ORIGINAL ARTICLE

Cumulative Hypoxemia During Sleep Predicts Vascular Endothelial Dysfunction in Patients With Sleep-Disordered Breathing

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BACKGROUND
Sleep-disordered breathing (SDB) is associated with repeated intermittent hypoxemia, and it is known as one of the risk factors for cardiovascular diseases. Previous studies assessing the effects of frequency and depth of hypoxemia on cardiovascular diseases have shown conflicting results. The aim of the current study was to clarify what SDB-related parameters most predict endothelial dysfunction to better understand the pathogenesis of endothelial dysfunction in patients with SDB.

METHODS
We conducted polysomnography (PSG) and measured flow-mediated vasodilation response (%FMD) in 50 outpatients suspected of SDB. Evaluated indices included: apnea-hypopnea index (AHI), 3% oxygen desaturation index (3%ODI), averaged arterial oxygen saturation (averaged SpO_2), lowest arterial oxygen saturation (lowest SpO_2), ratio of arterial oxygen saturation <90% (<SpO_2 90%), and averaged time desaturation summation index (TDS: [100%-averaged SpO_2] × total sleep time).

RESULTS
Significant differences were observed only in the TDS between the first and third (P = 0.03) and between the first and forth (P = 0.04) quartile groups, stratified by %FMD. The %FMD showed a significant relationship with TDS (β = −0.47, P = 0.001), even after adjusting for confounding factors (β = −0.33, P = 0.02). In contrast, AHI, 3%ODI, averaged SpO_2, lowest SpO_2, and <SpO_2 90% showed no significant relationships.

CONCLUSIONS
This study shows the validity of TDS in predicting endothelial damage in patients with SDB. Cumulative hypoxemia, rather than the frequency of hypoxemic events presented as AHI, may be a greater contributing factor in causing endothelial dysfunction. A simple index like TDS may be a useful and novel indicator of the influence of SDB on the vasculature.

Keywords: blood pressure; cardiovascular diseases; endothelial function; hypertension; hypoxemia; sleep-disordered breathing.

doi:10.1093/ajh/hpv135

Sleep-disordered breathing (SDB) is associated with recurrent hypoxemia and arousal during sleep. The prevalence of SDB is reported to be 1.2–7.5% in the general population.[1] Intermittent hypoxemia by SDB has been reported to produce oxygen-derived free radicals, which can impair endothelial function.[2] Previous studies have shown that endothelial dysfunction is strongly related to the pathogenesis of hypertension.[3,4] Furthermore, endothelial dysfunction leads to cardiovascular disorders.[5] Thus, hypoxemic stress via SDB may play a key role in inducing cardiovascular events in patients with SDB.[6–9]

However, although it is clear that SDB worsens prognosis and increases cardiovascular events, the previous studies on the relationship between the severity of SDB and cardiovascular events showed conflicting results.[10–13] Some studies suggest that the frequency of apnea and hypopnea is weakly related with rates of cardiovascular events.[10,12,13] In patients with chronic heart failure, complicated with Cheyne-Stokes respiration, mortality was shown to be related to the existence and severity of SDB, which is represented by the apnea-hypopnea index (AHI).[11] Thus far, most previous studies on the influence of SDB have been carried out using AHI, i.e., frequency of apneic/hypopneic episodes as a marker of SDB insult. We speculate that, as for evaluating cardiovascular outcome in patients with SDB, conventional indices of SDB, such as AHI or oxygen desaturation index (ODI), might not adequately indicate the effects of intermittent hypoxemia; as such indices do not contain enough information concerning the accumulation of desaturation. Therefore, we hypothesized that another index, which includes information on the

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severity and duration of hypoxemia caused by SDB, would be a better predictor of adverse impacts on the cardiovascular system compared with AHI. Thus, the aim of this study was to clarify what factors of SDB most relate to endothelial dysfunction to better understand the pathogenesis of endothelial dysfunction in patients with SDB.

METHODS

Subjects

We recruited 50 outpatients who were referred to our clinic to diagnose SDB using in-hospital polysomnography (PSG). Inclusion criteria for this study were as follows: (i) patients aged 20 years or older and (ii) AHI ≥5/hour by measurements using Type-1 PSG. Exclusion criteria were as follows: (i) patients diagnosed with pulmonary or respiratory diseases, (ii) with overt heart failure, and (iii) that had previously undergone or were currently undergoing treatment for SDB. This study was approved by the Ethics Committee of Kyushu University Graduate School of Medical Sciences. The aims of the study were explained and written informed consent was obtained from all subjects.

Measurements

We acquired data on clinical characteristics from medical records. These included sex, age, body mass index (BMI), history of smoking, underlying diseases (e.g., existence of diagnosed hypertension, diabetes mellitus, and dyslipidemia), use of medications (e.g., angiotensin receptor blocker (ARB), angiotensin-converting enzyme inhibitor (ACE-I), and calcium channel blocker), and overnight PSG data. We developed an index called the time desaturation summation index (TDS) to represent cumulative hypoxemia in a simple fashion. The formula for obtaining TDS is expressed as follows: TDS = (100%-averaged arterial oxygen saturation during sleep [averaged SpO_2]) × (multiplied by) total sleep time (hours). The flow-mediated vasodilation response (%FMD) was measured by a single technician blinded to patient information using automated recording, and an analysis system with a cross-sectional 10-MHz linear-array ultrasound probe (UNEX-EF, Unex, Nagoya, Japan) was used to provide the index of endothelial function.[5] The protocol of %FMD measurements followed published guidelines,[14] and has been described elsewhere.[15,16] Briefly, the cuff was placed distal to the probe, and we measured changes in arterial diameter during 2 minutes after cuff release. The arterial measurements were taken with electrocardiography gating. Systolic blood pressure and diastolic blood pressure were measured before %FMD measurements.

Polysomnography

Overnight PSG comprised 4-channel electroencephalography, electrooculogram, submental and leg electromyography, electrocardiography, nasal pressure sensor, thoracic and abdominal bands, oral thermistor, and oxygen saturation with fingertip pulse oximetry (Nihon-Koden, Tokyo, Japan). Respiratory events and sleep stages were scored using

criteria as set out by the 2007 guidelines of the American Academy of Sleep Medicine, and Rechtschaffen and Kales, respectively.[17,18] Briefly, an apnea event was defined as any reduction in nasal-oral thermistor ≥90% for over 10 seconds. A hypopnea event was defined as any reduction in nasal pressure ≥50% for over 10 seconds associated with 3% desaturation or arousal. AHI and 3%ODI were calculated as the number of respiratory events per hour, and the number of oxygen desaturations ≥3% per hour of sleep, respectively. The ratio of arterial oxygen saturation <90% (SpO_2 < 90%) was defined as the percentage of time of arterial oxygen saturation level <90% in total sleeping time.

Table 1. Clinical characteristics

Variable	
Subjects (N)	50
Age (years)	58 ± 13
Male (N [%])	43 [86]
BMI (kg/cm^2)	27.0 ± 5.1
Smoking (N [%])	16 [32]
Hypertension (N [%])	31 [62]
Diabetes mellitus (N [%])	16 [32]
Dyslipidemia (N [%])	15 [30]
ARB (N [%])	8 [16]
ACE-I (N [%])	2 [4]
Ca channel blocker (N [%])	6 [12]
SBP (mm Hg)	129.6 ± 18.9
DBP (mm Hg)	80.3 ± 10.4
HR (bpm)	72.2 ± 10.4
Polysomnography	
AHI (/hour)	44.6 ± 22.5
OAI (/hour)	16.0 ± 19.4
CAI (/hour)	1.1 ± 2.9
HI (/hour)	25.4 ± 15.2
TST (minute)	392 ± 81
3%ODI (/hour)	33.5 ± 19.9
Averaged SpO_2 (%)	94.0 ± 1.8
Lowest SpO_2 (%)	74.9 ± 9.6
SpO_2 < 90% (%)	8.1 ± 9.0
TDS (% × hour)	38.8 ± 11.5
FMD data	
Arterial diameter (mm)	4.3 ± 0.6
%FMD (%)	4.2 ± 2.3

Abbreviations: N, number; BMI, body mass index; ARB, angiotensin receptor blocker; ACE-I, angiotensin-converting enzyme inhibitor; Ca, calcium; FMD, flow-mediated vasodilation; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; PSG, polysomnography; AHI, apnea-hypopnea index, OAI, obstructive apnea index; CAI, central apnea index; HI, hypopnea index; TST, total sleep time; SpO_2, arterial oxygen saturation; ODI, oxygen desaturation index; TDS, time desaturation summation index.

Statistical analysis

After assessment of the Gaussian distribution of the data using the Kolmogorov–Smirnov test, the data were analyzed using analysis of variance with a Bonferroni *post hoc* test. The chi-square test was used to analyze binary data. Using general linear model analysis (i.e., single linear analysis and logistic regression analysis), we identified confounders for %FMD. If the significance of a variable was $P < 0.1$ in the general liner model analysis, we regarded it as a potential confounder. The identified confounders were included multivariate analysis as adjustments. To assess correlations, we used Pearson correlation analysis. All statistics were compiled using SPSS v21.0 (SPSS, Chicago, IL), and 2-sided tests were performed, with $P < 0.05$ being considered significant. Outcome values are expressed as mean ± SD, correlation coefficient (r), and standardized coefficient (β).

RESULTS

Clinical characteristics

A total of 50 patients with varying severity of SDB were included in this study. The clinical characteristics of these participants are shown in Table 1. The mean age was 58 years, with 86% being male. BMI ranged from 16 to 40 kg/m². With regards to underlying health conditions, 62% of the participants were hypertensive, 32% had diabetes, and 30% had dyslipidemia. A total of 16% of participants used ARB (4%) and ACE-I and calcium channel blocker (12%). The majority of patients were moderate to severe SDB (AHI: 44.6 ± 22.5/hour), and their %FMD was 4.2 ± 2.3%. The mean total sleep time, averaged SpO_2, and $SpO_2 < 90\%$ were 392 minutes, 94.0%, and 8.1%, respectively. The mean value of calculated TDS taken from these values was 38.8%/hour.

The clinical characteristics among quartile groups stratified by %FMD

Table 2 shows the differences in clinical characteristics among quartile groups stratified by %FMD. Although there were no significant differences in age, BMI, the existence of comorbidities (i.e., hypertension, diabetes mellitus, and dyslipidemia), use of medications, AHI, 3%ODI, averaged SpO_2, lowest arterial oxygen saturation (lowest SpO_2), and $SpO_2 < 90\%$ among any of the 4 groups, significant differences were observed in TDS between the 1st and 3rd and

Table 2. The clinical characteristics in the patients stratified by flow-mediated vasodilation

	1st quartile	2nd quartile	3rd quartile	4th quartile	*P* value
Number	12	13	12	13	—
%FMD (%)	7.3 ± 1.4	4.7 ± 0.3**	3.3 ± 0.4**,†	1.5 ± 0.9**,‡,§	<0.001
Age (years)	57 ± 15	59 ± 12	61 ± 10	57 ± 16	0.87
BMI (kg/cm²)	25.0 ± 6.2	27.0 ± 4.9	28.2 ± 4.3	27.8 ± 4.7	0.43
Smoking (*N* [%])	3 [25]	6 [46]	3 [25]	4 [31]	0.63
Hypertension (*N* [%])	6 [50]	5 [39]	9 [75]	11 [85]	0.06
Diabetes mellitus (*N* [%])	3 [25]	2 [15]	4 [33]	7 [54]	0.19
Dyslipidemia (*N* [%])	2 [17]	5 [39]	2 [17]	6 [46]	0.25
ARB (*N* [%])	1 [8]	1 [8]	1 [8]	5 [38]	0.09
ACE-I (*N* [%])	1 [8]	0 [0]	0 [0]	1 [8]	0.55
Ca channel blocker (*N* [%])	1 [8]	1 [8]	0 [0]	4 [31]	0.10
AHI (/hour)	49.3 ± 24.6	41.3 ± 21.2	50.0 ± 23.9	38.7 ± 20.9	0.51
OAI (/hour)	24.6 ± 26.1	13.4 ± 10.6	18.3 ± 25.2	8.4 ± 8.8	0.19
CAI (/hour)	0.6 ± 0.7	1.1 ± 1.7	2.3 ± 5.4	0.4 ± 1.0	0.36
HI (/hour)	22.0 ± 12.7	23.7 ± 16.2	26.5 ± 16.1	29.1 ± 16.4	0.67
TST (minute)	347 ± 80	425 ± 103	373 ± 55	418 ± 60	0.04
3%ODI (/hour)	33.3 ± 21.0	27.2 ± 13.4	43.7 ± 21.9	30.5 ± 20.9	0.19
Averaged SpO_2 (%)	94.5 ± 1.8	94.5 ± 1.8	93.2 ± 1.6	93.9 ± 1.7	0.22
Lowest SpO_2 (%)	73.7 ± 11.5	77.4 ± 7.7	75.5 ± 8.1	73.0 ± 11.0	0.66
$SpO_2 < 90\%$ (%)	7.9 ± 10.2	6.7 ± 6.9	9.5 ± 10.4	8.3 ± 9.1	0.90
TDS (% × hour)	30.6 ± 6.8	38.4 ± 11.8	43.2 ± 10.7*	42.8 ± 12.1*	0.02

Abbreviations: FMD, flow-mediated vasodilation; BMI, body mass index; ARB, angiotensin receptor blocker; ACE-I, angiotensin-converting enzyme inhibitor; Ca, calcium; AHI, apnea-hypopnea index; OAI, obstructive apnea index; CAI, central apnea index; HI, hypopnea index; TST, total sleep time; ODI, oxygen desaturation index; SpO_2, arterial oxygen saturation; TDS, time desaturation summation index.

*vs. 1st quartile group, $P < 0.05$. †vs. 2nd quartile group, $P < 0.01$. **vs. 1st quartile group, $P < 0.001$. ‡vs. 2nd quartile group, $P < 0.001$. §vs. 3rd quartile group, $P < 0.001$.

between the 1st and 4th quartile groups (F[49, 9] = 3.72, $P = 0.02$; 1st vs. 3rd quartile, $P = 0.03$; 1st vs. 4th quartile, $P = 0.04$).

The relationships between clinical characteristics and flow-mediated vasodilation response

%FMD was marginally related to BMI, the existence of hypertension, and use of ARB in a single regression model (BMI: $\beta = -0.26$, $P = 0.07$; hypertension: $\beta = -0.25$, $P = 0.07$; ARB: $\beta = -0.38$, $P = 0.08$; Table 3). %FMD showed a significant relationship with TDS only ($P = 0.001$; Table 3, Figure 1). Even in multiple regression analysis after adjusting for BMI, the existence of hypertension, and use of ARB, the relationship between %FMD and TDS remained significant ($\beta = -0.33$, $P = 0.02$).

Table 3. Unadjusted single regression analysis about the relationships between clinical characteristics and flow-mediated vasodilation

Variable	β	*P* value
Age	0.01	0.94
Sex	0.12	0.51
BMI	−0.26	0.07
Smoking	0.04	0.77
Hypertension	−0.25	0.07
Diabetes mellitus	−0.20	0.17
Dyslipidemia	−0.24	0.12
ARB	−0.38	0.08
ACE-I	0.16	0.58
Ca channel blocker	−0.35	0.14
SBP	−0.15	0.30
DBP	0.17	0.25
HR	0.02	0.91
AHI	0.06	0.68
OAI	0.20	0.18
CAI	−0.09	0.52
HI	−0.15	0.29
3%ODI	−0.13	0.37
Averaged SpO$_2$	0.29	0.11
Lowest SpO$_2$	0.10	0.70
SpO$_2$ < 90%	−0.10	0.48
Arterial diameter	−0.23	0.11
TDS	−0.47	0.001

Abbreviations: β, standardizing coefficient; BMI, body mass index; ARB, angiotensin receptor blocker; ACE-I, angiotensin-converting enzyme inhibitor; Ca, calcium; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; AHI, apnea-hypopnea index; OAI, obstructive apnea index; CAI, central apnea index; HI, hypopnea index; ODI, oxygen desaturation index; SpO$_2$, arterial oxygen saturation; TDS, time desaturation summation index.

DISCUSSION

The current study revealed that endothelial function was most strongly and inversely related to cumulative hypoxemia during sleep, as expressed by the TDS index in patients with SDB. In addition, we did not find any significant correlations between %FMD and traditional indices; AHI, 3%ODI, averaged SpO$_2$, or lowest SpO$_2$. This result suggests that cumulative hypoxemia during sleep is likely to be the most influential factor leading to cardiovascular diseases in patients with SDB.

Previous studies reported that hypertension is common in patients with SDB, ranging from 37% to 54%.[19] Moreover, a European-based large cohort study showed that the hypoxic state associated with SDB was significantly related to new onset hypertension.[20] An etiology of hypertension in patients with SDB is endothelial dysfunction.[21] The mechanisms of endothelial dysfunction associated with SDB, caused by hypoxemia during apneic sleep, involve the hypoxic state increasing the amount of inflammatory agents, such as hypoxia-inducible factor and nuclear factor-κB.[22,23] Hypoxia-inducible factor upregulation leads to loss of p27 induction, which is a cyclin-dependent kinase that plays a critical role in cell cycle arrest.[24] nuclear factor-κB upregulation contributes to the hypoxic state of cells through DNA binding activity, which leads to inflammation, increases in various cytokines, and adhesion of monocytes.[25] Finally, the actions of hypoxia-inducible factor and nuclear factor-κB can lead to endothelial cell apoptosis.[26,27] Thus, cumulative hypoxemia during sleep may have a strong impact on endothelial function. Specifically, hypoxia-inducible factor and nuclear factor-κB are known to increase according to the lowered oxygen saturation level and sustained hypoxemia.[28] Conventional indices of SDB such as AHI and 3%ODI do not directly reflect the degree of hypoxemia. This may be one of the possible reasons for the inconsistent results regarding the relationships between %FMD, AHI, and 3%ODI.[29–32] On the other hand, SpO$_2$ < 90% reflects the degree of hypoxemia to a limited extent, because this index measures the duration of atrial oxygen level under 90% only. In contrast, the TDS index contains information concerning oxygen saturation level and duration of hypoxemia during the entire sleep period. Although TDS may not perfectly reflect the actual amount of inflammatory agents, we believe that TDS would approximate actual cumulative hypoxemia more accurately compared with other conventional indices. We set the baseline saturation level as 100% in calculations of TDS, because the fluctuation of "baseline" oxygen level was quite large in some patients even while they were awake. It is likely difficult to set an accurate "baseline" level for these types of patients. Moreover, clinically, it would be easier to measure a total decrease from 100%.

Recently, SDB has been shown to have a strong, negative impact on the cardiovascular system through activation of the sympathetic nervous system, negative intra-thoracic pressure, and hypoxemia.[33,34] Since the endothelial layer is involved in critical functions such as preventing blood coagulation, controlling vascular tone, and anti-inflammatory actions,[35] its dysfunction can cause hypertension and arteriosclerosis,[36,37] leading to heart failure, coronary artery
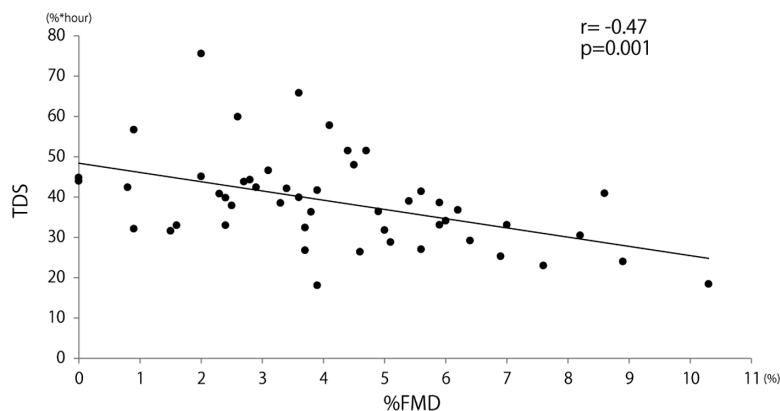
Figure 1. Correlation between averaged time desaturation summation index and flow-mediated vasodilation. Abbreviations: FMD, flow-mediated vasodilation; TDS, time desaturation summation index.

stenosis, and stroke.[33,38] Thus, a reliable index that correctly indicates cumulative hypoxemia during sleep is critical for the accurate estimation of the negative impact of SDB on the cardiovascular system.

There are some limitations of the current study. Endothelial function was likely strongly affected by plasma glucose and lipoprotein levels (i.e., hemoglobin A1c, low-density lipoprotein, and high-density lipoprotein).[39,40] In this study, we acquired data from a limited number (33 of 50) of patients. However, of these patients, %FMD was associated with BMI, hemoglobin A1c, high-density lipoprotein, hypertension, use of ARB, and TDS in a single regression analysis, but only %FMD was independently associated with TDS after adjusting for BMI, hemoglobin A1c, high-density lipoprotein, hypertension, and use of ARB (data not shown). Furthermore, %FMD was strongly correlated with TDS even in single regression analysis, indicating that TDS may be a powerful predictor.

We did not assess the nitroglycerin-induced vasodilation response. We recognize that this is a limitation that requires further study to clarify our findings. In addition, as this study was retrospective in nature, we were unable to eliminate the possibility of selection bias, though we collected patients as evenly as possible. Finally, we adopted all patients irrespective of total sleep time in this study, but patients with SDB with excessively short sleep times (e.g., 3 hours) during PSG might have significantly confounded the data because of the calculations involved in the TDS index formula. Future, large sample size, prospective studies are needed to address these issues.

In conclusion, cumulative hypoxemia during sleep, expressed by an index such as TDS in this study, may provide important information concerning endothelial function though the causality was not established, and such an index may more properly predict endothelial dysfunction in patients with SDB compared with traditional indices representing SDB severity.

DISCLOSURE

The authors declared no conflict of interest.

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