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Original article

Smoking interacts with sleep apnea to increase cardiovascular risk

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Abstract

Background: Sleep apnea syndrome is an important risk factor for atherosclerosis and cardiovascular morbidity and so is cigarette smoking. In both atherosclerosis and cardiovascular disease, oxidative stress and inflammation have been implicated as underlying pathophysiologic mechanisms. We investigated oxidative stress and inflammatory markers in 70 non-smoking and smoking patients with sleep apnea.

Methods: Thirty-five sleep apnea patients aged 20–60 years who smoke 20 or more cigarettes/day and for at least 5 years were individually matched by gender, age (±5 years), body mass index (BMI; categorized as, 'normal weight', 'overweight', and 'obese'), sleep apnea severity (categorized as 'mild', 'moderate', and 'severe'), and presence of cardiovascular diseases, with 35 patients who never smoked. Blood samples were drawn after an overnight fasting for determination of lipids profile, oxidative stress markers thiobarbituric acid reactive substances, peroxides and paraoxonase-1 and inflammatory markers C-reactive protein, ceruloplasmin, and haptoglobin.

Results: Smokers showed significantly higher levels of C-reactive protein, ceruloplasmin, and haptoglobin and triglycerides and lower levels of high-density lipoprotein (HDL) cholesterol than non-smokers. There was a significant interaction effect between smoking and apnea severity on ceruloplasmin and HDL levels. Smokers with severe sleep apnea had the highest level of ceruloplasmin and the lowest level of HDL.

Conclusion: There is a synergistic effect between cigarette smoking and sleep apnea on some of the biochemical cardiovascular risk markers. Patients with severe sleep apnea who smoke are at a greater cardiovascular risk than smokers with mild-moderate sleep apnea and patients who do not smoke.

Keywords: Sleep apnea; Smoking; Inflammation; Oxidative stress

1. Introduction

Obstructive sleep apnea (OSA) is an important risk factor for atherosclerosis and cardiovascular morbidity [1]. A large number of studies have demonstrated an association between sleep apnea and major underlying mechanisms predisposing to atherosclerosis. For instance, oxidative stress [2–5], activation of inflammatory cells [6], increased levels of systemic markers of inflammation [7], adhesion between leukocytes and endothelial cells [6], and cytotoxicity of lymphocytes against endothelial cells [8,9] were elevated in sleep apnea. Additionally, decreased levels of plasma nitric oxide [10], early signs of atherosclerosis [11] and endothelial dysfunction [12,13] further support this paradigm. Treatment with nasal continuous positive airway pressure (nCPAP) that normalizes the breathing disorder in sleep was shown to attenuate most of these measures. Cigarette smoking (CS) is also considered to be a risk factor for atherosclerosis and has been shown to be associated with oxidative stress [14], activation of the inflammatory system [15] and vascular abnormalities

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[16]. Thus, sleep apnea and CS appear to activate the same fundamental mechanisms implicated in atherosclerosis. Therefore, we investigated lipids' profile, oxidative stress markers, and circulating inflammatory markers in non-smoking and smoking patients with OSA matched for age, gender, body mass index (BMI), cardiovascular comorbidity, and sleep apnea severity.

2. Methods

A case-control design was employed to investigate the relationship between CS and sleep apnea. Cases were recruited from the patient population of the Technion Sleep Medicine Center (TSMC), using the following inclusion criteria: a diagnosis of sleep apnea syndrome based on a whole-night polysomnographic finding of apnea–hypopnea index (AHI)>10 associated with characteristic symptoms, age between 20 and 60 years, and smoking at least 20 cigarettes/day for at least 5 years. Each of the cases was individually matched with a patient similarly diagnosed who reported "never smoking". Non-smokers were matched by gender, age (±5 years), BMI (categorized as, "normal weight", "overweight", and "obese"), sleep apnea severity (categorized as "mild" if AHI was between 11 and 20, "moderate," AHI between 21 and 40 and "severe" AHI>40), and presence of cardiovascular disease (a history of ischemic heart disease verified by catheterization, previous myocardial infarction or previous cerebrovascular accident). Users of vitamins or antioxidants were excluded. Importantly, to ensure that none of the cases or controls had unknown major acute inflammation, only those having C-reactive protein (CRP) levels <10 mg/L were included [17]. Both cases and controls were drawn from a population of approximately 1000 consecutive patients referred to the TSMC during 2003–2004 because of suspected sleep apnea syndrome. The diagnostic procedure in our laboratory was described in details previously [3]. The study was approved by the local ethical committee and all participants signed an informed consent before being enrolled.

2.1. Blood collection

Ten milliliters venous blood were withdrawn upon awakening at 06:00–06:30 from the sleep study and overnight fast. Blood samples were collected to pre-cooled tubes containing ethylenediaminetetraacetic acid (EDTA) (vacutainers; Beckton–Dikinson, Plymouth, UK) and were kept on ice. Plasma was separated within an hour in a refrigerated centrifuge at 1000*g* for 15 min, aliquoted and stored at −80 °C until assayed. Serum was obtained for determination of cholesterol, triglycerides (TG), low-density lipoproteins (LDL), high-density lipoproteins (HDL), creatinine, glucose, and paraoxonase-1 (PON1) activity. The lipid profile, glucose and creatinine in serum were determined with routine laboratory techniques [3]. Thiobarbituric acid reactive substances (TBARS) and lipid peroxides (PD) assays were performed as described previously [3]. Serum PON1 activity was measured spectrophotometrically at 270 nm with phenyl acetate as the substrate as previously described [3]. CRP, haptoglobin, and ceruloplasmin were measured with a high-sensitivity assay using latex-enhanced immunoenphelometry on a Behring BN II Nephelometer (Dade Behring, Newark, DE).

2.2. Statistical analysis

Data are presented as means (standard deviation [SD]) for continuous variables or percentage for categorical data. First, we compared lipids' profile and markers of oxidative stress and inflammation between smokers and non-smokers using paired *t*-tests. Second, as both sleep apnea and CS were reported to be associated with increased levels of oxidative stress and systemic inflammation, we hypothesized that a relationship between them would be manifested in a significant statistical interaction. Thus, smokers and non-smokers were divided into two subgroups with AHI below and above the median of each group AHI, and data of the resultant four groups were analyzed by analysis of the variance to determine the independent influence of smoking, apnea severity and their interaction on the biochemical variables, using Bonferroni correction for multiple comparisons. As BMI, AHI, TBARS, PD, CRP, haptoglobin, and ceruloplasmin were not normally distributed, their natural log values were used instead. Spearman rank order correlations were calculated to determine the relationship between AHI and oxidative stress and inflammatory markers. Statistical analysis was performed with the SAS software program.

3. Results

Table 1 presents the demographic and clinical history of the 35 smokers who met the inclusion criteria and individually matched non-smokers. Smokers were similar to non-smokers with respect to gender, age, BMI, AHI, percent time below 90% arterial oxygen saturation (%T<90%Sa), and the rates of cardiovascular diseases, hypertension, and diabetes. Smokers, however, had significantly higher levels of TG ($p < 0.0007$), lnCRP ($p < 0.02$), Lnceruloplasmin ($p < 0.02$) and lnhaptoglobin ($p < 0.03$) and significantly lower levels of HDL ($p < 0.0008$). Across subjects of both groups, AHI was significantly correlated with TBARS ($r = 0.45$, $p < 0.0007$) and PON1 ($r = -0.35$, $p < 0.01$) but was not correlated with PD ($r = 0.01$, ns), CRP ($r = 0.03$, ns), haptoglobin ($r = 0.03$, ns), or ceruloplasmin ($r = 0.13$, ns).

Table 1
Demographic, clinical and biochemical data for smoking ($N = 35$) and non-smoking ($N = 35$) sleep apnea patients individually matched for gender, age, BMI, apnea severity, and cardiovascular morbidities

	Non-smokers		Smokers		
	Mean	SD	Mean	SD	*p*
% Male	91.4		91.4		NS
Age	46.9	9.5	45.9	11.0	NS
BMI	30.4	4.9	30.1	5.4	NS
AHI	31.8	20.5	29.8	13.8	NS
%T< 90% (%)	14.1	22.6	14.6	23.2	NS
Medical history (%)					
IHD	22.9		20.0		NS
s/p MI	11.4		2.9		NS
CVA	0.0		5.9		NS
Hypertension	42.8		51.4		NS
Diabetes	11.4		25.7		NS
Hypercholesterolemia	42.9		25.7		NS
Asthma	2.9		2.9		NS
Kidney disease	5.7		5.7		NS
Other diseases	14.3		8.6		NS
Use of medications (%)					
Aspirin	14.3		25.7		NS
Anti-diabetic	8.5		14.3		NS
Ace inhibitor	20		34.3		NS
Statins	25.7		20.0		NS
Beta-blockers	25.7		17.1		NS
Biochemical measures					
TBARS (nmol MDA/mL)	17.9	5.2	18.9	6.6	NS
PD (nmol/mL)	944.0	119.6	970.6	153.2	NS
PON1 (U/min/ml^{-1})	80.0	14.3	80.5	15.8	NS
Cholesterol (mg/dL)	195.7	35.2	209.3	42.4	NS
TG (mg/dL)	150.8	76.9	227.2	121.7	**.0007**
HDL (mg/dL)	42.7	11.3	34.2	8.7	**.0008**
LDL (mg/dL)	122.8	34.9	131.8	38.6	NS
Glucose (mg/dL)	100.7	15.6	108.6	35.0	NS
CRP (mg/L)	2.7	2.8	4.1	3.0	**.02**
Hatoglobin (g/L)	1.4	0.5	1.6	0.5	**.03**
Ceruloplasmin (mg/dl)	26.4	4.9	32.0	13.2	**.02**

The median AHIs were 28 and 29 for the non-smokers and smokers, respectively. Table 2 presents the relevant data for smokers and non-smokers with AHI above and below the median. As analysis of variance with smoking status and the AHI group revealed a borderline statistically significant interaction in age ($p < 0.06$), we used age as a covariate for the analysis of the biochemical data. Analysis of variance adjusted for age (Table 3 and Fig. 1) revealed significant main effect of AHI group for lnTBARS ($p < 0.0004$), lnceruloplasmin ($p < 0.02$) and a borderline significant effect for lnhaptoglobin ($p < 0.06$), that were higher in patients with AHI greater than the median, and for PON1 ($p < 0.005$) that was higher for patients with AHI lower than the median. Significant main effect of smoking was found for TG ($p < 0.0008$), lnCRP ($p < 0.02$), lnhaptoglobin ($p < 0.04$) and lnceruloplasmin ($p < 0.02$) that were higher in smokers than non-smokes, and for HDL ($p < 0.0004$) that was lower in smokers than non-smokers. Significant interactions between smoking and the AHI group were found for lnceruloplasmin ($p < 0.02$) and HDL ($p < 0.04$). Pre-planned post-hoc comparisons revealed that smokers with AHI>29 had significantly higher ceruloplasmin than smokers with AHI<29 ($p < 0.006$) and than non-smokers with AHI above or below the median ($p < 0.001$ for both). Conversely, smokers with AHI>29 had lower HDL levels than both non-smokers groups ($p < .004$, for both) and than smokers with AHI<29 ($p < 0.05$). Similar trends were found for TG, CRP, and haptoglobin but without statistical significance.

4. Discussion

The major findings of the present study are that sleep apnea patients who smoke 20 cigarettes or more per day and for at least 5 years have significantly higher levels of triglycerides and the inflammatory markers CRP, ceruloplasmin and haptoglobin, and significantly lower levels of HDL, than patients of the same age and BMI

Table 2
Demographic and clinical data of smoking and non-smoking sleep apnea patients with apnea–hypopnea index (AHI) below and above the group median

	Non-smokers		Smokers	
	AHI<28	AHI>28	AHI<29	AHI>29
N	17	18	17	18
Gender (M/F)	14/2	15/1	15/1	14/2
Age	48.1±9.3	45.7±9.8	42.4±10.8	49.2±10.4
BMI	29.7±4.1	31.0±5.6	29.0±5.8	31.2±4.9
AHI	17.4±2.9	45.4±20.7	19.4±4.9	39.7±12.1
%<90% O_2Sat	11.8± 23.0	16.2±22.7	11.4±23.2	17.6±23.5
Medical history (%)				
IHD	23.5	22.2	11.8	27.8
s/p MI	5.9	16.7	0	5.6
CVA	0	0	0	11.8
Hypertension	37.5	62.5	37.5	85.7
Diabetes	5.9	11.1	11.8	38.9
Hypercholesterolemia	58.8	27.8	23.5	27.8
Use of medications	52.9	66.7	35.3	72.2

Table 3
Markers of oxidative stress and inflammation and lipids' profile in smoking and non-smoking sleep apnea patients with apnea–hypopnea index (AHI) below and above the group median

	Non-smokers		Smokers		Main effects		Inter.AHIxCS
	AHI<28	AHI>28	AHI<29	AHI>29	AHI	CS	
TBARS (nmol MDA/mL)	15.7 ± 3.4	20.8 ± 5.4	17.0 ± 5.9	20.6 ± 6.8	.004	NS	NS
PD (nmol/mL)	940.6 ± 140.9	947.2 ± 99.5	967.9 ± 171.9	973.0 ± 139.6	NS	NS	NS
PON1 (U/min/ml^{-1})	85.0 ± 15.3	75.1 ± 11.7	85.9 ± 17.3	75.3 ± 12.6	.005	NS	NS
Cholesterol (mg/dL)	192 ± 33.2	199.2 ± 37.6	214.5 ± 47.2	204.4 ± 38.0	NS	NS	NS
TG (mg/dL)	146.9 ± 74.5	154.4 ± 80.9	188.9 ± 68.0	263.4 ± 149.7	NS	.0008	NS
HDL (mg/dL)	41.1 ± 10.8	44.1 ± 11.9	36.4 ± 7.9	32.1 ± 9.2	NS	.0009	.04
LDL (mg/dL)	121.3 ± 33.5	124.3 ± 37.1	140.4 ± 37.8	122.5 ± 38.5	NS	NS	NS
Glucose (mg/dL)	100.9 ± 15.6	100.5 ± 16.0	102.4 ± 27.7	114.4 ± 40.7	NS	NS	NS
CRP (mg/L)	2.8 ± 2.6	2.6 ± 3.0	3.6 ± 3.0	4.6 ± 3.0	NS	.02	.14
Haptoglobin (g/L)	1.4 ± 0.6	1.4 ± 0.5	1.5 ± 0.4	1.8 ± 0.6	.06	.04	NS
Ceruloplasmin (mg/dl)	26.5 ± 5.3	26.4 ± 4.5	26.6 ± 4.1	37.2 ± 16.6	.02	.02	.02

and with the same severity of sleep apnea who do not smoke. Furthermore, sleep apnea severity significantly interacted with smoking to influence the plasma levels of ceruloplasmin and HDL.

Smoking had significant effects on CRP, haptoglobin and ceruloplasmin which constitute three of the markers of the acute-phase response proteins to inflammation that are primarily synthesized by the liver [18]. Specifically, CRP, the most sensitive of all acute-phase proteins, serves as a powerful predictor of future cardiovascular events among individuals with no history of cardiovascular disease [17]. Haptoglobin is an α2-glycoprotein that binds free hemoglobin stochiometrically, prevents hemoglobin-mediated iron loss and cellular and renal damage [19], having both anti-inflammatory and antioxidant properties [20]. Ceruloplasmin is a plasma protein with anti-inflammatory properties that functions as a copper transporter and is essential for normal iron homeostasis [21] but may also act as a pro-oxidant. Importantly, ceruloplasmin was found to be associated with atherosclerosis [22].

Our results demonstrating that CS is associated with elevated systemic inflammatory markers in patients with sleep apnea are in agreement with previous reports in the literature. Significant increase in serum concentration of ceruloplasmin in smokers in comparison with non-smokers was reported previously [23]. Moreover, Lind et al. [24] who reported on elevated serum ceruloplasmin and haptoglobin with increasing consumption of cigarettes, showed that the incidence of mortality and morbidity in an 18-year follow-up in the same cohort were related to the levels of the inflammatory proteins. Likewise, increased levels of CRP [25,26], haptoglobin [27], interlukine-6, and TNF-α [28] were reported in smokers in comparison with non-smokers.

The effects of sleep apnea on inflammatory markers are less clear. While there are reports on increased levels of inflammatory markers – CRP, TNF-α, and

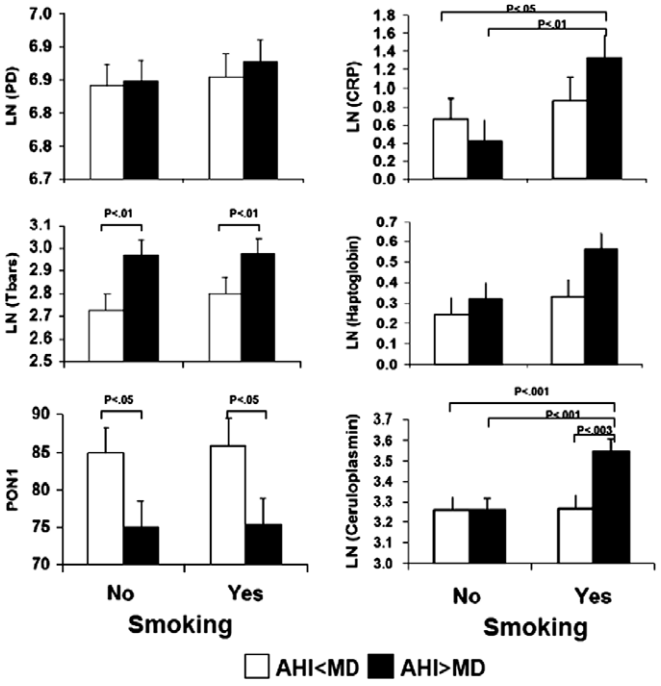

Fig. 1. Plasma levels of the oxidative stress markers TBARS, PD, and PON1 and the inflammation markers CRP, ceruloplasmin, and haptoglobin, in smoking and non-smoking sleep apnea patients with apnea–hypopnea index (AHI) above and below the group median (MD).

IL-6 – independently of smoking in sleep apnea patients [7], this was not observed in all studies [29,30]. In the present study, we found a significant effect of apnea severity only on ceruloplasmin, but this resulted mostly from elevated levels of ceruloplasmin in smoking patients with severe sleep apnea. There was also a borderline effect of AHI group on haptoglobin that can be explained in a similar way. Moreover, there were no significant correlations between AHI and the three inflammatory markers when calculated across all participating subjects, while AHI was significantly correlated with two of the three oxidative stress markers – TBARS and PON1. The finding that inflammatory markers are not correlated with sleep apnea severity is in agreement with recent results from our laboratory demonstrating that BMI rather than apnea severity is the most important determinant of the levels of the markers of systemic inflammation in sleep apnea patients [31].

Our present results demonstrate, however, significant interaction effect between sleep apnea and CS on ceruloplasmin levels with the highest levels observed in smokers with severe sleep apnea. Similar trends, but without statistical significance, were found for haptoglobin and CRP. For all inflammatory markers, the highest concentrations were found in patients with severe sleep apnea who smoked. Given the reported associations between the levels of the inflammatory markers and cardiovascular risk, this puts patients with severe sleep apnea who smoke at least a package of cigarettes per day at a higher cardiovascular risk than patients with milder sleep apnea who smoke or non-smoking sleep apnea patients.

The increased levels of TG and decreased levels of HDL in smoking patients are also in agreement with previous results [32]. Similar to the effect on cerulopasmin, here, too, we found a statistically significant interaction effect between sleep apnea and CS on HDL with the lowest HDL levels in smokers with severe sleep apnea. Although not significantly so, TG showed the same trend with the highest values in the group of smokers with severe sleep apnea. These findings also attest to the increased cardiovascular risk in smokers with severe sleep apnea.

Our present findings support the claim that CS and sleep apnea are associated with activation of similar pathophysiologic mechanisms. This is also evident from the finding that both are associated with increased levels of adhesion molecules such as VCAM-1, ICAM-I, E-selectin [33], and sE-selectin [34]. Moreover, exposure of human monocytes to cigarette extract was associated with a significant increase in adherence between the monocytes and HUVECs in vitro [35,36], similar to the increased adherence between monocytes obtained from untreated sleep apnea patients and endothelial cells [6]. Finally, both CS and sleep apnea were associated with endothelial dysfunction, a sub-clinical condition of atherosclerosis that is prognostic of future cardiovascular events [12,13,37].

The lack of effect of CS on the oxidative stress markers PD and TBARS and the antioxidant enzyme PON1 was unexpected. Cigarette smoking was reported to be associated with a production of a large amounts of free radicals, both with long and with short half-life times

[38]. Exposure of human plasma to the gas phase of cigarette smoke caused oxidative modification of LDL [39], and cigarette smoke extract decreased plasma activity of PON1 [40]. In our study, only the severity of sleep apnea significantly affected the level of the oxidative stress marker TBARS and the antioxidative enzyme PON1, which is in agreement with our previous results [3,31]. The lack of effect of sleep apnea on PD may be explained by the fact that we compared mild with severe sleep apnea patients rather than non-apneic controls used in our previous studies. One possible explanation for the lack of effect of CS on oxidative stress is that blood samples were collected in the morning after at least eight hours of smoking cessation. It is still possible that acute effects of smoking will increase oxidative stress in sleep apnea patients. Another possibility is that the added burden of oxidative stress caused by smoking was not sufficient to increase its level above what was already contributed by sleep apnea. In order to properly address these questions, matched groups of smokers and non-smokers without sleep apnea should be investigated as well. This will be an interesting research agenda for further studies.

In conclusion, our present data suggest that sleep apnea patients who smoke at least a package of cigarettes per day for at least 5 years may be at a greater cardiovascular risk than sleep apnea patients who do not smoke and than smokers with only moderate sleep apnea. Sleep apnea patients should be aware of this association.

Potential conflict of interests

P.L. is a board member and consultant of Itamar Medical and SLP that produce ambulatory devices for the diagnosis of sleep apnea syndrome.

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