Original Article

Effects of Obstructive Sleep Apnea with Intermittent Hypoxia on Platelet Aggregability

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Aim: Obstructive sleep apnea (OSA) is a risk factor for cardiovascular diseases. Platelets play key roles in the development of atherothrombosis. Several studies assessing platelet activation in patients with OSA have been published; however, there have been only a few studies with a small number of patients with OSA investigating platelet aggregability, which evaluates platelet aggregation more directly than the platelet activation status. We aimed to investigate the effects of OSA and nasal continuous positive airway pressure (nCPAP) therapy, a well-established treatment for OSA, on platelet aggregability.

Methods and Results: We examined 124 consecutive patients with snoring in whom the 3% oxygen desaturation index (3%ODI), a severity marker of OSA, and ADP- and collagen-induced platelet aggregability measured with the optical aggregometer were analyzed. ADP-induced platelet aggregability was increased more in patients with moderate-to-severe OSA (3%ODI > 15) than in patients with non-to-mild OSA (p = 0.029). In multiple linear models, 3%ODI significantly contributed to increased platelet aggregability induced by both ADP and collagen among 59 subjects with one or more risk factors for vascular diseases, such as smoking, hypertension, diabetes mellitus or hyperlipidemia. In 23 patients treated by nCPAP, collagen-induced platelet aggregability was ameliorated on Day 90, compared to at the baseline.

Conclusion: The severity of OSA significantly contributed to platelet aggregability, which was improved by nCPAP treatment partially at three months.


Key words: Nasal continuous positive airway pressure, Oxygen desaturation index, Total hypoxic time

Introduction

Obstructive sleep apnea (OSA) is a common respiratory disorder in which an individual stops breathing repeatedly during sleep. OSA is associated with an increased risk for cardiovascular diseases and premature death from vascular events; however, the mechanism through which OSA increases the risk is not completely understood.

OSA is characteristically associated with repetitive oscillations in oxyhemoglobin saturation during sleep, thus resulting in chronic exposure to intermittent hypoxia and reoxygenation. Based on the results of clinical and basic research, a hypothesis has been proposed that the main cause of vascular diseases in
OSA is exposure to intermittent hypoxia, which is thought to be responsible for oxidative stress, inflammation, atherosclerosis, endothelial dysfunction and hypertension; however, how intermittent hypoxia and reoxygenation contribute to vascular diseases as compared to the total hypoxic period during sleep remains unknown.

It is believed that atheromatous plaque rupture or endothelial erosion in arteries leads to the formation of an occlusive thrombus in which the initial step is platelet aggregation on exposed collagen in the subendothelial tissues. The involvement of platelets is relevant because many studies have demonstrated that anti-platelet therapy reduces vascular events. It is therefore possible that enhanced platelet aggregability in OSA patients contributes to their increased risk for cardiovascular diseases. Presently, nasal continuous positive airway pressure (nCPAP) therapy is a well-established treatment for OSA. nCPAP therapy has been shown to reduce the increased risk for fatal and non-fatal cardiovascular events in OSA patients; therefore, nCPAP therapy may affect platelet aggregability in OSA patients, if our hypothesis is the case.

Recent reviews have indicated that studies on platelet dysfunction, hypercoagulability, thrombosis and thrombus formation in OSA patients have specific priority. There are several reports assessing the platelet activation status in patients with OSA; however, platelet aggregability is a more direct way of investigating platelet aggregation. There have been only two small-scale studies on platelet aggregability including 32 or 23 OSA patients, respectively, and these studies reported that the maximal aggregation rates of platelets at baseline between the OSA and control groups exhibited no differences. Here, we systematically analyzed platelet aggregability by the optical aggregometer method in 124 OSA-suspected subjects at baseline and in 23 nCPAP-treated patients among them until 90 days. We found that patients with OSA had increased platelet aggregability, which was affected more strongly by the severity of intermittent hypoxia and reoxygenation than by total hypoxic time during sleep, and that continuous treatment with nCPAP improved increased platelet aggregability in patients with OSA.

**Methods**

**Study Design and Subjects**

This study included 124 consecutive patients who came to our hospital for evaluation of sleep apnea/hypopnea. Arterial oxygen saturation was continuously monitored during sleep with a pulse oximeter (Pulsox-24; Minolta, Osaka, Japan) over two consecutive nights. The severity of sleep apnea was quantified by the 3% oxygen desaturation index (3%ODI), which is the number of oxygen desaturation events of 3% or more below the baseline level per hour during sleep. This index is the principal marker of the severity of intermittent hypoxia and reoxygenation in OSA patients. Patients who had a 3%ODI of more than 5 events/hour were diagnosed as having OSA. The oxygen saturation monitor records were visually inspected and scored by at least two experienced medical doctors who specialize in respiratory medicine.

In the present study, patients at risk for vascular diseases were defined as those possessing one or more of a smoking habit, hyperlipidemia, diabetes mellitus and hypertension. Hyperlipidemia was defined as taking anti-hyperlipidemic drugs or having a serum level of total cholesterol of >220 mg/dL, high density cholesterol of <40 mg/dL, low density cholesterol of >140 mg/dL or triglyceride of >150 mg/dL. Diabetes mellitus was defined as therapy with antidiabetic medicine or having a fasting plasma glucose level of >110 mg/dL or hemoglobin A1c level of >6.5%. Hypertension was defined as taking antihypertensive drugs or having a systolic blood pressure of >140 mmHg or a diastolic blood pressure of >90 mmHg.

We prescribed nCPAP therapy to 58 OSA patients with a 3%ODI of more than 15 events per hour. We obtained complete data of nCPAP and platelet aggregability in 23 OSA patients at baseline and on day 4, 30, and 90 after the start of nCPAP therapy. The main reason for the reduction in the number of patients was that some patients refused frequent fasting blood drawing and others took drugs affecting platelet function, such as non-steroidal anti-inflammatory drugs, at which point follow-up was discontinued. Compliance with the prescribed nCPAP therapy was determined by checking the periods of CPAP usage recorded in the integrated time recorder.

None of the subjects was taking an anti-platelet drug or anti-coagulant during the study. This study was approved by the institutional ethics committee of Kyoto University, and all participants provided written informed consent.

**Measurement of Platelet Aggregability**

Fasting blood samples were drawn in the supine position from an antecubital vein using a 21G needle with a quick tourniquet into a glass tube containing 0.313% sodium citrate in the morning at 8–10 o’clock. The effect of the tourniquet is negligible when blood sampling is performed smoothly (data not shown). The aggregability of platelet-rich plasma (PRP) was

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measured within 120 minutes after blood sampling, using the MCM HEMATRACER 212 optical aggregometer (MC Medical, Tokyo, Japan). The light transmission of platelet-poor plasma, which was obtained as the supernatant after centrifugation of blood at 2,000 x g at 25°C for 10 min, was set as 100% and that of PRP, which was obtained after centrifugation of blood at 200 x g at 25°C for 10 min, was set as 0%. A 20 μL solution of adenosine 5'-diphosphate (ADP) or collagen was added to 180 μL PRP under stirring conditions at 37°C and this was allowed to react for 5 min. The ADP (Sigma, St. Louis, Missouri, USA) concentrations were 0, 0.3, 1, 3, 9, and 27 μM, and the collagen (Horm, Germany) concentrations were 0, 0.025, 0.1, 0.4, 1.6 and 6.4 μg/mL. The putative concentration of ADP or collagen giving half maximal light transmission at 5 min after the start of stimulation was calculated and defined as the PRP-PATI (platelet-aggregation threshold index) for ADP or collagen, respectively. Given that the hypertriglycerideric level observed in many OSA patients would be a hindrance to correct measurement due to a narrower range of changes, we used PATI values which represent the agonist concentration giving half maximal aggregation, as described previously. It is noted that a lower PRP-PATI value indicates enhanced aggregability.

Statistical Analysis

Results are presented as the mean ± SE. Platelet aggregability was expressed as the logarithm of the PRP-PATI value because the PRP-PATI values were skewed. The unpaired t-test and chi-square test were used to compare backgrounds between groups. To evaluate predictive factors for the PRP-PATI value, multiple linear models were developed. Independent variables included gender, age (years), body mass index (BMI) (kg/m²), current smoking status, 3%ODI (events/hour), the percentage of time of arterial O₂ saturation <90% during sleep (%time of SpO₂ <90%) (%), hypertension, hyperlipidemia and diabetes mellitus. In the analysis of the effect of nCPAP therapy on the PRP-PATI value, we used repeated-measures analysis of variance (ANOVA) after adjusting for gender, age, BMI, current smoking status, hypertension, hyperlipidemia, and diabetes mellitus. A p value < 0.05 was defined as significant. Analyses were conducted using SAS software version 8.02 (SAS Institute Inc., Cary, NC).

Table 1. Profiles of the 124 subjects

<table>
<thead>
<tr>
<th></th>
<th>All subjects (n = 124)</th>
<th>Non-to-mild OSA (n = 66)</th>
<th>Moderate-to-severe OSA (n = 58)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>104 (83.8%)</td>
<td>54 (81.8%)</td>
<td>50 (86.2%)</td>
<td>0.51</td>
</tr>
<tr>
<td>Age (year)</td>
<td>46.4 ± 1.3</td>
<td>38.9 ± 1.4</td>
<td>54.8 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.2 ± 0.4</td>
<td>23.1 ± 0.3</td>
<td>27.5 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3%ODI (events/hour)</td>
<td>20.3 ± 1.9</td>
<td>4.2 ± 0.6</td>
<td>38.6 ± 2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>%time of SpO₂ &lt;90% (%)</td>
<td>5.6 ± 0.9</td>
<td>0.4 ± 0.1</td>
<td>11.6 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current smoker</td>
<td>13 (10.5%)</td>
<td>4 (6.1%)</td>
<td>9 (15.5%)</td>
<td>0.086</td>
</tr>
<tr>
<td>Hypertension</td>
<td>32 (25.8%)</td>
<td>5 (7.6%)</td>
<td>27 (46.6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>46 (37.1%)</td>
<td>8 (12.1%)</td>
<td>38 (65.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>12 (9.7%)</td>
<td>1 (1.5%)</td>
<td>11 (19.0%)</td>
<td>0.0010</td>
</tr>
</tbody>
</table>

OSA, 3%ODI and %time of SpO₂ <90% indicated obstructive sleep apnea, the number of events in oxygen saturation by 3% or more every hour and the percentage of time of arterial O₂ saturation <90% during sleep, respectively.

Results

Baseline Characteristics

The characteristics of the 124 patients in the present study are shown in Table 1. Among the 124 patients enrolled, 59 were categorized as being at established risk for vascular diseases.

Baseline characteristics were compared based on the severity of OSA. Patients were divided into two groups of 66 patients with no OSA or mild OSA (3%ODI ≤ 15) and 58 patients with moderate-to-severe OSA (3%ODI > 15) (Table 1). The moderate-to-severe OSA group was older (p < 0.001) and had a higher BMI (p < 0.001) than the non-to-mild OSA group. Compared with the non-to-mild OSA group, the moderate-to-severe OSA group had significantly higher 3%ODI (38.6 ± 2.2 vs. 4.2 ± 0.6; p < 0.001) and %time of SpO₂ < 90% (11.6 ± 1.7 vs. 0.4 ± 0.1; p < 0.001). Patients in the moderate-to-severe OSA group had significantly higher rates of hypertension (p < 0.001), hyperlipidemia (p < 0.001) and diabetes mellitus (p = 0.0010).
OSA and Platelet Aggregability

Log PRP-PATI values for ADP were 0.78 ± 0.09 μM in the moderate-to-severe OSA group and 1.04 ± 0.07 μM in the non-to-mild OSA group, indicating that the platelets of patients with moderate-to-severe OSA were in a significantly hyperaggregable state in response to ADP ($p = 0.029$) (Fig. 1A). In contrast, log PRP-PATI values for collagen were −1.41 ± 0.13 μg/mL in the moderate-to-severe OSA group and −1.20 ± 0.10 μg/mL in the non-to-mild OSA group. This difference did not reach a statistically significant level in spite of showing a tendency ($p = 0.17$) (Fig. 1B).

The multiple linear model for predicting log PRP-PATI values for ADP showed that 3%ODI was a significant predictive factor ($p = 0.013$) after adjusting for gender, age, BMI, smoking and comorbidities (Table 2); however, %time of SpO₂ < 90% was not a significant factor ($p = 0.46$). The multiple linear model for predicting log PRP-PATI values for collagen showed that neither 3%ODI nor %time of SpO₂ < 90% was a significant factor ($p = 0.22$ and 0.49, respectively) (Table 2).

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Table 2. Multiple linear models to predict log PRP-PATI values for ADP and collagen in the 124 subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>ADP</th>
<th>Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>SE</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.425</td>
<td>0.475</td>
</tr>
<tr>
<td>Gender</td>
<td>0.537</td>
<td>0.152</td>
</tr>
<tr>
<td>Age (years)</td>
<td>−0.013</td>
<td>0.005</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>0.031</td>
<td>0.016</td>
</tr>
<tr>
<td>Current smoking</td>
<td>−0.178</td>
<td>0.189</td>
</tr>
<tr>
<td>3%ODI (events/hour)</td>
<td>−0.012</td>
<td>0.005</td>
</tr>
<tr>
<td>%time of SpO₂ &lt; 90% (%)</td>
<td>0.006</td>
<td>0.009</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.269</td>
<td>0.158</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>0.122</td>
<td>0.144</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>−0.253</td>
<td>0.204</td>
</tr>
</tbody>
</table>

PRP-PATI, 3%ODI and %time of SpO₂ <90% indicated platelet-rich plasma platelet-aggregation threshold index, the number of events in oxygen saturation by 3% or more every hour and the percentage of time of arterial O₂ saturation <90% during sleep, respectively.
Vascular Risk and Platelet Aggregability

Next, we examined how OSA affected platelet aggregability in patients with vascular risk factors. Log PRP-PATI values for ADP were $0.84 \pm 0.10 \, \mu M$ among the 59 patients who had one or more vascular risk factors and $0.99 \pm 0.07 \, \mu M$ among the 65 patients who had no risk factors, indicating that platelet hyperaggregability in response to ADP was not significantly different between patients who were or were not at vascular risk ($p = 0.22$) in spite of some hyperaggregable tendency in the risk group (Fig. 2A). Log PRP-PATI values for collagen exhibited a similar tendency ($-1.43 \pm 0.12 \, \mu g/mL$ in the vascular-risk group versus $-1.18 \pm 0.10 \, \mu g/mL$ in the non vascular-risk group; $p = 0.11$) (Fig. 2B).

To investigate the relative strength for predicting log PRP-PATI values in the 59 patients who were at vascular risk, a multiple linear model was used. Regarding the response to ADP, 3%ODI was a strongly significant predictive factor ($p < 0.001$) after adjusting

<table>
<thead>
<tr>
<th>Variables</th>
<th>ADP Coefficient</th>
<th>ADP SE</th>
<th>ADP $p$</th>
<th>Collagen Coefficient</th>
<th>Collagen SE</th>
<th>Collagen $p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.146</td>
<td>0.969</td>
<td></td>
<td>-3.335</td>
<td>1.242</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>0.877</td>
<td>0.274</td>
<td>0.0024</td>
<td>1.441</td>
<td>0.351</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.017</td>
<td>0.007</td>
<td>0.031</td>
<td>-0.015</td>
<td>0.010</td>
<td>0.13</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>0.056</td>
<td>0.024</td>
<td>0.025</td>
<td>0.076</td>
<td>0.031</td>
<td>0.018</td>
</tr>
<tr>
<td>Current smoking</td>
<td>-0.262</td>
<td>0.216</td>
<td>0.23</td>
<td>-0.371</td>
<td>0.277</td>
<td>0.19</td>
</tr>
<tr>
<td>3%ODI (events/hour)</td>
<td>-0.024</td>
<td>0.006</td>
<td>&lt;0.001</td>
<td>-0.026</td>
<td>0.008</td>
<td>0.0026</td>
</tr>
<tr>
<td>%time of SpO$_2$ &lt; 90% (%)</td>
<td>0.012</td>
<td>0.010</td>
<td>0.21</td>
<td>0.016</td>
<td>0.013</td>
<td>0.20</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.308</td>
<td>0.185</td>
<td>0.10</td>
<td>0.421</td>
<td>0.237</td>
<td>0.082</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>-0.045</td>
<td>0.209</td>
<td>0.83</td>
<td>0.113</td>
<td>0.268</td>
<td>0.68</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>-0.339</td>
<td>0.218</td>
<td>0.13</td>
<td>-0.544</td>
<td>0.280</td>
<td>0.058</td>
</tr>
</tbody>
</table>

PRP-PATI, 3%ODI and %time of SpO$_2$ < 90% indicated platelet-rich plasma platelet-aggregation threshold index, the number of events in oxygen saturation by 3% or more every hour and the percentage of time of arterial O$_2$ saturation <90% during sleep, respectively.
for gender, age, BMI, smoking and comorbidities (Table 3); however, %time of SpO₂ < 90% was not a significant factor (p = 0.21). Regarding the response to collagen, 3%ODI was a strongly significant predictive factor (p = 0.0026) (Table 3); however, %time of SpO₂ < 90% was not a significant factor (p = 0.20).

Thus, although platelet aggregability was not so different between patients with and without vascular risk factors, the effects of the severity of OSA on platelet aggregability were strong in patients with vascular risk factors.

**nCPAP Therapy and Platelet Aggregability**

The 3%ODI of the patients who were treated with nCPAP therapy fell to less than 5 within two or three days after starting nCPAP treatment, as observed by oximeter monitoring. The length of nCPAP usage was 5.1 ± 0.4 hours per night during the 1-month period after nCPAP therapy was started, and 4.8 ± 0.4 hours per night during the period from 1 to 3 months. BMI did not significantly change during follow-up, and the medication regimen was not changed during the study period.

Among the 23 OSA patients who were treated with nCPAP therapy, log PRP-PATI values for ADP and collagen on day 0, 4, 30 and 90 are shown in Table 4. nCPAP therapy increased ADP-induced platelet aggregability from day 0 to day 30 (p = 0.035), and then significantly improved this increased platelet aggregability from day 30 to day 90 (p = 0.011). In contrast, collagen-induced platelet aggregability remained unchanged up until day 30, and then improved on day 90, compared with day 0, 4 and 30 (p = 0.026, 0.011 and 0.055, respectively).

**Discussion**

We found that ADP-induced platelet aggregability was significantly increased in patients with moderate-to-severe OSA compared with patients with non-to-mild OSA. Multiple regression analysis reveals that OSA-related intermittent hypoxia and reoxygenation frequency, but not total hypoxic time during sleep, significantly contributed to platelet aggregability for ADP. Among patients with one or more vascular risk factors, the severity of intermittent hypoxia and reoxygenation strongly contributed to platelet hyperaggregability for both ADP and collagen. Ninety days after the initiation of nCPAP therapy, platelet aggregability improved.

OSA is an independent risk factor for cardiovascular diseases. Although there is growing evidence that OSA is associated with increased rates of hypertension, arrhythmia and tachycardia, we here found increased platelet aggregability in patients with moderate-to-severe OSA, which could contribute to the increase in cardiovascular risk in OSA patients. There is some evidence for a hypercoagulable state in patients with OSA; however, no previous studies explained this from the point of platelet aggregability by evaluating platelet aggregation directly by aggregometer.

Multiple linear models revealed that 3%ODI, a principal index of the severity of intermittent hypoxia and reoxygenation, was a significant contributor to ADP-induced platelet hyperaggregability. Many studies showed increased levels of proinflammatory mediators in OSA patients. Recent studies have demonstrated significant relationships between sleep-related intermittent hypoxia and reoxygenation and elevated expression levels of nuclear factor-kappaB (NF-kappaB)-dependent inflammatory genes, such as tumor necrosis factor (TNF)-α, which has been shown to promote platelet aggregation in vitro and in vivo. Therefore, TNF-α may be one of the factors causing platelet hyperaggregability. Augmented sympathetic activity with increased concentrations of epinephrine and norepinephrine as a result of hypoxemia, or repetitive arousals from sleep in OSA patients, may also enhance platelet aggregability, since elevated circulating catecholamine causes platelet aggregation in vitro and in vivo. Although such humoral factors might contribute to platelet hyperaggregability in OSA, it is also possible that factors inside platelets might be affected by intermittent hypoxia and reoxygenation, which should be elucidated in the future.

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 4</th>
<th>Day 30</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log PRP-PATI for ADP (µM)</td>
<td>0.91 ± 0.14</td>
<td>0.81 ± 0.15</td>
<td>0.62 ± 0.16*</td>
<td>0.97 ± 0.15‡</td>
</tr>
<tr>
<td>Log PRP-PATI for collagen (µg/mL)</td>
<td>-1.47 ± 0.18</td>
<td>-1.56 ± 0.22</td>
<td>-1.47 ± 0.24</td>
<td>-1.04 ± 0.16*†</td>
</tr>
</tbody>
</table>

nCPAP, OSA and PRP-PATI indicated nasal continuous positive airway pressure, obstructive sleep apnea and platelet-rich plasma platelet-aggregation threshold index, respectively.

* p < 0.05, vs Day 0; † p < 0.05, vs Day 4; ‡ p < 0.05, vs Day 30.

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**Table 4.** Effects of nCPAP therapy on platelet aggregation in response to ADP and collagen in 23 patients with OSA.
Among patients with vascular risk, the level of 3%ODI strongly contributed to increased platelet aggregability; therefore, in the management of patients with conventional vascular risk factors, it would be more important to assess the co-existence of OSA as this can synergistically make patients vulnerable to thrombosis due to increased platelet aggregation.

Beneficial effects of nCPAP on collagen-induced platelet aggregability were observed at day 90 which might be due to reduction of systemic inflammation \(^{(27)}\) or sympathetic activity \(^{(28)}\) by nCPAP therapy. We recently demonstrated that adequate nCPAP therapy decreases the diastolic blood pressure and heart rate \(^{(21, 29)}\) which might indicate that nCPAP therapy stabilized the augmented sympathetic nerve activity induced by OSA, and that the improvement in sympathetic nerve activity ameliorated platelet hyperaggregability.

nCPAP did not improve platelet aggregability at day 30. Short-term treatment of CPAP within 1 month tends to fail to show significant effects on many activated coagulation factors raised in untreated OSA \(^{(30, 31)}\). Rather, unexpectedly, platelet aggregability for ADP worsened at first in the present study. Although the mechanism causing an early ADP-induced hyperaggregable status after nCPAP therapy is not clear, it should be noted for the development of cardiovascular events immediately after nCPAP therapy. This should be confirmed carefully in future studies.

As shown in Table 4, we observed a discrepancy between the changes in ADP- and collagen-induced platelet aggregation after starting nCPAP therapy. Although several different agonists induce platelet aggregation, ADP and collagen are considered to act at different receptors on platelets: ADP binds to two classes of purinergic G-coupled seven transmembrane receptors (P2Y1 and P2Y12), and collagen binds to a GP VI immunoglobulin superfamily member and integrin \(a_\mu b_3\) \(^{(32)}\); therefore, it is probable that medical interventions might have discrepant effects on platelet aggregation induced by different agonists, depending on how they affect these receptor-mediated signaling pathways.

Recently, the evaluation of whole blood aggregability has become popular, although the present methodology using PRP still seems to be the standard method; therefore, we may have to investigate platelet aggregability in OSA patients using whole blood samples in the future.

One limitation of the present study was that we did not perform polysomnography. Although a consensus has not been reached, using oximetry alone to diagnose OSA was useful in some studies \(^{(30)}\). To verify the accuracy of the data, the oxygen saturation monitor records were visually inspected and scored by at least two experienced medical doctors specializing in respiratory medicine. In addition, ODI has been proposed as a principal marker of the severity of intermittent hypoxia and reoxygenation in OSA patients; therefore, from the point of hypoxemia with apnea and hypopnea, we here evaluated the severity of OSA with ODI.

In conclusion, we showed that platelet aggregability was increased in patients with moderate-to-severe OSA partly through an intermittent hypoxia and reoxygenation phenomenon, which is pathophysiologically characteristic of OSA. As the effects of the severity of OSA on platelet aggregability were stronger in patients with vascular risk factors, the co-existence of OSA should be examined for the treatment of patients with multiple risk factors. Continuous nCPAP therapy improved platelet aggregability at 90 days. Thus, one of the reasons for the increase in cardiovascular events in OSA could be increased platelet aggregability.

**Acknowledgements**

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