Microvascular endothelial function in patients with obstructive sleep apnea syndrome

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Abstract:
Background: Obstructive sleep apnea syndrome (OSAS) is a novel risk factor for cardiovascular disease (CVD) and thought to be associated with endothelial dysfunction. Although many reports have demonstrated endothelial dysfunction of large conduit artery by flow-mediated dilatation in patients with OSAS, only a few reports have assessed microvascular endothelial function in OSAS patients. In this study, we investigated microvascular endothelial function by reactive hyperemia-peripheral arterial tonometry (RH-PAT) in patients with OSAS and assessed the effects of continuous positive airway pressure (CPAP) treatment on microvascular endothelial function. Methods: Twelve male patients with OSAS (51±13 yrs, body mass index [BMI]: 27.6±7.1 kg/m², apnea hypopnea index [AHI]: 44±18/hr) and 12 male patients with CVD (54±13 yrs, BMI: 25.5±3.0 kg/m²) underwent an endothelial function test by RH-PAT using Endo-PAT 2000 before and 12 months after induction of CPAP treatment. Results: The baseline reactive hyperemia index (RHI) in patients with OSAS was similar to that in patients with CAD (1.78±0.48 vs 1.79±0.36, P=0.878). In patients with OSAS, the RHI increased 12 months after CPAP treatment (to 2.32±0.40; P=0.003). The change in RHI from baseline after CPAP treatment tended to have a negative correlation with the baseline 3% oxygen desaturation index (3% ODI) (R=-0.526, P=0.079) and was correlated negatively with the baseline AHI (R=-0.607, P=0.036). Conclusion: Microvascular endothelial function is impaired in patients with OSAS, but improves after CPAP treatment. In addition, the improvement of microvascular endothelial function through CPAP treatment was driven by mainly patients with mild to moderate OSAS at baseline.

Key words: Obstructive sleep apnea, Vascular endothelial function, Microvasculature, Reactive hyperemia-peripheral arterial tonometry, Continuous positive airway pressure

Introduction
Obstructive sleep apnea syndrome (OSAS) has recently been acknowledged as a novel risk factor for cardiovascular disease (including atherosclerosis) that is independent of conventional risk factors¹-³. OSAS is thought to be associated with vascular endothelial dysfunction, possibly mediated by intermittent hypoxia and sleep disruption⁴. Brachial artery flow-mediated dilation (FMD) and reactive hyperemia-peripheral arterial tonometry (RH-PAT) are established methods to assess vascular endothelial function. Although FMD assesses vascular endothelial function of the large conduit arteries⁵, the reactive hyperemia index (RHI) measured by RH-PAT reflects endothelial function of the microvasculature (i.e., resistance vessels)⁶. The FMD and RHI both predict cardiovascular events, although each measures vascular function in different vessels⁷,⁸. There are many reports showing that OSAS is associated with vascular endothelial dysfunction, as demonstrated by a decrease in FMD⁹-¹⁰; however, only a few reports have assessed RH-PAT-based microvascular endothelial function in patients with OSAS¹¹. In this study, we investigated endothelial...
function of the microvasculature by RH-PAT in patients with OSAS and assessed the effects of continuous positive airway pressure (CPAP) on the RHI measured by RH-PAT.

**Methods**

**Subjects**

We enrolled 12 male patients (age: 51±13 yrs, body mass index [BMI]: 27.6±7.1 kg/m²) with OSAS but without any cardiovascular diseases (CVD), who successfully underwent CPAP treatment for 12 months. In addition, the patients had an RH-PAT test both before and 12 months after CPAP treatment. The presence of OSAS was diagnosed by the sleep study. Successful CPAP treatment was based on patients’ declaration and was defined as more than 4 hr/night of CPAP adherence. In these patients, the background treatments were not changed during 12 months of the CPAP treatment. We also enrolled 12 male CVD patients who underwent the RH-PAT test, and their age and body mass index (BMI) were matched to the OSAS patients (age: 54±13 yrs, BMI: 25.5±3.0 kg/m²), to serve as a control group. In these patients, CVD included coronary artery disease (n=5), dilated cardiomyopathy (n=3), atrial fibrillation (n=3) and aortic stenosis (n=1). These patients did not undergo a sleep study; thus, the possibility of subclinical OSAS could not be excluded. The local institutional review board of Dokkyo Medical University approved the study protocol, and written informed consent was obtained from each patient.

**Sleep study**

Overnight pulse oximetry was performed while the patients were breathing room air under stable conditions as a screening test for sleep-related breathing disorders. An oxygen saturation monitor (Pulsox-M24², Konica Minolta Sensing Inc., Osaka, Japan) was attached to the left fourth finger to determine oxygen saturation (SpO₂) and pulse rate from 10 pm to 6 am. The frequency of reduction of SpO₂ by ≥3% per hour (3% oxygen desaturation index, 3% ODI) and the lowest SpO₂ were used as parameters to identify sleep-related breathing disorders. For the patients who had ≥ 5 oxygen desaturation index events, portable polysomnography with electroencephalography (Sleep Watcher®, Compumedics Ltd, Abbotsford, Australia) was performed to assess obstructive sleep apnea. The parameters were analyzed by 2 experienced technicians who were unaware of the study design. A respiratory amplitude reduction ≥50% was defined as hypopnea and ≥80% as apnea, and the number of apnea or hypopnea events/hour was determined as the apnea-hypopnea index (AHI). Obstructive sleep apnea was defined as an AHI ≥5, based on the recommendation of the American Academy of Sleep Medicine Task Force³⁰.

**RH-PAT test**

The RH-PAT test was performed using an EndoPAT 2000 device (Itamar Medical, Caesarea, Israel), as described previously³⁰. Briefly, vasoactive drugs were withheld on the day of measurement. They then rested in the supine position for at least 15 min in a quiet, temperature-controlled room. A blood pressure cuff was placed on one upper arm, while the contralateral arm served as a control. PAT probes were placed on a finger of each hand. After a 5-min equilibration period, the cuff was inflated to 60 mmHg above systolic pressure (if systolic blood pressure was <140 mmHg) or to 200 mmHg (if systolic blood pressure was >140 mmHg) for 5 min and then deflated to induce reactive hyperemia. The RHI was calculated as the ratio between the signals measured at baseline and 5 min after upper-arm occlusion, relative to the response in the contralateral arm.

**Statistical analysis**

Values for continuous variables are expressed as the mean ± standard deviation or the number (percent) of patients for categorical variables. Intergroup and intragroup comparisons for continuous variables were performed using an unpaired and a paired t-test, respectively. Categorical variables were assessed using a chi-square test. A P value <0.05 was considered significant.

**Results**

In patients with OSAS, the proportion of patients who had 3% ODI at baseline was 41±28%, and the AHI at baseline was 44±18/hr. Baseline characteristics of the patients with OSAS and those with CVD are shown in Table 1. The prevalence of diabetes tended to be lower and diastolic blood pressure tended to be higher in the OSAS patients, compared with the CVD patients. Usage of statin was significantly higher in the CVD patients, compared with the OSAS patients. There were no significant differences in age, BMI, the prevalence of hypertension, dyslipidemia, smoking habit and systolic blood pressure, the levels of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride, and the usage of renin-angiotensin system inhibitors or anti-diabetic agents between the 2 groups. The RHI in patients with OSAS at baseline before CPAP treatment was similar to that in patients with CVD (1.78±0.48 vs 1.79±0.36, P=0.878) (Figure 1A). In patients with OSAS, the RHI significantly increased after 12 months of CPAP treatment (to 2.14±0.45, P=0.003) (Figure 1B). In patients with OSAS, the baseline RHI was not correlated with either the baseline 3% ODI (R=0.291, P=0.359) or the baseline AHI (R=0.382, P=0.221) (Figure 2A). However, the change in RHI after CPAP treatment tended to show a negative correlation with the baseline 3% ODI (R=-0.526, P=0.079) and was correlated negatively with the baseline AHI (R=-0.607, P=0.036) (Figure 2B).

**Discussion**

In the present study, we demonstrated that RHI measured by RH-PAT was similar between patients with OSAS and
Figure 1. RHI in patients with OSAS. (A) The baseline RHI before CPAP treatment in patients with OSAS was similar to that in patients with cardiovascular disease (CVD). (B) In patients with OSAS, RHI significantly increased after 12 months of CPAP treatment. RHI, reactive hyperemia index; OSAS, obstructive sleep apnea syndrome; CVD, cardiovascular disease.

Table 1. Baseline characteristics in patients with OSAS and those with CVD

<table>
<thead>
<tr>
<th></th>
<th>Patients with OSAS (n=12)</th>
<th>Patients with CVD (n=12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>51±13</td>
<td>54±13</td>
<td>0.486</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.6±7.1</td>
<td>25.5±7.1</td>
<td>0.313</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>6 (50)</td>
<td>5 (42)</td>
<td>0.680</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>1 (8)</td>
<td>5 (42)</td>
<td>0.059</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>2 (17)</td>
<td>6 (50)</td>
<td>0.110</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>6 (50)</td>
<td>5 (42)</td>
<td>0.680</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>125±13</td>
<td>126±20</td>
<td>0.980</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>83±10</td>
<td>75±10</td>
<td>0.076</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dl</td>
<td>119±22</td>
<td>100±34</td>
<td>0.113</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dl</td>
<td>58±15</td>
<td>54±19</td>
<td>0.604</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>114±51</td>
<td>88±25</td>
<td>0.196</td>
</tr>
<tr>
<td>ACE inhibitors/ARB, n (%)</td>
<td>5 (42)</td>
<td>8 (67)</td>
<td>0.219</td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td>2 (17)</td>
<td>8 (67)</td>
<td>0.013</td>
</tr>
<tr>
<td>Anti-diabetic agents, n (%)</td>
<td>1 (8)</td>
<td>3 (25)</td>
<td>0.423</td>
</tr>
</tbody>
</table>

OSAS, obstructive sleep apnea syndrome; CVD, cardiovascular disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker

those with CVD, and that 12 months of treatment with CPAP significantly increased RHI. The results suggest that microvascular endothelial function was impaired in patients with OSAS, similar to the impairment in patients with CVD, but that it improved by CPAP treatment.

OSAS is characterized by the presence of apnea-hypopnea during sleep that leads to intermittent hypoxia. A relationship between OSAS and cardiovascular disease has been shown in a large clinical trial, the Sleep Heart Health Study. However, the underlying mechanisms linking OSAS and vascular pathology are not well understood. Several studies have suggested that patients with OSAS have vascular endothelial dysfunction, which is the initial step in the progression of atherosclerosis and plays a pivotal role.
in the pathophysiology of cardiovascular disease\(^\text{19}\). Impaired vascular endothelial function in patients with OSAS is believed to be caused by intermittent hypoxemia, subsequent oxidative stress, inflammatory reaction, coagulation abnormalities and lipid metabolism disorders\(^\text{20}\). In addition, increased sympathetic nerve activity in patients with OSAS may also lead to endothelial dysfunction\(^\text{21,22}\). FMD is an established method to assess \textit{in vivo} vascular endothelial function, in which the ability of the brachial artery to release endothelial nitric oxide (NO) during reactive hyperemia after a 5-minute occlusion of the artery with a blood pressure cuff is measured\(^\text{5}\). FMD predicts future cardiovascular events that may exceed the predictive ability of traditional risk factors\(^\text{25}\), and there have been many reports showing an association between OSAS and FMD\(^\text{26,27}\). On the other hand, RH-PAT has also been introduced as a surrogate marker to evaluate vascular endothelial function. RH-PAT is a noninvasive, automatic, and quantitative method of clinical assessment based on digital measurements of the hyperemic response\(^\text{14}\). Although FMD reflects endothelial function of the large conduit arteries\(^\text{5}\), the RHI measured by RH-PAT reflects endothelial function of the microvasculature\(^\text{6}\) and depends more on endothelium-derived hyperpolarizing factor (EDHF) than NO\(^\text{28}\). Endothelial function measured by RH-PAT also predicts cardiovascular events\(^\text{29}\). However, unlike FMD, RH-PAT has rarely been investigated in patients with OSAS.

Borel et al\(^\text{12}\) demonstrated that RHI measured by RH-PAT was lower in 14 patients with OSAS compared with 39 obese patients without OSAS. Afterward they performed a randomized controlled trial in 35 patients with OSAS and showed that CPAP treatment for one month (n=18) did not improve RHI, compared with lifestyle counseling in the control group (n=17)\(^\text{30}\). This result is contrary to our study, which showed improved RHI by CPAP treatment. Although the AHI in patients undergoing CPAP in the trial by Borel et al. was 40±37/hr, which seems to be nearly equivalent to 44±18/hr in OSAS patients in our present study, this discrepancy may be due to the difference in the duration of treatment (one month vs 12 months). Thus, long-term but not short-term CPAP treatment may improve impaired microvascular endothelial function in patients with OSAS.

In our results, baseline RHI before CPAP treatment was not correlated with either 3% ODI or AHI. These findings suggest that vascular endothelial dysfunction, as assessed by RH-PAT, might be independent of the severity of OSAS. Since FMD is related to the severity of OSAS\(^\text{26}\), this difference may be due to difference in the vessels that are assessed by FMD and RH-PAT (conduit arteries or microvasculature). In addition, we also demonstrated in the present study that the change in RHI after CPAP treatment tended to be negatively correlated with baseline 3% ODI and was negatively correlated with baseline AHI. In other words, a
negative correlation was observed between the effect of CPAP on improving microvascular endothelial function and the severity of OSAS. The result suggests that improvement of microvascular endothelial function through CPAP treatment was driven by mainly patients with mild to moderate OSAS at baseline and that baseline severity of OSAS can predict the response of microvascular endothelial function through CPAP treatment in patients with OSAS. From this result, we can envision that we should start CPAP treatment from early stage OSAS to improve microvascular endothelial function.

**Potential limitations**

The study has several potential limitations. The biggest limitation is that the sample size was too small for our results to be scientifically established. Especially, the lack of a correlation between baseline RHI and the baseline severity of OSAS might be a type II error. In our study, we demonstrated a correlation between the change in RHI after CPAP treatment and baseline OSAS severity. However, we could not assess whether the improvement in RHI depended on the severity of OSAS after CPAP treatment, because we did not perform sleep studies after CPAP treatment in all patients. We discussed the possible mechanism of impaired vascular endothelial function in OSAS, but we could not establish the mechanism from the present study alone. Further comprehensive assessments on the association of endothelial function with the hypoxic state during sleep, oxidative stress and autonomic nerve activity in OSAS are required. In addition, we could not determine the different roles of vascular endothelial function measured by RH-PAT from those measured by FMD (large conduit vessels vs microvasculature), and their effect on the pathophysiology of OSAS. Simultaneous measurement of both FMD and RH-PAT in patients with OSA is needed in future studies.

**Conclusions**

In this study, we demonstrated that microvascular endothelial function was impaired in patients with OSAS in a similar manner to patients with CVD. Furthermore, the impairment of endothelial function in the OSAS patients was improved by 12 months of treatment with CPAP. Since the effect of CPAP on improving microvascular endothelial function was negatively correlated with the severity of OSAS, we should start CPAP treatment from early stage OSAS to improve microvascular endothelial function.

**Conflicts of Interest**

T.I. has received honorariums from Mochida, research grants from Shionogi, Daiichi Sankyo, Takeda, Mitsubishi Tanabe, Teijin, Boehringer Ingelheim, Bayer, Abbott Vascular, Kaatsu Japan, Goodman, Clinico, St. Jude Medical, Public Health Research Center, Boston Scientific, Union Tool, and Research Institute for Production Development. The remaining authors declare no conflicts of interest.

**References**

18. Nieto FJ, Herrington DM, Redline S, Benjamin EJ, Robbins JA. Sleep apnea and markers of vascular endothelial function in a


