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Effects of the presence of hypertension on the relationship between obstructive sleep apnoea and sleepiness

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SUMMARY Obstructive sleep apnoea (OSA) plays a significant role in increasing blood pressure. Significant decreases were reported in blood pressure of hypertensive OSA patients with sleepiness who underwent continuous positive airway pressure (CPAP) treatment, but not in non-sleepy hypertensive OSA patients. More recently, however, significant decreases in blood pressure in non-sleepy hypertensive OSA patients following CPAP were shown. Effects of sleepiness on hypertension in OSA patients have been investigated, but not the effects of hypertension on sleepiness in OSA patients. We investigated the relationships between hypertension and sleepiness in patients with OSA. We analysed data on 275 middle-aged male subjects from a cross-sectional epidemiological health survey. We measured blood pressure and sleep duration objectively using an actigraph for 7 days and the respiratory disturbance index (RDI) with a type 3 portable device for 2 nights, and assessed sleepiness using the Epworth Sleepiness Scale (ESS). The RDI correlated significantly with ESS scores in the 88 hypertensive subjects ($r = 0.33$, $P = 0.0024$), but not in the 187 non-hypertensive subjects ($r = -0.01$, $P = 0.91$). Short sleep duration correlated significantly with ESS scores in both groups. Both the RDI and short sleep duration were related independently to sleepiness in only hypertensive subjects. Furthermore, the RDI was related negatively significantly to sleep duration in hypertensive subjects. Although short sleep duration was related significantly to sleepiness in both groups, hypertension may be important for the sleepiness in OSA patients. Detailed mechanisms of the difference in the relationship between sleepiness and the severity of OSA with or without hypertension should be studied further.

KEYWORDS hypertension, sleep apnoea, sleep duration, sleepiness

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INTRODUCTION

A large body of work has shown that obstructive sleep apnoea (OSA) plays a significant role in increasing blood pressure (Garvey *et al.*, 2009). Systemic hypertension in OSA, often underdiagnosed, is a large clinical problem, as it may increase the cardiovascular risk of OSA. Currently, continuous positive airway pressure (CPAP) treatment is the first-line treatment of OSA. However, there has been some conflict over whether CPAP can reduce systemic blood pressure in patients with OSA (Robinson *et al.*, 2004). There may be subgroups of patients with OSA who benefit from CPAP treatment in terms of blood pressure control (Robinson *et al.*, 2004). From this viewpoint, several studies suggested that OSA patients with prior daytime sleepiness were likely to experience a lowering of blood pressure after CPAP treatment (Barbé *et al.*, 2001; Robinson *et al.*, 2004, 2006, 2008), while Barbé *et al.* (2010) recently reported a decrease in blood pressure in non-sleepy hypertensive patients with OSA after CPAP treatment. Thus, the effects of sleepiness on hypertension in OSA patients have been investigated, but the effects of hypertension on sleepiness in these patients have not been studied.

We hypothesized that hypertension and sleepiness in OSA patients have a significant relationship. To investigate this relationship, using information from a cross-sectional epidemiological health survey in a group of middle-aged male employees in Japan (Chin *et al.*, 2010; Nakayama-Ashida *et al.*, 2008), we analysed the relationship between sleepiness and OSA in subjects with or without hypertension, taking into account objectively measured sleep duration by actigraph. Accurate measurements of sleep duration are important in investigating the effects of hypertension on sleepiness in OSA patients, as sleep duration has been shown to be an important factor for sleepiness in OSA patients (Vakulin *et al.*, 2009). Although most epidemiological studies used subjective self-reported sleep duration, such self-reports may be inaccurate and cause misclassification of sleep duration (Lauderdale *et al.*, 2008; Van Den Berg *et al.*, 2008). Thus, actigraphic measurements used in this study will ensure greater accuracy in sleep duration data than in previous reports.

METHODS

Subjects

Study subjects were male employees of an urban wholesale company in Japan, as reported previously elsewhere in detail (Chin *et al.*, 2010; Nakayama-Ashida *et al.*, 2008). Of the 322 male employees who were first entered into the study (Nakayama-Ashida *et al.*, 2008), 275 were investigated further to examine the relationship between OSA and metabolic syndrome (Chin *et al.*, 2010). In the present study, we analysed data on those 275 subjects regarding the relationship between sleepiness, sleep duration and OSA with or without hypertension. The study protocol was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee. Written informed consent was obtained from all subjects.

Measurements of weight, waist circumference and blood pressure

Trained research staff performed measurements of weight, waist circumference and blood pressure. Blood pressure was measured seven times using OMRON HEM-759P (Kyoto, Japan) after subjects were seated and rested for 1–2 min, with the average of the last three measurements used for the analyses. Individuals who had systolic blood pressure readings of more than 140 mmHg, diastolic readings of more than 90 mmHg, a history of a diagnosis of hypertension before the study measurements or were currently using anti-hypertensive medications were defined as having hypertension (Joint National Committee, 1993).

Home monitoring of sleep

We determined sleep duration by actigraphy (Littner *et al.*, 2003), in conjunction with a sleep diary. Each subject was asked to wear an actigraph (Actiwatch AW-Light; Mini Mitter, Brend, OR, USA) (Littner *et al.*, 2003) for 7 days to estimate sleep–wake time, and a type 3 portable monitor (PM) (Morpheus; Teijin, Tokyo, Japan, which is the same as Somté; Compumedics, Vic., Australia) (Chesson *et al.*, 2003), an alternative for polysomnography in the diagnosis of OSA (Kushida *et al.*, 2005), for 2 nights at home.

Actigraph and PM data analysis

The respiratory disturbance index (RDI: number of apnoea and hypopnoea episodes per hour of the analysed time) was calculated from both the actigraphy and PM. Records of the PM were inspected visually and scored by at least two medical doctors specialized in respiratory medicine. Apnoea is defined as the cessation of breathing for at least 10 s and hypopnoea as a more than 50% reduction in the amplitude of nasal pressure or respiratory effort associated with more than 3% reduction in oxyhaemoglobin saturation for at least 10 s. Apnoea and hypopnoea were scored while blinded to other information, except for sleep–wake time by actigraphy. Data without oxygen saturation values and illegible recordings were excluded from analysis. Data for <2 h were also excluded, because the Medicare guidelines require at least 2 h of documented sleep time. When data from both recorded nights were available, records from the second night were analysed further.

Assessment of sleepiness

The modified Japanese version of the Epworth Sleepiness Scale (ESS) was used to assess subjective sleepiness (Takegami *et al.*, 2009). A separate sleep diary was also completed during the survey period (Nakayama-Ashida *et al.*, 2008).

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Unpaired *t*-tests were used to compare the backgrounds

between the hypertensive and non-hypertensive subjects and between treated and untreated hypertensive subjects. Relationships between the two sets of data were analysed by Pearson's correlation coefficient tests. Multiple regression analyses were performed to identify those variables that could best predict sleepiness by the ESS scores using the RDI and sleep duration as explanatory variables. *P*-values < 0.05 were considered to be statistically significant. All analyses were performed using Statview 5.0 (SAS Institute, Inc. Cary, NC, USA).

RESULTS

Characteristics of the subjects

Characteristics of the subjects are presented in Table 1. A total of 88 subjects (32.0%) had hypertension; among these, 25 (28.4%) were being treated with anti-hypertensive medicine. From our examination of the subjects, we could not identify any subject who took medicine during the daytime that could affect sleepiness. RDI, sleep duration and ESS scores of the hypertensive subjects treated with anti-hypertensive medicine and the untreated hypertensive subjects did not differ significantly ($P = 0.20$, 0.061 and 0.87, respectively). A total of 161 subjects (58.5%) had OSA ($5 \leq$ RDI). The hypertensive subjects were older ($P < 0.001$) and had a higher RDI than those without hypertension ($12.2 \pm 12.0\ h^{-1}$ versus $9.3 \pm 10.0\ h^{-1}$, $P = 0.034$), while the body mass index (BMI), waist circumference, sleep duration and ESS scores did not differ between the two groups.

Among the 88 hypertensive subjects, 59 (67.0%) had OSA ($5 \leq$ RDI) and 23 (26.1%) had moderate-to-severe OSA ($15 \leq$ RDI). Among the 187 non-hypertensive subjects, 102 (54.5%) had OSA and 35 (18.7%) had moderate-to-severe OSA.

Relationships between sleepiness and OSA, and between sleepiness and sleep duration

Fig. 1 shows the relationships between sleepiness estimated by ESS scores and OSA in the hypertensive and non-hypertensive groups. The RDI was correlated significantly but weakly with ESS scores in the hypertensive subjects [correlation coefficient (r) = 0.33, $P = 0.0024$] but not in the non-hypertensive subjects ($r = -0.01$, $P = 0.91$) (Table 2a). With regard to relationships between sleepiness and sleep duration in the hypertensive and non-hypertensive groups, as well as in all subjects taken together, although sleep duration was correlated significantly with ESS scores in the hypertensive subjects ($r = -0.30$, $P = 0.0050$) this relationship was also seen in the non-hypertensive subjects ($r = -0.18$, $P = 0.014$) (Table 2a) and in the entire subject population ($r = -0.22$, $P < 0.001$). Lastly, multiple regression analyses to predict sleepiness estimated by ESS scores were performed using the RDI and sleep duration as explanatory variables in the hypertensive and non-hypertensive groups. These analyses revealed that both the RDI and sleep duration explained ESS scores independently only in the hypertensive group [contribution rate (R^2) = 8.6% and 6.9%, respectively] but, in contrast, sleep duration alone explained the ESS scores independently in the non-hypertensive group ($R^2 = 3.2\%$) (Table 2b,c).

Relationship between RDI and sleep duration

We examined further the relationship between RDI and sleep duration. As a whole, RDI and sleep duration had a negative relationship ($r = -0.19$, $P = 0.0017$). As shown in Fig. 2, in the hypertensive group the RDI was related negatively significantly to sleep duration ($r = -0.29$, $P = 0.0056$), but there was no significant relationship in the non-hypertensive group ($r = -0.13$, $P = 0.084$) (Table 2a).

DISCUSSION

In the present cross-sectional epidemiological survey in an urban company in Japan, we compared the relationships between sleepiness and OSA in subjects with or without hypertension. We found that short sleep duration was related to sleepiness both in the hypertensive and non-hypertensive subjects, but that the RDI was also related significantly to sleepiness independently of sleep duration only in the hypertensive subjects. In addition, we observed an inverse

Table 1 Characteristics of the subjects

	All subjects	*Subjects with hypertension*	*Subjects without hypertension*	P-*value*
Number of subjects (%)	275	88 (32.0)	187 (68.0)	
Age (years)	44 ± 8	48 ± 7	42 ± 8	< 0.001
BMI ($kg\ m^{-2}$)	23.9 ± 3.1	24.2 ± 3.6	23.8 ± 2.9	0.42
Waist circumference (cm)	83.6 ± 8.5	85.0 ± 9.3	83.0 ± 8.0	0.075
RDI (h^{-1})	10.2 ± 10.7	12.2 ± 12.0	9.3 ± 10.0	0.034
Sleep duration (h)	6.0 ± 0.8	6.0 ± 0.8	6.0 ± 0.8	0.51
ESS score	8.2 ± 4.3	7.9 ± 4.3	8.3 ± 4.3	0.43
Systolic blood pressure (mmHg)	129 ± 14	143 ± 12	122 ± 8	< 0.001
Diastolic blood pressure (mmHg)	81 ± 11	91 ± 9	76 ± 8	< 0.001

Values are presented as mean ± standard deviation or n (%) unless stated otherwise. BMI, body mass index; ESS, Epworth Sleepiness Scale; RDI, respiratory disturbance index.

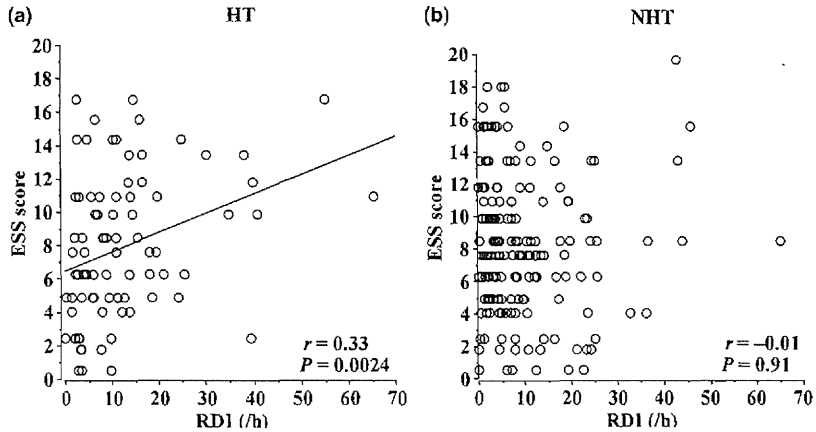

Figure 1. Relationship between Epworth Sleepiness Scale scores and respiratory disturbance index in the subjects with hypertension (HT) (a) and in the subjects without hypertension (NHT) (b). ESS, Epworth Sleepiness Scale; RDI, respiratory disturbance index.

Table 2 (a) Univariate analyses of correlation coefficient among sleepiness assessed by ESS scores, the severity of OSA assessed by RDI and sleep duration. Multiple regression analyses to predict sleepiness assessed by ESS scores in subjects (b) with hypertension (n = 88) and (c) without hypertension (n = 187)

	Subjects with HT	*Subjects without HT*
(a)		
ESS score and RDI	0.33*	−0.01
ESS score and sleep duration	−0.30*	−0.18*
RDI and sleep duration	−0.29*	−0.13

	β	γ	R^2 (%)
(b)			
RDI (h^{-1})	0.26	0.33	8.6
Sleep duration (h)	−0.23	−0.30	6.9
Cumulative R^2			15.5
(c)			
RDI (h^{-1})	NA	NA	NA
Sleep duration (h)	−0.18	−0.18	3.2
Cumulative R^2			3.2

ESS, Epworth Sleepiness Scale; OSA, obstructive sleep apnoea; RDI, respiratory disturbance index; HT, hypertension; NA, not applicable. In (a), data are expressed as correlation coefficient and *$P < 0.05$; in (b and c), β, standard regression coefficient, r, correlation coefficient and R^2, contribution rate.

relationship between the RDI and sleep duration in the hypertensive subjects.

In the present study, insufficient sleep was an important determinant of sleepiness regardless of the existence of hypertension. Pack *et al.* (2006) also reported that among commercial driver's licence holders, chronic short sleep duration rather than sleep apnoea was a risk factor for sleepiness. In addition, a recent report showed that OSA patients were more vulnerable than healthy subjects to the effects of sleep restriction (Vakulin *et al.*, 2009). Indeed, the patients with OSA made more mistakes in the driving simulation test following sleep restriction than the subjects without OSA (Vakulin *et al.*, 2009). Thus, adequate sleep duration would be necessary for patients with OSA as well as for subjects without OSA. In this study, sleep duration was measured in home settings. Although, in many studies, sleep duration was assessed by overnight polysomnography in laboratory settings, in that situation subjects tended to sleep more poorly than at home (Kapur *et al.*, 2005). In addition, sleep duration was measured by actigraph for a week in this study. Although most epidemiological studies use self-reported sleep duration, which may not be accurate, differences between actigraph-measured and subjective reported sleep durations were detected, and objectively measured sleep duration is recommended (Lauderdale *et al.*, 2008; Van Den Berg *et al.*, 2008). Thus, in our study, using both actigraphy and a sleep diary under usual circumstances for a week, we could examine sleep duration accurately to explore the importance of adequate sleep.

This study also showed that the significant but weak relationship between RDI and sleepiness was dependent on the existence of hypertension. Despite some conflicting results in interventional trials of treatment of OSA and hypertension, pre-treatment sleepiness is an important factor in determining a reduction in blood pressure after CPAP treatment (Robinson *et al.*, 2004). Thus, it is suggested that there is some relationship between sleepiness in OSA and presence of hypertension. However, in this study there was a significant difference in age between subjects with and without hypertension. Therefore, including objective assessment of sleepiness, such as with the multiple sleep latency test (MSLT), further studies would be needed to investigate the difference in sleepiness in OSA subjects with and without hypertension.

We noticed an inverse relationship between the RDI and sleep duration. There has not been sufficient evidence to suggest that patients with OSA sleep more or less than average, although short sleep duration or sleep fragmentation is reported to be associated with obesity (Knutson *et al.*, 2007; Van Den Berg *et al.*, 2008), a main risk factor for OSA. Interestingly, this relationship was observed with actigraphically determined sleep duration, as in our study, and was undetectable with self-reported sleep duration (Van Den Berg *et al.*, 2008) which indicated the advantage of our study, which included actigraph data.

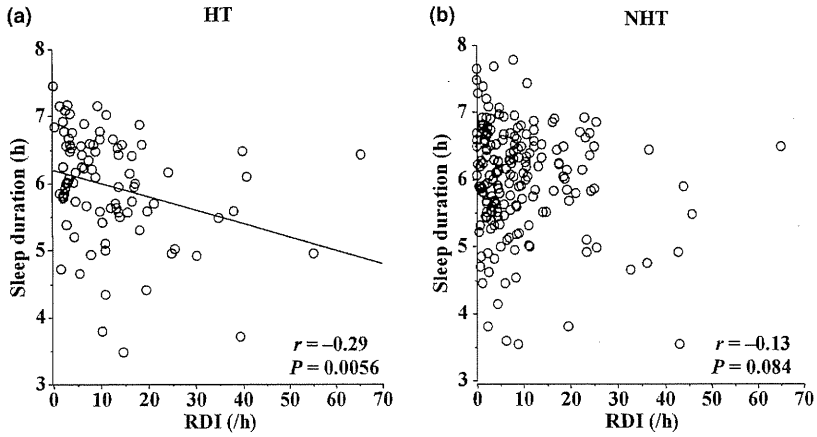

Figure 2. Relationship between respiratory disturbance index and sleep duration in the subjects with hypertension (HT) (a) and in the subjects without hypertension (NHT) (b). RDI, respiratory disturbance index.

The prevalence of males with moderate-to-severe OSA in this study was 21.1%, which is equivalent to the data from the Sleep Heart Health Study showing a prevalence of 25.0% (Baldwin *et al.*, 2004). Among the hypertensive and non-hypertensive subjects, 26.1% and 18.7% had moderate-to-severe OSA, respectively, which is equivalent to findings of another study (Hla *et al.*, 1994). In addition, the prevalence of hypertension among Japanese males in their 40s was 35.5% in 2006 (Ministry of Health, Labor and Welfare, 2006; updated 2008), which is similar to that in this study (32.0%). Therefore, our data are applicable to reflect the current background in Japan.

There are some limitations to this study. First, this was a cross-sectional study and it was difficult to exclude completely the influence of confounders such as age, obesity, and medication, etc., as mentioned above. Secondly, we did not perform polysomnography, partly because we wanted to perform the study under usual lifestyle conditions. However, the interscorer and night-to-night reliability of the RDI were excellent (inter-class correlation coefficients of 0.98 and 0.95, respectively) (Nakayama-Ashida *et al.*, 2008). In addition, it has been reported that the non-attached type 3 PM is reliable under the specified conditions in which our study was conducted (Collop *et al.*, 2007). Thirdly, we did not assess sympathetic activity, nor did we administer the MSLT to estimate sleepiness objectively.

In conclusion, although this study had several limitations, we showed that sleepiness was related to the severity of OSA in the hypertensive subjects, but not in the non-hypertensive subjects. Also, we showed that sleepiness was related to short sleep duration in both groups. Furthermore, we observed a relationship between short sleep duration and the severity of OSA in the hypertensive subjects. Thus, in addition to sleep duration, OSA accompanied by hypertension may be important in sleepiness. Further study is needed to determine details of the mechanism of the difference in the relationship between sleepiness and the severity of OSA with or without hypertension.

CONFLICTS OF INTEREST

No authors have indicated any financial conflicts of interest.

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Comparison of biomarkers of subclinical lung injury in obstructive sleep apnea

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KEYWORDS
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KL-6;
Surfactant protein-D;
C-reactive protein;
Lung injury

Summary
Background: Obstructive sleep apnea (OSA) has both systemic and local effects partly through the increased oxidative stress caused by intermittent hypoxia and reoxygenation. However, lung-specific biomarkers in OSA have not been fully assessed in comparison with systemic biomarkers such as C-reactive protein (CRP), although results of a recent study having a small sample size indicated KL-6 as one candidate.
Methods: Subjects of the present study were 197 patients suspected to have OSA. In addition to polysomnography, we also measured serum levels of KL-6, surfactant protein-D (SP-D) and CRP and pulmonary function. We examined the relationships of different biomarkers with OSA severity and pulmonary function.
Results: The apnea/hypopnea index (AHI) was significantly positively correlated with serum KL-6 levels even after adjustment for body mass index (BMI) and smoking ($p = 0.03$), but not with SP-D and CRP. Also, a significant trend for an increase in serum KL-6 was noted in accordance with the severity of OSA even after adjustment for BMI and smoking (β coefficient $= 0.18$, $p = 0.02$). Additionally, elevated KL-6 levels were significantly associated with restrictive lung function disturbance and gas exchange derangement after adjustment for obesity and smoking, which contrasted with CRP whose elevations were significantly associated with worsened airflow limitation and increased lung volume.
Conclusions: Serum KL-6 levels may reflect the degree of subclinical lung injury associated with OSA independently of obesity or smoking, unlike CRP. We consider that KL-6 can be a potential candidate as a lung-specific biomarker of OSA and might provide complementary information on systemic biomarkers in assessing OSA.

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Introduction

Obstructive sleep apnea (OSA) is characterized by repeated episodes of upper airway obstruction accompanied by intermittent hypoxia and reoxygenation, which can cause oxidative stress.[1] It contributes not only to endothelial dysfunction in the peripheral circulation, but possibly also contributes to epithelial and endothelial cell injury in the alveolus causing increased alveolar wall permeability in the lungs.[2,3]

KL-6 is a mucin-like glycoprotein with a molecular weight of 200 kd and is mainly expressed on alveolar type II and bronchiolar epithelial cells in human lungs.[4] Elevated levels of circulating KL-6 have been reported in patients with lung injury.[5,6] Recently, Lederer et al. reported that circulating levels of KL-6 were elevated in some patients with OSA in association with greater endothelial dysfunction, suggesting that subclinical lung injury may be present in OSA.[7] However, the sample size in that study was very small, comprising only 11 OSA patients and 10 controls, and the relationships of elevated levels of KL-6 with other biomarkers or pulmonary function were not examined. Therefore, the role of KL-6 as a biomarker of lung injury in OSA has not been fully elucidated.

In addition to KL-6, circulating levels of surfactant protein-D (SP-D) are elevated in patients with lung injury.[8,9] SP-D is also exclusively produced by alveolar type II cells in the lungs and its values change with the clinical status of patients or with relevant exposures.[10] Therefore, recently, much attention has been paid to SP-D as a potential lung-specific biomarker in diseases such as chronic obstructive pulmonary disease (COPD) where both local and systemic inflammation play respective important roles in disease progression.[10,11] In OSA, increased levels of various systemic inflammatory biomarkers, including C-reactive protein (CRP), tumor necrosis factor-α, interleukin-6, and interleukin-8, have been reported to be associated with future cardiovascular risk.[12] Considering that OSA has respiratory and systemic effects, a lung-specific blood biomarker would be attractive and informative.

We hypothesized that higher serum levels of KL-6 and SP-D would be associated with more severe OSA and more severe impairments in pulmonary function, reflecting the degree of subclinical lung injury unlike CRP, which is the most widely studied systemic biomarker in OSA.[12] Although the magnitude of the injury might not be great, it may not only cause respiratory symptoms such as chronic cough[13] but also may worsen other possible comorbid disorders such as asthma,[14] COPD[15] or idiopathic pulmonary fibrosis (IPF).[16] Therefore, in the present study, we examined the relationships between these three different biomarkers, OSA and pulmonary function and assessed their clinical relevance.

Methods

Study subjects

A total of 197 patients were consecutively recruited from the Sleep Unit of Kyoto University Hospital between April 2009 and April 2010. All had been referred to our sleep unit with symptoms such as habitual snoring or daytime sleepiness. None had been previously diagnosed with or treated for OSA. Patients with pulmonary diseases such as asthma ($n = 14$), COPD ($n = 5$) or interstitial lung diseases ($n = 2$) and who were diagnosed as having central sleep apnea ($n = 6$) were excluded based on clinical history, spirometry, chest radiograph and polysomnography, and a total of 170 patients were examined further. This study was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee, and informed consent was obtained from all patients. Arterial blood gas analysis, including arterial partial tension of oxygen (PaO_2) and arterial partial tension of carbon dioxide ($PaCO_2$), was performed while patients were breathing room air at rest in the supine position. The alveolar-arterial oxygen tension difference ($A\text{-}aDO_2$) was calculated according to the standard formula, using the respiratory exchange ratio of 0.8.

Polysomnography

The diagnosis of OSA was confirmed by polysomnography (SomnoStar pro, Cardinal Health, Dublin, OH, USA), which was started at 22:00 and ended at 6:00 the following morning. Surface electrodes were attached using standard techniques to obtain an electrooculogram, electromyogram of the chin, and 12-lead electroencephalograph. Sleep stages were defined according to the criteria of Rechtschaffen and Kales.[17] Ventilation was monitored by inductive plethysmography (Respitrace QDC, Viasys Healthcare, Palm Springs, CA, USA). Airflow was monitored by a nasal pressure transducer (PTAFlite, Pro-Tech Services Inc., Mukilteo, WA, USA) and supplemented by an oronasal thermal sensor (Sleepmate Technologies, Midlothian, VA, USA). Arterial oxygen saturation (SpO_2) was monitored continuously with a pulse oximeter (Adult Flex System, Nonin Medical, Plymouth, MN, USA).

Apnea was defined as the complete cessation of airflow and hypopnea as a clear decrease in airflow of 50% or more lasting for 10 s or more accompanied by a decrease in SpO_2 of at least 3%.[18] All AHI values were expressed as the number of episodes of apnea and hypopnea per hour over the total sleep time. The lowest SpO_2 during sleep was calculated in each patient. OSA severity was defined by the AHI as follows: non OSA ($AHI<5$), mild OSA ($5 \leq AHI<15$), moderate OSA ($15 \leq AHI<30$) and severe OSA ($30 \leq AHI$).

Blood sample collection and measurements of serum biomarkers levels

Following overnight polysomnography, samples of peripheral venous blood were collected in the morning after an overnight fast. Serum KL-6 levels were measured by a sandwich-type electrochemiluminescence immunoassay kit (Picolumi KL-6; Sanko Junyaku, Tokyo, Japan), serum SP-D levels were measured by a sandwich-type enzyme immunoassay kit (SP-D kit Yamasa EIA II; Yamasa Shoyu, Chiba, Japan), and serum CRP levels were measured using a high sensitive assay kit (N-Assay LA CRP-S kit; Nittobo Medical, Tokyo, Japan).

Pulmonary function tests

Pulmonary function tests were performed using CHESTAC (Chest M.I. Inc., Tokyo, Japan). Residual volume (RV) and total lung capacity (TLC) were measured by the closed-circuit helium method, and diffusing capacity for carbon monoxide (DL_{CO}) was measured using a single-breath technique. Percent-predicted values were used for analyses.

Statistics

All statistical analyses were performed using StatView version 5.0 for Windows (Abacus Concepts, Berkeley, CA, USA). Continuous variables are expressed as means ± standard deviation (SD). The unpaired *t*-test was used to compare AHI levels between men and women. The significance of intergroup differences based on the severity of OSA was determined by an analysis of variance (ANOVA). When a significant difference was observed, we used the Fisher's PLSD method to identify where the differences were significant. A chi-square test was used to compare dichotomous variables. Relationships between 2 variables were analyzed by Pearson's correlation coefficient tests. The trend of serum biomarker levels across OSA groups was examined by two models. Model 1 is a linear model with serum biomarker levels as the dependent variable and with three indicator variables for mild, moderate, and severe OSA as the independent variables. We reported the β coefficients for the three OSA variables as mean differences in biomarker levels versus the non OSA group (reference). Model 2 is also a linear model wherein the indicator variables are replaced with a single ordinal variable equal to the median AHI in the mild, moderate and severe OSA groups, respectively and 0 in the non OSA group. Multiple regression analyses were performed to adjust for the confounders. A p value less than 0.05 was considered to indicate statistical significance.

Results

Relationships of clinical indices and serum biomarker levels to AHI

Patient characteristics and polysomnographic data are shown in Table 1. The study group of 170 patients was comprised of 20 non OSA, 25 mild OSA, 52 moderate OSA and 73 severe OSA patients. Significant differences were observed in sex, age, body mass index (BMI) and smoking among the groups. Serum KL-6 levels were also significantly different among the groups ($p = 0.04$), but not SP-D ($p = 0.65$) or CRP ($p = 0.53$). There were significant differences among the groups in PaO_2 and A-aDO_2.

We investigated the relationships between subjects' background, serum biomarker levels and AHI. With regard to clinical characteristics, the AHI was not significantly different between men and women ($p = 0.12$), and had significant positive correlations with BMI [r (correlation coefficient) $= 0.50$, $p < 0.001$] and smoking ($r = 0.23$, $p = 0.003$), but not with age ($r = 0.06$, $p = 0.44$). On the other hand, the AHI had a significant positive correlation with serum levels of KL-6 ($r = 0.20$, $p = 0.01$) and CRP ($r = 0.17$, $p = 0.03$), although a significant correlation was not found between the AHI and serum levels of SP-D ($r = 0.06$, $p = 0.48$). After adjustment for BMI and smoking, which were significantly associated with the AHI as mentioned above, the correlation of the AHI with serum KL-6 levels remained weak but statistically significant (β coefficient $= 0.14$, $p = 0.03$), whereas its correlation with serum CRP levels was far from significant (β coefficient $= 0.00$, $p = 0.97$).

Table 2 shows trends of serum biomarker levels across OSA groups. A significant trend for an increase in serum KL-6 levels was noted in accordance with the severity of OSA even after adjustment for BMI and smoking (β coefficient $= 0.18$, $p = 0.02$ for both Model 1 and 2), whereas no significant trend was found in serum levels of SP-D and CRP.

As to the lowest SpO_2, no significant relationships with serum levels of KL-6, SP-D and CRP were found. Regarding the inter-relationships among the three serum biomarkers examined, there was a significant correlation between serum levels of KL-6 and SP-D ($r = 0.24$, $p = 0.002$), but serum CRP levels did not correlate with either KL-6 or SP-D levels.

Relationships of pulmonary function and arterial blood gas data to serum biomarker levels

Next, to assess the clinical relevance of KL-6 and CRP, which were significantly related to AHI, we compared their relationships with pulmonary function and arterial blood gas data. After adjustment for BMI and smoking, serum KL-6 levels were significantly positively correlated with A-aDO_2 and negatively correlated with vital capacity (VC), forced vital capacity (FVC), TLC and DL_{CO} (Table 3). In contrast, serum CRP levels were significantly positively correlated with TLC and negatively correlated with forced expiratory volume in 1 s (FEV_1) (Table 3).

Discussion

We analyzed the inter-relationships of different serum biomarkers with OSA and pulmonary function. We found that, after adjustment for obesity and smoking, the serum KL-6 levels significantly correlated with the AHI, unlike serum levels of SP-D and CRP. Additionally, elevated serum KL-6 levels were significantly associated with increased A-aDO_2 and decreased VC, FVC, TLC and DL_{CO} even after adjustment for obesity and smoking, whereas elevated serum CRP levels were significantly associated with increased TLC and decreased FEV_1.

Our results indicate the significance of circulating levels of KL-6 as a marker of lung injury associated with OSA. Although Lederer et al. reported elevated circulating levels of KL-6 in some patients with OSA in comparison with controls in a study having a small sample size,[7] we expanded knowledge in this area by confirming the significant relationship between serum KL-6 levels and the severity of OSA with a relatively large number of subjects and, in addition, compared values for serum KL-6 with

Table 1 Clinical characteristics and polysomnographic data on 170 patients.

	non OSA (n = 20)	mild OSA (n = 25)	moderate OSA (n = 52)	severe OSA (n = 73)	p value
Sex (male/female)	12/8	16/9	37/15	62/11[a,c]	0.04
Age (years)	43.6 ± 17.7	56.7 ± 15.7[a]	57.5 ± 15.6[a]	57.3 ± 13.5[a]	0.003
BMI (kg/m^2)	24.8 ± 3.4	25.7 ± 4.1	25.6 ± 5.3	28.7 ± 6.1[a,b,c]	0.002
Smoking history (current/ex/never)	12/6/2	9/9/7	26/18/8	18/39/16[a,c]	0.02
Smoking (pack years)	5.4 ± 12.7	18.0 ± 23.3	21.1 ± 34.2[a]	28.3 ± 30.9[a]	0.02
Serum KL-6 (U/ml)	198.7 ± 56.0	227.2 ± 90.0	246.8 ± 117.1	285.7 ± 170.8[a]	0.04
Serum SP-D (ng/ml)	41.6 ± 26.4	48.4 ± 22.3	53.0 ± 43.7	51.9 ± 36.1	0.65
Serum CRP (mg/dl)	0.14 ± 0.27	0.20 ± 0.40	0.13 ± 0.22	0.21 ± 0.32	0.53
AHI (events/hour)	2.2 ± 1.5	9.8 ± 2.8	22.1 ± 4.3[a,b]	54.3 ± 21.6[a,b,c]	<0.0001
Lowest SpO_2 (%)	89.7 ± 6.4	84.4 ± 8.3	80.3 ± 11.3[a]	73.9 ± 9.3[a,b,c]	<0.0001
VC (% predicted)	107.3 ± 13.3	107.8 ± 19.0	113.0 ± 15.5	110.6 ± 17.4	0.46
ERV (% predicted)	82.8 ± 26.1	81.6 ± 36.9	83.1 ± 38.5	71.1 ± 35.8	0.23
FVC (% predicted)	107.7 ± 14.0	105.4 ± 19.7	111.8 ± 16.3	108.5 ± 17.9	0.46
FEV_1 (% predicted)	99.4 ± 13.6	98.9 ± 20.7	107.2 ± 15.5	106.3 ± 17.9	0.10
FRC (% predicted)	106.9 ± 24.4	103.2 ± 30.2	111.5 ± 47.9	125.4 ± 41.9	0.71
RV (% predicted)	108.4 ± 37.6	113.2 ± 38.9	104.9 ± 26.9	106.4 ± 41.2	0.81
TLC (% predicted)	95.3 ± 13.8	102.0 ± 25.9	99.3 ± 18.9	96.6 ± 15.4	0.50
DL_{CO} (% predicted)	89.4 ± 15.7	82.0 ± 17.3	81.1 ± 19.3	85.7 ± 15.1	0.21
$PaCO_2$ (kPa)	5.7 ± 0.5	5.6 ± 0.5	5.7 ± 0.5	5.6 ± 0.6	0.61
PaO_2 (kPa)	12.0 ± 1.7	11.6 ± 1.7	11.5 ± 1.4	10.9 ± 1.6[a,c]	0.01
A-aDO_2 (kPa)	0.9 ± 1.7	1.5 ± 1.6	1.3 ± 0.4	2.1 ± 1.5[a,c]	0.003

Data presented as number or mean ± SD.
[a] $p < 0.05$ versus non OSA.
[b] $p < 0.05$ versus mild OSA.
[c] $p < 0.05$ versus moderate OSA. OSA: obstructive sleep apnea; BMI: body mass index; SP-D: surfactant protein-D; CRP: C-reactive protein; AHI: apnea/hypopnea index; VC: vital capacity; ERV: expiratory reserve volume; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s; FRC: functional residual capacity; RV: residual volume; TLC: total lung capacity; DL_{CO}: diffusing capacity for carbon monoxide; $PaCO_2$: arterial partial tension of carbon dioxide; PaO_2: arterial partial tension of oxygen; A-aDO_2: alveolar-arterial oxygen tension difference.

other potential biomarkers. Moreover, elevated KL-6 levels were also significantly associated with restrictive lung function disturbance and gas exchange derangement, which are characteristic features in lung injury. Lederer et al. speculated that the possible mechanisms whereby OSA leads to lung injury include chronic oxidative stress associated with repeated intermittent hypoxia and reoxygenation and mechanical stretch caused by dynamic changes in intrathoracic pressure.[7] In addition to these factors, snoring-induced vibratory force might be a candidate as another factor causing lung injury.[19] Although both comorbid obesity and smoking exposure could affect plasma cytokine levels[20] or the subjects' lung diffusion capacity,[21,22] we showed that even after adjustment for these confounders the association between KL-6, pulmonary function impairment and OSA remained significant, indicating the possible pathogenetic role of OSA itself in lung injury.

KL-6 is a well-known biomarker for the diagnosis of IPF and determination of the severity of this condition.[23,24]

Table 2 Trends of serum biomarker levels across OSA groups.

	Model 1		Model 2	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
Serum KL-6 (U/ml)	$\beta = 0.22$	$\beta = 0.18$	$\beta = 0.22$	$\beta = 0.18$
	$p = 0.004$	$p = 0.02$	$p = 0.004$	$p = 0.02$
Serum SP-D (ng/ml)	$\beta = 0.08$	$\beta = 0.04$	$\beta = 0.07$	$\beta = 0.02$
	$p = 0.28$	$p = 0.67$	$p = 0.39$	$p = 0.80$
Serum CRP (mg/dl)	$\beta = 0.06$	$\beta = -0.02$	$\beta = 0.08$	$\beta = -0.01$
	$p = 0.41$	$p = 0.84$	$p = 0.32$	$p = 0.88$

Model 1: Linear model with three indicator variables for mild, moderate, and severe OSA as independent variables. Model 2: Linear model in which indicator variables were replaced with a single ordinal variable equal to the median AHI in the mild, moderate and severe OSA groups, respectively, and 0 in the non OSA group. OSA: obstructive sleep apnea; SP-D: surfactant protein-D; CRP: C-reactive protein.
[a] Adjusted for body mass index and smoking.

Table 3 Relationships between serum biomarker levels and pulmonary function and arterial blood gas data.

	Serum KL-6 (U/ml)		Serum CRP (mg/dl)	
	β coefficient	p value	β coefficient	p value
VC (% predicted)	−0.20	0.01	−0.08	0.24
ERV (% predicted)	−0.15	0.07	−0.03	0.75
FVC (% predicted)	−0.22	0.003	−0.11	0.14
FEV_1 (% predicted)	−0.11	0.17	−0.19	0.008
FRC (% predicted)	0.15	0.11	−0.02	0.79
RV (% predicted)	0.05	0.53	0.12	0.11
TLC (% predicted)	−0.14	0.049	0.18	0.01
DL_{CO} (% predicted)	−0.22	0.005	−0.14	0.06
$PaCO_2$ (kPa)	−0.12	0.12	0.10	0.16
PaO_2 (kPa)	−0.13	0.11	−0.11	0.16
A-aDO_2 (kPa)	0.18	0.02	0.06	0.45

These analyses were adjusted by body mass index and smoking. VC: vital capacity; ERV: expiratory reserve volume; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s; FRC: functional residual capacity; RV: residual volume; TLC: total lung capacity; DL_{CO}: diffusing capacity for carbon monoxide; $PaCO_2$: arterial partial tension of carbon dioxide; PaO_2: arterial partial tension of oxygen; A-aDO_2: alveolar-arterial oxygen tension difference.

Although the pathophysiological link between IPF and OSA remains unclear, as a new topic in the field of IPF, recent studies have reported a high prevalence of OSA in IPF patients.[16,25] Our findings may partially explain potential mechanisms underlying this association and also provide a warning that their combination as a comorbid condition may additively worsen lung injury.

Serum levels of CRP did not correlate with the severity of OSA independently of BMI in our study, which is consistent with some reports.[26,27] Obesity was shown to be associated with chronic low-grade inflammation as indicated by raised serum CRP levels.[28] Considering that CRP reflects obesity-related inflammation, it could be anticipated that CRP might have an association with reduced lung volume due to obesity, particularly visceral adiposity. However, notably, we found that elevated CRP levels were significantly associated with worsened airflow limitation and increased lung volume independently of obesity and smoking, as contrasted with the significant relationship between KL-6 and restrictive defects. Increased levels of CRP also have been reported in obstructive and restrictive lung disease,[29] and were shown to be correlated with lower levels of FEV_1[30–32] and a decline in FEV_1.[33] Moreover, a recent large-scale population-based study showed that abdominal obesity was positively related to both obstructive and restrictive ventilatory patterns regardless of BMI.[34] Thus, the inter-relationships among OSA, pulmonary function, obesity and inflammatory markers are complicated, and further studies are needed.

Contrary to our hypothesis, unlike KL-6, SP-D was not significantly associated with the severity of OSA, although both are mainly produced by alveolar type II cells. Leakage of these markers into the bloodstream may be dependent on an alteration of production by alveolar type II cells and, in addition, on the intensity, extent, and type of injuries that precipitate the increase in alveolar wall permeability.[24] Thus, the magnitude of lung injury in patients with OSA might be too subtle to increase the production of SP-D by alveolar type II cells, although we did not assess the local concentrations of SP-D in lung compartments. SP-D was recently reported to be a promising lung-specific biomarker in COPD.[10] However, results of previous studies suggested that KL-6 was a more discriminative biomarker than SP-D in IPF and collagen vascular disease-associated interstitial pneumonitis,[24] idiopathic pulmonary alveolar proteinosis[35] or sarcoidosis.[36] Madsen et al. reported that SP-D was widely distributed in epithelial cells in a variety of tissues and was not restricted to the respiratory system.[37] Only a weak, though significant, relationship between serum levels of KL-6 and SP-D in the present study indicates that the blood level of these two cytokines may not necessarily reflect the same pathological state of the disease. Thus, although KL-6 appears to be superior to SP-D in evaluating subclinical lung injury associated with OSA, further study is needed to explain this discrepancy.

OSA is associated with significant morbidity and mortality. Analyses of biomarkers in OSA have been extensively performed in relation to its cardiovascular effects through systemic inflammation.[12] However, as well as systemic inflammation, local inflammation or injury is also implicated in the pathophysiology of OSA.[38,39] Local inflammation or injury has been assessed by surgical specimens, exhaled breath condensate (EBC), induced sputum, exhaled breath and oral air,[38,39] but more studies with a lung-specific biomarker in OSA are necessary for future use of such biomarkers in clinical practice. A recent study has also reported that the combination of two biomarkers, SP-D (lung epithelial barrier injury) and interleukin-8 (inflammation and neutrophil chemotaxis), had significant prognostic value, reflecting the two important pathogenetic pathways of acute lung injury.[40] Thus, KL-6 in association with a systemic biomarker such as CRP might provide complementarily useful information in assessing OSA.

In our study, there was a significant positive relationship between AHI and smoking. Airway inflammation and damage due to cigarette smoke could alter the mechanical and neural properties of the upper airway and increase its collapsibility during sleep, which may be related to sleep apnea.[41]

The prevalence of OSA in our population was high (88.2%). Since all participants in our study were referred to

our sleep unit with suspicious symptoms, there was a high clinical probability of OSA among these patients. Actually, the diagnostic rates in some other sleep laboratories were comparable to ours.[42–44]

The present study has some limitations. First, as we did not directly assess local expressions of KL-6 in the lung and alveolar wall permeability in OSA patients, our results cannot clearly establish the presence of lung injury in OSA. Measurements of KL-6, albumin concentration or cell count in bronchoalveolar lavage fluid, EBC or induced sputum would provide additional information and supporting evidence for our suggestions regarding the implications of these results. Second, as we did not assess serum biomarker levels after treatment of OSA, we could not confirm the specific association between OSA and serum biomarkers. Third, as the values of AHI and biomarkers tended to be skewed, we tried to transform them logarithmically. However, in 57 patients the CRP values could not be transformed logarithmically (CRP value was '0'). We then made calculations using absolute values. However, significance of the result between KL-6 and AHI remained even after logarithmic transformation ($r = 0.20$, $p = 0.01$).

In summary, serum levels of KL-6 but not SP-D were correlated with subclinical lung injury associated with OSA independently of obesity or smoking, and KL-6 could be a potential candidate as a lung-specific biomarker in OSA. KL-6 values may pathologically reflect epithelial and endothelial injury in the alveolus and pulmonary function impairment in OSA. This is in contrast to CRP, which is a well-studied systemic biomarker and may preferably cause an obstructive defect. A combination of two different types of biomarkers might be complementary and be superior to each biomarker alone from a pathogenesis perspective.

Conflict of interest disclosure

Kazuo Chin received Grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology (nos. 20590921 and 22590860), Respiratory Failure Research Group and Health Science Research Grants (Comprehensive Research on Life-Style Related Diseases including Cardiovascular Diseases and Diabetes Mellitus: nos. 22110201 and 22111201) from the Ministry of Health, Labor and Welfare of Japan, and the Japan Vascular Disease Research Foundation.

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Original Article

Self-Reported Snoring Frequency and Incidence of Cardiovascular Disease: The Circulatory Risk in Communities Study (CIRCS)

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ABSTRACT

Background: Although associations between snoring and cardiovascular disease have been reported in several prospective studies, there is limited evidence from Asian populations. The objective of this study was to determine if there is an association between self-reported snoring frequency and the incidence of cardiovascular disease in Japanese.

Methods: The subjects were 2350 men and 4163 women aged 40 to 69 years who lived in 3 communities in Japan. All subjects were participants in the Circulatory Risk in Communities Study (CIRCS) and were followed for 6 years. Incidence of cardiovascular disease during the follow-up period comprised events of myocardial infarction, angina pectoris, sudden cardiac death and stroke.

Results: During the 6-year follow-up period, 97 participants (56 men and 41 women) had cardiovascular events. After adjustment for potential confounding factors, self-reported snoring frequency was associated with an increased risk of cardiovascular events among women but not men. The hazard ratios (95% CI) for cardiovascular events were 0.9 (0.4–2.0) for sometimes snoring and 2.5 (1.0–6.1) for everyday snoring in women and 0.7 (0.3–1.3) and 1.0 (0.5–2.1), respectively, in men. Further adjustment for body mass index attenuated the association in women; the respective hazard ratios for cardiovascular events were 0.9 (0.4–1.9) and 2.1 (0.9–5.4).

Conclusions: Self-reported habitual snoring was associated with increased risk of cardiovascular events among Japanese women. Overweight may partly mediate this association.

Key words: cardiovascular events; obstructive sleep apnea; population-based study; prospective cohort study

INTRODUCTION

Sleep-disordered breathing (SDB) is characterized by repeated episodes of apnea and hypopnea events during sleep.[1] Recently, SDB was identified as a risk factor for various disorders and diseases such as hypertension,[2–5] insulin and glucose abnormalities,[6] and cardiovascular disease.[7] Evidence has shown that self-reported snoring is a surrogate marker for SDB.[8] Associations of snoring and SDB with cardiovascular disease were examined cross-sectionally[9,10] in clinical and large-scale epidemiologic studies, most of which clearly showed an independent positive association between the 2 conditions, even after adjustment for potential confounding factors such as age, sex, and body mass index (BMI).[11]

This causal relationship has also been observed in studies of Western populations. As compared with non-snorers, the relative risk of developing cardiovascular disease among habitual snoring American women was 30% higher in the Nurses' Health Study,[12] and the risk among habitual and frequent snoring Finish men was 2.1-fold higher.[13] However, evidence from Asian populations is still very limited, and these results from Western studies cannot simply be extrapolated to Asian populations, because substantial differences exist between Asian and white populations, such

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as disparities in physique and prevalence of cardiovascular disease subtypes.[14]

In our previous large cross-sectional study,[15] we reported that the prevalence of snorers among Japanese men was about 70%, despite the low prevalence of obesity. The study revealed that alcohol consumption and cigarette smoking increased snoring risk among Japanese, especially among those who were not overweight. We also reported differences in the distribution of cardiovascular disease subtypes among Japanese versus whites, eg, among Japanese, the proportion of stroke was 2 times higher,[16] and the proportion of ischemic heart disease was one quarter, compared to the respective values reported in the United Kingdom and United States.[17] It is thus important to assess the risks of habitual snoring in Asians, because snoring is affected not only by obesity but also other factors, and Asian populations have different distributions of cardiovascular disease subtypes. We therefore examined the risk of cardiovascular events among habitual snorers compared with non-snorers in a large community-based prospective study of Japanese adults.

METHODS

Study subjects

Subjects were recruited from participants in the Circulatory Risk in Communities Study (CIRCS, see Appendix), a prospective community-based study of cardiovascular disease in 5 communities across Japan that was launched in 1963.[18] The subjects of the present sleep study comprised Japanese men and women aged 40 to 69, and none had previously received a diagnosis of SDB. Baseline data on snoring frequency and cardiovascular risk factors were obtained during annual surveys between 2001 and 2005 in the district of Yao City (midwestern suburban community, $n = 1994$), Ikawa (northeastern rural community, $n = 1446$), and between 2000 and 2004 in Kyowa (mideastern rural community, $n = 3209$). We excluded participants with incomplete data on sleep questionnaires ($n = 82$), those with missing data for BMI or other parameters ($n = 10$), and those with a history of ischemic heart disease ($n = 17$) or stroke ($n = 19$). A total of 2350 men and 4163 women were enrolled in the present study. All subjects had the protocol explained in detail and gave their informed consent for participation. The study protocol was approved by the Medical Ethics Committees of the University of Tsukuba.

Determination of endpoint

Follow-up lasted until the end of 2009 for Ikawa, until the end of 2008 for Yao, and until the end of 2006 for Kyowa. The criteria for ischemic heart disease were modified from those established by the World Health Organization (WHO) Expert Committee.[19] Definite myocardial infarction was defined as characteristic severe chest pain (lasting for ≥30 min) together with the appearance of new abnormal and persistent Q or QS waves and/or consistent changes in cardiac enzyme activity. Probable myocardial infarction was defined as characteristic chest pain in the absence of electrocardiographic findings or findings related to enzyme activity. Angina pectoris was defined as repeated episodes of chest pain during effort, especially when walking, that usually rapidly disappeared after cessation of effort or use of sublingual nitroglycerin. Sudden cardiac death was defined as death within 1 hour of symptom onset, a witnessed cardiac arrest, or abrupt collapse not preceded by symptoms persisting 1 hour or longer. Stroke was defined as a focal neurological disorder of rapid onset that persisted at least 24 hours or until death. Determination of incident strokes was conducted based on clinical criteria.[20] A panel of 3 or 4 physician-epidemiologists who were blinded to data from the risk factor surveys made the final diagnoses for these suspected cases of ischemic heart disease and stroke. Cardiovascular disease included events related to ischemic heart disease (definite and probable myocardial infarction, angina pectoris, and sudden cardiac death) and stroke during the follow-up period.

For case ascertainment, histories of cardiovascular events were obtained from annual cardiovascular risk surveys, national insurance claims, ambulance records, reports of local physicians, and public health nurses. To confirm the diagnosis, all living patients were telephoned or visited to obtain a medical history, and their medical records were reviewed. For deaths, we obtained histories from families and reviewed medical records. The protocol has been described in detail elsewhere.[20,21]

Risk factor measurements

At the annual cardiovascular surveys, each participant was asked about their snoring frequency. Answer options for the question "Did you snore during the past 3 months?" were almost every day, sometimes, never, and unknown. Information on smoking and drinking habits, menopausal status (for women), and measurements of blood pressure, serum glucose concentration, and physique were obtained according to the CIRCS protocol.[22] Hypertension was defined as systolic blood pressure of 140 mm Hg or higher, diastolic blood pressure of 90 mm Hg or higher, or antihypertensive treatment; diabetes mellitus was defined as fasting blood glucose of 126 mg/dl or higher, non-fasting blood glucose of 200 mg/dl or higher, or antihyperglycemic treatment; and hypercholesterolemia was defined as serum total cholesterol of 220 mg/dl or higher or treatment with lipid-lowering medication.

Statistical analyses

Person-years for cardiovascular events were calculated as the sum of individual follow-up time until cardiovascular event, emigration, or the end of the follow-up period. Age- and community-adjusted and multivariable-adjusted hazard ratios (HRs) and 95% CIs for cardiovascular events were calculated according to baseline snoring frequency by using the Cox

proportional hazards model. The interaction of snoring with sex in relation to cardiovascular events was tested using their cross-product terms.

Confounding variables included age (continuous), BMI (continuous), alcohol consumption (never, ex-, <23 g and ≥23 g ethanol per day), cigarette smoking (never, ex-, <20 and ≥20 cigarettes per day), community (categorical), and menopausal status (for women; yes, no). To confirm the hypothesis that snoring induces cardiovascular events by increasing the risk of hypertension and metabolic disorders, we further adjusted for systolic blood pressure (continuous), use of antihypertensive medication (dichotomous), diabetes mellitus (dichotomous), and hypercholesterolemia (dichotomous). All statistical analyses were performed using SAS version 9.1 software (SAS Institute Inc., Cary, NC, USA). All statistical tests were 2-tailed, and P values less than 0.05 were regarded as statistically significant.

RESULTS

Demographic characteristics at baseline

The mean (± SD) age of participants was 56.3 ± 8.3 years, mean BMI was 23.5 ± 3.2 kg/m^2, mean systolic blood pressure was 132.3 ± 18.6 mm Hg, and mean diastolic blood pressure was 80.4 ± 11.1 mm Hg.

Analysis revealed that 42.5% of participants had hypertension, 17.5% were using antihypertensive medication, 5.9% had diabetes mellitus, 44.7% had hypercholesterolemia, 37.6% were current drinkers, 21.1% were current smokers, and 69.1% of women were post-menopausal. Baseline demographic characteristics, by sex, are shown in Table 1.

Table 1. Sex-specific mean values (SD) and prevalence of selected cardiovascular risk characteristics among 2350 men and 4163 women aged 40–69 years

	Men n = 2350	Women n = 4163	P value
Age (years)	57.5 (8.2)	55.6 (8.3)	<0.001
Body mass index (kg/m^2)	23.9 (3.0)	23.3 (3.3)	<0.001
Systolic blood pressure (mm Hg)	135.1 (17.6)	130.7 (18.9)	<0.001
Diastolic blood pressure (mm Hg)	83.3 (10.9)	78.7 (10.8)	<0.001
Antihypertensive use (%)	19.2	16.5	0.005
Hypertension (%)[a]	51.1	37.6	<0.001
Diabetes mellitus (%)[b]	9.1	4.1	<0.001
Hypercholesterolemia (%)[c]	34.5	50.5	0.28
Alcohol consumption (%)			
never	19.6	78.5	<0.001
ex-drinker	7.3	4.0	
<23 g ethanol per day	22.6	15.1	
≥23 g ethanol per day	50.6	2.4	
Cigarette smoking (%)			
never	17.0	90.5	<0.001
ex-smoker	35.4	3.3	
<20 cigarettes per day	13.2	4.4	
≥20 cigarettes per day	34.3	1.8	
Menopause (%)	—	69.1	—

[a]Hypertension was defined as blood pressure ≥140/90 mm Hg or current treatment.
[b]Diabetes mellitus was defined as fasting blood glucose ≥126 mg/dl, non-fasting blood glucose ≥200 mg/dl, or current treatment.
[c]Hypercholesterolemia was defined as total cholesterol ≥220 mg/dl or current treatment.

Prevalence of snoring and its correlates

The distribution of snoring frequency was as follows: 14.0% (23.9% in men and 8.5% in women) reported snoring almost every day, 46.7% (48.7% in men and 45.6% in women) reported snoring sometimes, 28.9% (20.7% in men and 33.6% in women) reported never snoring, and 10.3% (6.8% in men and 12.3% in women) reported that their snoring frequency as unknown. Sex-specific, age-adjusted characteristics classified according to snoring frequency are shown in Table 2. The mean values and proportions of baseline risk characteristics tended to be higher with increasing snoring frequency, except for mean age in men and women and prevalence of diabetes mellitus in men. As compared with women who reported a snoring frequency, those who answered "unknown" were 2.6 years older ($P < 0.001$), 0.5 points lower in mean BMI ($P = 0.001$), and 4.3% higher in the mean proportion of current smokers ($P < 0.001$) in women. There was no significant difference among men.

Incidence of cardiovascular events

During the 6-year median follow-up duration, 97 participants (56 men and 41 women) experienced cardiovascular events, including 30 (22 in men and 8 in women) incident cases of ischemic heart disease and 67 (34 in men and 33 in women) strokes. The numbers of ischemic heart disease and stroke events, according to snoring frequency and sex, are shown in Table 3.

Association of snoring with cardiovascular events

As compared with never snorers, the age- and community-adjusted HR of ischemic heart disease and stroke combined was higher among female but not male everyday snorers; the HRs (95% CI) for cardiovascular events were 0.9 (0.5–2.0) for sometimes snoring and 2.6 (1.1–6.3) for everyday snoring in women and 0.7 (0.4–1.4) and 1.1 (0.5–2.2), respectively, in men (Table 3). These associations in men did not vary by age group (40–59 and 60–69 years), smoking status (current and non-current), or drinking status (current and non-current) (data not shown in table). After adjustment for age, community, and other confounding variables, the association between self-reported snoring frequency and cardiovascular events was unchanged in women: the HRs (95% CI) for cardiovascular events were 0.9 (0.4–2.0) for sometimes snoring and 2.5 (1.0–6.1) for everyday snoring in women (Table 3, Model 1). Further adjustment for BMI attenuated the association in women: the respective HRs for cardiovascular events were 0.9 (0.4–1.9) and 2.1 (0.9–5.4; Table 3, Model 2). To confirm

Table 2. Sex-specific, age-adjusted, mean values (standard error) and prevalence of selected cardiovascular risk characteristics according to snoring frequency among 2350 men and 4163 women aged 40–69 years

Snoring frequency	Men				Women			
	Never $n = 486$	Sometimes $n = 1144$	Daily $n = 561$	Unknown $n = 159$	Never $n = 1399$	Sometimes $n = 1900$	Daily $n = 352$	Unknown $n = 512$
Age (years)[a]	59.0 (0.4)	57.1 (0.2)	56.8 (0.3)	57.9 (0.6)	55.1 (0.2)	55.2 (0.2)	55.9 (0.4)	57.9 (0.4)
Body mass index (kg/m^2)	23.1 (0.1)	23.8 (0.1)	24.9 (0.1)	23.6 (0.2)	22.6 (0.1)	23.6 (0.1)	24.9 (0.2)	22.8 (0.1)
Systolic blood pressure (mm Hg)	133.4 (0.8)	135.1 (0.5)	136.5 (0.7)	135.0 (1.3)	128.3 (0.5)	132.4 (0.4)	133.5 (0.9)	129.0 (0.8)
Diastolic blood pressure (mm Hg)	81.9 (0.5)	83.0 (0.3)	85.3 (0.5)	83.2 (0.9)	77.0 (0.3)	79.6 (0.2)	80.8 (0.6)	78.6 (0.5)
Antihypertensive use (%)	18.6	17.6	22.1	22.8	13.3	18.1	22.3	15.0
Hypertension (%)[b]	48.3	49.3	56.8	52.2	31.5	41.4	46.0	34.8
Diabetes mellitus (%)[c]	9.2	9.2	8.8	9.3	3.4	4.3	6.2	3.8
Hypercholesterolemia (%)[d]	30.2	35.6	37.4	28.9	46.7	51.8	55.4	52.7
Cigarette smoking (%)								
never	22.4	15.6	15.0	18.2	92.5	91.1	87.4	84.8
ex-smoker	34.5	35.8	36.1	33.7	2.6	3.2	4.3	5.2
<20 cigarettes per day	13.6	14.1	10.9	13.7	3.9	4.2	4.6	6.2
≥20 cigarettes per day	29.5	34.6	38.1	34.3	1.0	1.6	3.7	3.8
Alcohol consumption (%)								
never	23.2	19.0	17.4	20.2	82.5	77.8	72.5	74.8
ex-drinker	10.5	6.2	6.1	9.9	3.1	3.8	6.6	5.1
<23 g ethanol per day	21.9	24.2	21.0	18.4	13.2	16.1	16.1	16.1
≥23 g ethanol per day	44.4	50.6	55.6	51.5	1.2	2.3	4.9	4.0
Menopause (%)	—	—	—	—	67.1	70.0	71.5	69.2

[a]Age was not included in the adjustment variables.
[b]Hypertension was defined as blood pressure ≥140/90 mm Hg or current treatment.
[c]Diabetes mellitus was defined as fasting blood glucose ≥126 mg/dl, non-fasting blood glucose ≥200 mg/dl, or current treatment.
[d]Hypercholesterolemia was defined as total cholesterol ≥220 mg/dl or current treatment.

Table 3. Sex-specific age- and community-adjusted and multivariable-adjusted hazard ratios (95% CI) for incidence of cardiovascular events according to snoring frequency

Snoring frequency	Men				Women			
	Never	Sometimes	Daily	Unknown	Never	Sometimes	Daily	Unknown
Person-years	2792	7014	3427	951	8462	11715	2119	3268
Subjects (*n*)	486	1144	561	159	1399	1900	352	512
Incident cardiovascular event (*n*)	15	22	16	3	13	16	8	4
Incident ischemic heart disease (*n*)	7	10	7	2	1	4	2	1
Incident stroke (*n*)	8	12	9	1	12	12	6	3
Age- and community-adjusted HR (95% CI)	(Reference)	0.7 (0.4–1.4)	1.1 (0.5–2.2)	0.7 (0.2–2.3)	(Reference)	0.9 (0.5–2.0)	2.6 (1.1–6.3)	0.8 (0.2–2.4)
Model 1 HR (95% CI)[a]	(Reference)	0.7 (0.3–1.3)	1.0 (0.5–2.1)	0.7 (0.2–2.3)	(Reference)	0.9 (0.4–2.0)	2.5 (1.0–6.1)	0.8 (0.2–2.4)
Model 2 HR (95% CI)[b]	(Reference)	0.6 (0.3–1.3)	1.0 (0.5–2.0)	0.6 (0.2–2.3)	(Reference)	0.9 (0.4–1.9)	2.1 (0.9–5.4)	0.8 (0.2–2.4)
Model 3 HR (95% CI)[c]	(Reference)	0.6 (0.3–1.2)	0.9 (0.4–1.9)	0.6 (0.2–2.1)	(Reference)	0.8 (0.4–1.7)	1.9 (0.8–4.9)	0.7 (0.2–2.2)

HR: hazard ratio; CI: confidence interval.
[a]Model 1 was adjusted for age, alcohol consumption, cigarette smoking, community, and, for women, menopausal status at baseline.
[b]Model 2 was adjusted for factors in Model 1 plus body mass index.
[c]Model 3 was adjusted for factors in Model 2 plus systolic blood pressure, antihypertensive medication use, diabetes mellitus, and hypercholesterolemia.

the hypothesis that snoring induces cardiovascular events by increasing the risk of hypertension and metabolic disorders, we further adjusted for systolic blood pressure, antihypertensive medication use, diabetes mellitus and hypercholesterolemia. The respective HRs (95% CI) for cardiovascular events were 0.8 (0.4–1.7) and 1.9 (0.8–4.9; Table 3, Model 3). The association between everyday snoring and risk of cardiovascular events was not significantly modified by sex (P for interaction = 0.12). The risk of cardiovascular events associated with unknown snoring was not increased and was similar to the risk associated with sometimes snoring in both men and women.

DISCUSSION

In the present prospective study, snoring frequency was associated with an increased incidence of cardiovascular events among community-dwelling middle-aged Japanese

women. This association was independent of age and other confounding factors. As compared with never snorers, 'everyday snoring' women had a 2.5-fold higher risk of cardiovascular events during 6 years of follow-up. The association of everyday snoring with cardiovascular events was attenuated after adjustment for BMI and after further adjustment for systolic blood pressure, antihypertensive medication use, diabetes mellitus, and hypercholesterolemia. This suggested that overweight partly mediated the association and that hypertension and metabolic abnormalities partly caused by snoring contribute to the risk of cardiovascular events in women who snore every day. This is the first study to show a relationship between habitual snoring and risk of cardiovascular events among a population in Asia, which has a low prevalence of obesity.

The biological mechanisms that link habitual snoring to the development of cardiovascular disease remain to be fully elucidated, but a number of mechanisms have been proposed. Habitual snoring is often accompanied by sleep apnea or hypopnea. Repetitive episodes of intermittent complete and partial airway collapse during sleep result in hypoxemia, hypercapnia, changes in intrathoracic pressure, and repeated arousal from sleep. Episodes of snoring and apneic events can cause acute hemodynamic changes[11] (such as increased cardiac output, enhanced cardiac arrhythmia, patent foramen ovale appearance,[23] increased intracranial pressure, and decreased cerebral blood flow),[24] increased platelet aggregation[25] and fibrinogen concentrations,[26] and decreased fibrinolysis, which directly affect the cardiovascular system. Abnormal metabolic conditions such as hypertension, diabetes mellitus, and hypercholesterolemia may also increase the risk of cardiovascular disease via elevation of sympathetic activation,[27,28] oxidative stress,[29] activation of the hypothalamic-pituitary-adrenal axis due to sleep fragmentation,[30,31] and endothelial dysfunction.[32]

In the present study, habitual snorers were more likely to be overweight, hypertensive, and diabetic than non-snorers, and the association between snoring frequency and the risk of cardiovascular events was attenuated when we further adjusted for these factors. This suggests that overweight partly mediates the association and that habitual snoring increases the risk of cardiovascular events partly through increasing the risk of hypertension and metabolic disorders.

The present results are consistent with those from studies of Western women[12] but not men[13]; however, this study is the first to note an independent association in a population with a different distribution of cardiovascular disease subtypes and a low prevalence of obesity. Among Japanese, 70% of cardiovascular events are strokes—whereas in Western countries ischemic heart disease is the largest cause of such events—and the risk factors for cardiovascular events among populations with low obesity are hypertension and metabolic abnormalities rather than overweight.[33]

In contrast to the significant association of habitual snoring with cardiovascular events in women, no such association was observed in men. Large population-based prospective studies of middle-aged men,[13] women,[12] and a population of men and women aged 20 years or older[34] have reported positive associations between habitual snoring and cardiovascular events. However, no study has reported a sex difference in the association. Recent reports from the Sleep Heart Health Study (a large population-based study of American residents aged 40 or older) have noted sex differences in the association between SDB, as defined by the apnea-hypopnea index (AHI), and the risk of coronary heart disease, heart failure, and stroke.[35,36] The multivariable HRs associated with a 10-unit increase in AHI were 1.1 (1.0–1.3) for incident heart failure in men and 1.1 (1.0–1.2) for incident coronary heart disease in men aged 70 years or younger, whereas no such associations were observed in women.[35] Similarly, the multivariable HR for ischemic stroke incidence was 2.9 (1.1–7.4) in men and 1.2 (0.7–2.2) in women for the highest (>19) as compared with the lowest (≤4) AHI quartiles.[36] The reasons for the present lack of association between habitual snoring and risk of cardiovascular events in men are unknown. We found no association in men when the analysis was stratified by age, smoking, or drinking status. Further research is necessary to elucidate this sex difference.

The strengths of the present study include the use of systematic surveillance of cardiovascular events and complete data collection on incident stroke and ischemic heart disease, including sudden cardiac death. Our large population-based prospective cohort study enabled us to examine sex-specific associations between snoring frequency and risk of cardiovascular events and provides the first evidence of a positive association between these conditions in an Asian population.

The limitations of the present study are as follows: first, our data on snoring frequency were obtained from a self-reported questionnaire, so lack of awareness of snoring or the absence of a sleep partner may have resulted in misclassification. However, in our simultaneous subsample study (1564 men and 2806 women aged 40–69 years) using a 3% oxygen desaturation index (ODI) measured by pulse oxymetry (PULSOX-3Si; Minolta, Osaka, Japan) during 1 night of sleep at a participants' homes, we found that the proportion of SDB (ODI ≥5 events/hours) was 22% in never snorers, 36% in sometime snorers, and 50% in everyday snorers among men. The respective proportions for women were 9%, 19%, and 34%. Thus, self-reported snoring seemed to be reliable. Second, data on sleep duration were not obtained in this study. According to a recent meta-analysis, both short (≤5–6 hours per night in most studies) and long sleep duration (≥8–9 hours per night in most studies) were associated with increased risks of coronary heart disease and stroke.[37] Further studies of the effects of sleep quality and quantity on the risk of cardiovascular disease will be necessary to confirm an effect of habitual snoring.

In summary, the present large cohort study showed that habitual snoring was associated with an increased risk of cardiovascular events among community-dwelling middle-aged Japanese women and that overweight, snoring-related hypertension, and metabolic disorders may partly mediate the association. The present study provides epidemiologic evidence for physicians and other health professionals that habitual snoring should be considered in the prevention of cardiovascular disease among middle-aged Japanese.

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Conflicts of interest: None declared.

APPENDIX

CIRCS investigators

The Circulatory Risk in Communities Study (CIRCS) is a collaborative study managed by the Osaka Medical Center for Health Science and Promotion, Osaka University, University of Tsukuba, and Ehime University. CIRCS investigators who contributed to this study are as follows: Hiroyasu Iso, Tetsuya Ohira, Hironori Imano, Renzhe Cui, Ai Ikeda, Hiroyuki Noda, Satoyo Ikehara, Isao Muraki, and Kotatsu Maruyama, Osaka University, Suita; Tomoko Sankai and Kazumasa Yamagishi, Mitsumasa Umesawa, Choy-Lye Chei, Kimiko Yokota, and Minako Tabata, University of Tsukuba, Tsukuba; Masamitsu Konishi, Yoshinori Ishikawa, Masakazu Nakamura, Akihiko Kitamura, Masahiko Kiyama, Takeo Okada, Kenji Maeda, Masatoshi Ido, Masakazu Nakamura, Takashi Shimamoto, Minoru Iida, and Yoshio Komachi, Osaka Medical Center for Health Science and Promotion, Osaka; Shinichi Sato, Chiba Prefectural Institute of Public Health, Chiba; Takeshi Tanigawa, Isao Saito, Susumu Sakurai, and Shinichi Hitsumoto Ehime University, Toon; Masayuki Yao, Ranryoen Hospital, Ibaraki.

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