RESEARCH LETTER

Activity of serum matrix metalloproteinase 9 in patients with obstructive sleep apnea

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Introduction Obstructive sleep apnea (OSA) is the most common sleep-related breathing disorder. It exposes the cardiovascular system to various noxious stimuli (such as oxidative stress, inflammation, and endothelial dysfunction), which can mediate the development or progression of cardiovascular disease (CVD). Finding a biomarker for assessing OSA burden and early identification of patients with OSA at higher risk of adverse comorbidities are some of the main priorities in sleep apnea research.

We have previously shown that serum matrix metalloproteinase (MMP)-2 activity is associated with OSA severity, and level of hypoxemia in patients with OSA.[1] However, the majority of previous studies on MMPs were focused on MMP-9.[2] MMP-9 is a proteolytic enzyme which is involved in a variety of physiological processes, but its dysregulation leads to the development of many pathological conditions, including CVD.

The MMP-9 level can be increased by oxidative stress, the hallmark of OSA. Although MMP-9 has recently been a subject of growing interest in sleep apnea research, the available studies are limited by their experimental design, small numbers of participants, and/or lack of adjustment for potential confounders.[2] None of the previous studies on MMP-9 in OSA controlled for the white blood cell (WBC) count which is the main source of MMP-9[3] and an indicator of subclinical inflammation.[4]

Therefore, in this study, we aimed to determine: 1) if serum MMP-9 activity differs in patients with different levels of OSA severity and in controls, and 2) if serum MMP-9 activity correlates with the total WBC count in OSA.

Patients and methods **Patients** Participants were recruited among patients referred for polysomnography to the Sleep Disorders Center, Saskatoon City Hospital, Saskatoon, Saskatchewan, Canada. The study was approved by the University of Saskatchewan Ethics Committee. Written consent was obtained from all participants.

The detailed inclusion and exclusion criteria as well as a thorough description of the undertaken procedures were described in our previous study.[1]

Severity of OSA was established based on the apnea-hypopnea index (AHI) categorized according to the American Academy of Sleep Medicine criteria. The clinical cutoffs for the AHI were as follows: ≥5 events/h of sleep (mild OSA), ≥15 events/h of sleep (moderate OSA), ≥30 events/h of sleep (severe OSA). Patients who were not diagnosed with OSA (AHI <5 events/h of sleep) were classified as controls. The severity of hypoxemia was established based on the oxygen desaturation index (ODI) with 3% desaturation as a cutoff.

All patients self-completed a questionnaire which included sociodemographic characteristics and medical history. Total WBC counts were analyzed at the hospital laboratory and the results were obtained from the online database.

Blood sampling and measurement of MMP-9 activity Blood samples have been collected from participants in the morning after polysomnography. Gelatin zymography, used for the measurement of MMP-9 activity, was performed as previously described.[1]

Statistical analysis Descriptive statistics of participant baseline characteristics were calculated using the χ^2 test and one-way analysis of variance with post hoc Scheffe tests. The comparisons of MMP activity between groups were performed using the Mann–Whitney test and the Kruskal–Wallis test (with the Bonferroni correction) as MMP-9 distribution did not meet

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the assumption of normality. Following the descriptive analyses, multiple regression analysis was completed to adjust for potential confounders. The outcome (MMP-9 activity) was transformed to its natural logarithm. First, the univariable analysis was conducted to determine the candidate variables for the multivariable models.

In our analyses, we fitted 2 multivariable models (a parsimonious model and a full model) separately for each sleep indicator (AHI and ODI) to adjust for potential confounders. The variable selection process was analogical to the one described in our previous study.[1] The models with the AHI as the primary predictor are labeled as Model 1, whereas the models with the ODI as the primary predictor, as Model 2.

Data analysis was performed using SPSS version 25 (IBM Corp, Armonk, New York, United States). Analyses were 2-tailed and a *P* value of less than 0.05 was considered significant.

Results **Baseline characteristics of study participants** The study groups did not differ in terms of sex (46.2%, 46.5%, 61.8%, 66% of participants were men in control, mild, moderate, and severe OSA groups, respectively; $P = 0.18$) and height distribution (mean, 171.7 cm; $P = 0.87$). The participants with more severe OSA tended to have greater body mass (mean [SD], 113.1 [34.4] kg) than those with less severe disease (mean [SD], 91.7 [15.9] and 96.9 [19.6] kg for mild and moderate OSA groups, respectively) or the controls (mean [SD], 83.6 [15.4] kg) ($P < 0.001$). The prevalence of CVD and hyperlipidemia increased along with OSA severity. Severe OSA cases had the highest prevalence of CVD (63.8%) while in both mild and moderate OSA groups, the prevalence of CVD was approximately 48%, and in the control group, it was 19.2% ($P = 0.004$). There were no significant differences between the groups in terms of prevalence of obesity, inflammatory airway diseases, connective tissue diseases, use of statins, and smoking status. There were differences in the total WBC count between the groups ($P = 0.04$). The total WBC count was higher in the severe OSA group than in controls (7.1 [1.8] $\times 10^3/\mu l$ vs 5.8 [1.5] $\times 10^3/\mu l$).

Serum MMP-9 activity There was no difference in serum MMP-9 activity between OSA participants and controls (mean [95% CI], 2381 [1533–3229] and 1800 [1037–2564] arbitrary units, respectively; $P = 0.78$). MMP-9 activity differed between the study groups ($P = 0.04$). MMP-9 activity in serum was lower in participants with moderate OSA compared with those with severe and mild OSA.

Simple linear regression showed associations with MMP-9 activity in serum for AHI (β [SE] = 0.008 [0.004]; $P = 0.04$), ODI (β [SE] = 0.007 [0.003]; $P = 0.03$), total WBC count (β [SE] = 0.356 [0.067]; $P < 0.001$), age (β [SE] = –0.017 [0.009]; $P = 0.049$), and smoking status (β [SE] = 0.828 [0.379]; $P = 0.03$ for current smoker group). Serum MMP-9 activity was associated with the WBC count, independently of AHI / ODI, age, sex, body mass index (BMI), CVD, and smoking status (the parsimonious model), and additionally diabetes, inflammatory airway diseases, connective tissue diseases, hyperlipidemia, and statin use (the full model) (TABLE 1).

Discussion There has been growing evidence of associations between MMP-9 and OSA, making MMP-9 a potential candidate biomarker of adverse OSA comorbidities. It has been previously shown that the peripheral level of MMP-9 was increased in participants with OSA, and the increase was relevant to OSA severity[2,5] However, any of the previous studies on MMP-9 in OSA did not control for the WBC count. Our results are in accordance with the study carried out in a healthy population suggesting that the main source of circulating MMP-9 are WBCs.[3]

Causation cannot be inferred from the results of any observational study; however, inflammation (measured as the WBC count)[4] seems to be the putative mechanism contributing to changes in circulating MMP-9 activity in OSA. MMP-9, unlike MMP-2, is highly inducible by inflammatory processes. The association between OSA severity and the total WBC count has been previously shown.[6] Further, the WBC count (or subclinical inflammation it represents) could be on a causal pathway linking OSA severity with MMP-9 levels. However, multicollinearity between AHI / ODI and WBC was not found in our analysis (variance inflation factor values for AHI and WBC were 1.15 and 1.36, respectively), thus both variables could be included as predictors in the multivariable models. Since MMP-9 was associated with WBCs independently of other predictors, it is important to include WBC in the future studies on MMP-9 in OSA.

It is possible that inflammation mediated via MMP-9 could be a link between OSA and CVD. MMP-9 causes a degradation of extracellular matrix, by which it may promote the atherosclerotic plaque instability and enhance the infiltration of inflammatory cells. Moreover, MMP-9 release is an indicator of leukocyte activation. Activated leukocytes are crucial contributors to atherogenesis, which consequently can lead to other cardiovascular complications. The release of MMP-9 from leukocytes can be influenced by oxidative stress related to intermittent hypoxia in OSA. Interestingly, MMP-9 transcription can be upregulated in response to hypoxia-inducible factor 1α,[7] which was recently proposed as a promising diagnostic marker of OSA.[8]

It has been shown that plasma MMP-9 was significantly associated with C-reactive protein (CRP; indicator of inflammation) and BMI, but not with the AHI.[9] The positive correlation between CRP and serum MMP-9 levels was also shown[10] (however, MMP-9 was associated with AHI). Except for the 2 studies mentioned above in which CRP was an additional variable in the analysis,[9,10] and the studies in

TABLE 1 Results from multivariable linear regression analysis for the total white blood cell count with serum matrix metalloproteinase 9 as the outcome

Variable[a]		Parsimonious model				Full model			
		Model 1[b]		Model 2[b]		Model 1[b]		Model 2[b]	
		Parameter estimate β (SE)	*P* value	Parameter estimate β (SE)	*P* value	Parameter estimate β (SE)	*P* value	Parameter estimate β (SE)	*P* value
WBC count, × 10^3/μl		0.310 (0.083)	<0.001	0.302 (0.085)	0.001	0.312 (0.086)	<0.001	0.306 (0.088)	0.001
Risk factor of primary interest, n/h	AHI	0.007 (0.005)	0.16	–	–	0.007 (0.005)	0.18	–	–
	ODI	–	–	0.005 (0.004)	0.26	–	–	0.005 (0.005)	0.32
Age, y		–0.013 (0.011)	0.24	–0.012 (0.011)	0.28	–0.011 (0.012)	0.36	–0.010 (0.012)	0.41
Female sex (reference: male)		–0.255 (0.247)	0.31	–0.281 (0.247)	0.26	–0.256 (0.264)	0.34	–0.272 (0.266)	0.31
BMI, kg/m^2		–0.01 (0.024)	0.67	–0.003 (0.024)	0.91	–0.009 (0.025)	0.72	–0.001 (0.025)	0.96
CVD[c]: yes		0.002 (0.268)	0.99	0.007 (0.27)	0.98	0.056 (0.293)	0.85	0.056 (0.294)	0.85
Smoking status (reference: never smoker)	Former smoker	–0.063 (0.263)	0.81	–0.101 (0.265)	0.71	–0.087 (0.285)	0.76	–0.129 (0.288)	0.66
	Current smoker	0.156 (0.464)	0.74	0.159 (0.467)	0.73	0.114 (0.497)	0.82	0.105 (0.5)	0.83
Type 2 diabetes: yes		–	–	–	–	0.087 (0.407)	0.83	0.033 (0.409)	0.94
Inflammatory airway diseases[d]: yes		–	–	–	–	–0.086 (0.303)	0.78	–0.101 (0.304)	0.74
Connective tissue diseases[e]: yes		–	–	–	–	–0.13 (0.418)	0.76	–0.15 (0.422)	0.72
Hyperlipidemia: yes		–	–	–	–	–0.056 (0.418)	0.89	–0.032 (0.42)	0.94
Use of statins: yes		–	–	–	–	–0.144 (0.474)	0.76	–0.118 (0.476)	0.8

a No is the reference category for yes/no binary variable

b Model 1, with the apnea-hypopnea index as the primary predictor; Model 2, with oxygen desaturation index as the primary predictor. Both indexes were introduced into the models as continuous variables

c Cardiovascular disease was defined as arterial hypertension, coronary artery disease, heart failure, stroke, and atrial fibrillation

d Inflammatory airway disease was defined as asthma and chronic obstructive pulmonary disease

e Connective tissue disease was defined as ankylosing spondylitis, osteoarthritis, and rheumatoid arthritis

Abbreviations: AHI, apnea-hypopnea index; BMI, body mass index; CVD, cardiovascular diseases; ODI, oxygen desaturation index; WBC, white blood cell

which patients with systemic or respiratory inflammation were excluded,[11,12] the authors did not address the potential confounding of inflammation in their analysis on circulating MMP-9 in patients with OSA.

Apart from controlling for WBC count, our study differs from others in several other aspects: a larger sample size, in-laboratory polysomnography performed in each participant, classification of OSA according to the American Academy of Sleep Medicine criteria, and controlling for potential confounders including CVD, diabetes, smoking status, connective tissue diseases, hyperlipidemia, and use of statins.

We need to acknowledge that the study groups were heterogenous with respect to age, BMI, and prevalence of CVD. However, this reflects the situation commonly observed in the clinical setting which arguably made the results more applicable. Moreover, despite statistical adjustment for a number of potential confounders, due to observational design of our study, there is a possibility of residual confounding.

In conclusion, our study showed a significant association between WBCs and MMP-9 activity in OSA. It suggests that the WBC count is an important factor affecting MMP-9 changes in OSA. WBC count should be monitored in future OSA studies of MMP-9, as both oxidative stress and inflammation (the important hallmarks of OSA) could affect WBC count/activation and in turn lead to changes in MMP-9 activity. Larger studies with longitudinal designs are needed to confirm our results and to study the potential use of MMP-9 as a biomarker of CVD in OSA.

ARTICLE INFORMATION

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CONFLICT OF INTEREST RS reports grants from AstraZeneca and a grant from the Canadian Sleep and Circadian Network during the conduct of the study as well as grants from GSK, BI, and Roche outside the submitted work. AstraZeneca had no involvement in this study. Other authors declare no conflict of interest.

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