>Model 4: ODI, adjusted R² = 15.6%</i>		
ODI	-0.067 (0.033)	0.004
MCP-1*	-0.061 (0.027)	0.027
Pack-year group	-0.080 (0.040)	0.048
<i>Model 5: Minimum O₂, adjusted R² = 18.3%</i>		
Minimum O ₂	0.142 (0.040)	0.001
MCP-1*	-0.062 (0.027)	0.023
Pack-year group	-0.092 (0.039)	0.020

Sleep parameters (AHI, duration of SaO₂ <90%, arousal index, ODI or minimum SaO₂) were entered separately in the regression models to avoid colinearity. The individual sleep parameter included in each regression model is specified in italics. Independent variables with statistical significance are presented. Other independent variables included in each model: age, BMI, pack-year group, sleep parameters (AHI*, duration of SaO₂ <90%, arousal index, ODI or minimum SaO₂), MCP-1*, AOPP* and 8-isoprostane*.

SaO₂ = Oxygen saturation. *Log-transformed.

Endothelium-dependent vasodilation has been reported by a number of clinical studies to be impaired in OSA subjects and has been demonstrated by multiple randomized studies to be reversible with continuous positive airway pressure (CPAP) treatment [26–33]. The consistent impact of CPAP therapy in improving endothelial function in OSA subjects is summarized in a recent systematic review [34]. A majority of these studies utilized brachial artery ultrasound with measurement of flow-mediated dilation. Among the studies assessing PAT, a clinical trial of 46 sleep apnea subjects showed that the RH-PAT index was lower in those with severe OSA (AHI ≥30/h) as compared to those with an AHI <30/h [26]. In a cohort of over 400 men and women in Norway, sampled from a larger population-based study, only

women with sleep apnea demonstrated dose-dependent worsening of the reactive hyperemia index compared to their non-apneic counterparts of the same gender [27]. Recently, Seif et al. [23] demonstrated a non-linear relationship between OSA severity and endothelial function measured by the Framingham index in a cohort of predominantly male subjects at high cardiovascular risk. Similar to that study, we found a significant association between PAT ratio and OSA severity, in particular intermittent hypoxia, albeit the relationship weakened after adjustment for age and BMI/waist circumference as confounding variables (table 2). Of note, the relationship appears to be largely linear in our cohort of younger subjects who are free of known medical diseases.

Smokers are at higher risk of having OSA [35], which could be related to aggravated inflammation of the airway mucosal layers predisposing to upper airway collapse [36]. In line with previous studies, we found that a positive smoking history is associated with presence of moderate-severe OSA. While tobacco smoking undoubtedly leads to endothelial dysfunction and premature atherosclerosis [37], the concurrent presence of other risk factors could further increase the propensity to early formation of atherosclerotic plaques and cardiovascular complications. The effects of smoking and OSA on acute phase reactants (ceruloplasmin) and lipid profile (high-density lipoprotein) have been investigated in one study, which found that both factors were independently associated with higher levels of serum ceruloplasmin and lower levels of high-density lipoprotein, and that the presence of both conditions had a synergistic effect [13]. Our study now provides direct evidence on the adverse impact of concurrent OSA and smoking exposure on endothelial function in subjects free of other cardiovascular risk factors. An incremental impairment of endothelial dysfunction was observed in those subjects who had both moderate-severe OSA and smoking exposure, compared to those who had only one or neither risk factor (fig. 1). Interaction of smoking and sleep parameters did not achieve significance in the regression model of PAT, indicating that the smoking variables at whatever values did not affect the relationship between OSA and PAT ratio, and vice versa.

Imbalance in the redox system, as imposed by oxidative stress, is directly linked to vascular injury and atherosclerosis [38]. Intermittent hypoxia and re-oxygenation in OSA subjects result in the formation of excessive free radicals [12, 29], which in turn leads to the peroxidation of fatty acids (8-isoprostane) and proteins (AOPP). Indeed, our results consistently suggest that the indices of

severity of hypoxemia correlate most closely with endothelial dysfunction. Most studies have found an elevated blood 8-isoprostane level in OSA subjects compared to non-OSA controls [39, 40], and a randomized controlled study showed that levels were subsequently decreased after CPAP therapy for 3 months [41]. Similarly, AOPP levels were higher in untreated OSA subjects [17]. We found that elevated levels of 8-isoprostane correlated with OSA severity independent of age and BMI, and that AOPP also showed a similar correlation, but that the association became non-significant on adjustment for age and BMI. Neither oxidative stress biomarker was independently related to smoking.

The expression of MCP-1, an inflammatory mediator responsible for monocyte recruitment, is upregulated in diseased vascular endothelium and atheroma [42]. Two previous small-scale studies found elevated level of MCP-1 in OSA subjects [43, 44]. However, in contrast to the other two oxidative stress markers, MCP-1 levels showed no association with OSA severity in our study. It was reported that smokers with hypertension had higher MCP-1 levels than smokers who were normotensive [45]. It has also been suggested that serum MCP-1 levels are significantly higher in COPD patients than in healthy smokers [46]. We were therefore careful to exclude COPD subjects as a potential confounding factor in this study, and we further note that smoking contributed to elevated serum MCP-1 levels when compared to non-smoking. MCP-1 level was an independent determinant of the PAT ratio, alongside smoking quantity and OSA severity. Our findings in the different biomarker profiles of oxidative stress and inflammation, in relation to OSA and smoking respectively, speak for the diverse and interactive pathways linking to endothelial dysfunction.

This study has several limitations. Smoking exposure was based on self-reporting and could be associated with both under-reporting or recall bias. Only male subjects were included in this study, and therefore the results cannot be generalized to women. Subjects were recruited from those presenting for sleep studies, which may pose a potential bias. However, as one recent study highlighted the importance of comorbidities and medications on measurement of arterial function in OSA [47], meticulous effort was made to exclude those with comorbidities to allow more focused investigation on the impact of the two factors on endothelial function. The findings of our study should encourage physicians to target both smoking cessation and treatment of sleep apnea to attain more effective prevention of cardiovascular morbidities.

Conclusion

Our findings illustrate the additive impact of tobacco smoking and OSA on endothelial dysfunction. Effective interventions, including smoking cessation and CPAP for sleep apnea, should be implemented timely to achieve more benefits on prevention of cardiovascular complications. Further longitudinal studies and randomized trials are eagerly awaited to delineate the relationship of both risk factors and the effects of interventions on overall cardiovascular health.

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Statement of Ethics

This study was approved by the Institutional Review Board of the University of Hong Kong and the Hospital Authority Hong Kong. All subjects gave informed written consent for participation.

Financial Disclosure and Conflicts of Interest

M.S.M.I. received lecture honoraria from Philips Respironics Inc. in 2009 and 2011 and travel sponsorship from Celki Ltd. to attend WASM in 2015. M.M.S.L. received conference sponsorship from ResMed Ltd. in 2013. A.Y.K.L. received conference sponsorship from ResMed and Philips Respironics Inc. in 2013. J.C.M.L. received conference sponsorship from ResMed, Philips Respironics Inc. and Homecare Medical Ltd. in 2011, 2012 and 2013, respectively. C.K.M.H., D.C.L.L. and J.C.W.M. have no competing interests to declare.

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