Plasma Levels of Platelet-Derived Microparticles in Patients with Obstructive Sleep Apnea Syndrome

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**Aim:** Obstructive sleep apnea syndrome (OSAS) has been associated with high cardiovascular morbidity and mortality, and patients suffer from repeated episodes of hypoxia. Platelet-derived microparticles (PDMPs) are released via platelet activation by various agonists, including inflammatory cytokines or high shear stress. Plasminogen activator inhibitor -1 (PAI-1) is a fibrinolytic marker and soluble fibrin (SF) is a coagulation activation marker. We examined plasma levels of PDMPs, PAI-1 and SF in patients with OSAS. We also examined the effects of continuous positive airway pressure (CPAP) on plasma levels of PDMPs.

**Methods:** Full polysomnography (PSG) monitoring was performed on 27 patients. The apnea-hypopnea index (AHI) of 5 events/h or less than 30 events/h indicated mild to moderate OSAS, and an AHI of 30 events/h or more indicated severe OSAS. Plasma levels of PDMPs were measured using an ELISA kit, and PAI and SF were determined by a latex immunoassay. In addition, the effects of CPAP treatment were studied in 7 patients.

**Result:** The plasma level of PDMPs was significantly higher in patients with severe OSAS (15.8 ± 10.4 U/mL) than normal controls (10.8 ± 7.1 U/mL, p < 0.05) and patients with mild to moderate OSAS (9.2 ± 3.5 U/mL, p < 0.05). The plasma levels of PDMPs correlated with the AHI (r = 0.39, p < 0.05). In addition, CPAP treatment decreased the plasma level of PDMPs (11.9 ± 5.6 U/mL to 6.7 ± 3.2 U/mL, p < 0.05).

**Conclusions:** Patients with OSAS might be at increased cardiovascular risk due to elevated PDMPs. Moreover, a decrease in the plasma level of PDMPs by treatment with CPAP might reduce cardiovascular risk.


**Key words:** Obstructive sleep apnea syndrome, Platelet-derived microparticles, Apnea-hypopnea index, Continuous positive airway pressure

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**Introduction**

Obstructive sleep apnea syndrome (OSAS) is a disorder that is characterized by episodic apnea/hypopnea due to airway obstruction during sleep, and is associated with increased cardiovascular morbidity and mortality¹ ². Compared to patients with no OSAS, patients with OSAS have higher levels of plasma thrombin-antithrombin complexes (TAT), a known procoagulant molecular marker, and plasminogen activator inhibitor -1 (PAI-1), a known fibrinolytic marker³ ⁴. Furthermore, a previous study reported that markers of platelet activation, such as soluble CD40 ligand (sCD40L) and soluble P-selectin (sP-selectin), are also increased in patients with OSAS⁵ ⁶;
Therefore, OSAS may increase the thrombotic tendency via activation of the coagulation system and platelet activation. Moreover, previous studies suggested that continuous positive airway pressure (CPAP) might reduce apnea-related hypoxia and cardiovascular risk. CPAP treatment has been shown to lead to a significant decrease in plasma levels of fibrinogen, PAI-1, sCD40L and sP-selectin; therefore, CPAP treatment might decrease the activation of coagulation and platelets by improving apnea-related hypoxia.

Platelet activation by various agonists such as thrombin, collagen and inflammatory cytokines, or physical stimuli such as hypoxia and shear stress, results in the shedding of submicroscopic membrane vesicles, platelet-derived microparticles (PDMPs). These are defined as vesicles less than 1.5 μm in diameter that are enriched in procoagulant platelet proteins. PDMPs have a negatively charged phospholipid surface, allowing them to bind to activated coagulation factors and expose tissue factors under various conditions. Several studies have shown that PDMPs that form during platelet activation have procoagulant activity. PDMPs are formed by platelets upon activation, and hence their membrane should possess all the properties of the activated platelet membrane. In addition, PDMPs can bind to the components of procoagulant complexes, such as factors V (Va) and VIII (VIIIa), moreover, the binding-site densities of these proteins on PDMP membranes may even exceed those observed on platelet membranes. The addition of PDMPs to platelet-free recalcified plasma without the addition of any coagulation activators accelerates the initiation of thrombin generation. The procoagulant activity of PDMP membranes is approximately 50- to 100-fold higher than that of activated platelets. PDMPs are not only platelet activation markers, but may also promote clot formation. Unlike soluble CD40 and P-selectin, which were described in a previous study, elevated plasma levels of PDMPs might therefore be a trigger of hypercoagulability.

In recent years, plasma PDMP levels have been measured using enzyme-linked immunosorbent assay (ELISA) methods, and are used as a marker of platelet activation. A previous study reported that plasma PDMP levels are increased in patients with acute coronary syndrome, suggesting that PDMPs may play a role in the pathogenesis of arterial thrombosis and atherosclerosis.

On the other hand, plasminogen activator inhibitor-1 (PAI-1), a member of the serine protease inhibitor family, inhibits fibrinolytic activity by binding to tissue-type plasminogen activator (t-PA). A previous study demonstrated that a higher apnea-hypopnea index (AHI) and lower mean nighttime oxyhemoglobin saturation (SpO2) were both associated with a higher concentration of circulating PAI-1. Soluble fibrin (SF) appears in plasma during the early stage of blood coagulation, and is a well-known indicator of thrombogenic conditions, such as disseminated intravascular coagulation (DIC). Patients with OSAS have been reported to have higher levels of plasma TAT, a known procoagulant molecular marker than patients without OSAS.

The purpose of the present study was to examine the relationship between OSAS and platelet activation, as well as the relationship between OSAS and thrombotic tendency. For this propose, we examined the plasma levels of PDMPs, PAI-1 and SF in OSAS patients and control subjects. Furthermore, we examined whether CPAP therapy in OSAS patients reduces plasma levels of PDMPs, PAI-1 and SF by improving apnea-related hypoxia.

Subjects and Methods

Subjects
Twenty-seven patients, in whom OSAS had been diagnosed by full polysomnography (PSG) at Kanazawa Municipal Hospital, were enrolled in this study (Table 1). Nineteen healthy subjects served as controls. The control subjects were eligible for the study if they had no history of OSAS. A current smoker was defined as a person who had smoked within the past year. The body mass index (BMI) was calculated as an index of obesity. Blood pressure was measured twice and averaged. Hypertensive patients were defined as those with systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or those receiving antihypertensive agents. Hyperlipidemic patients were defined as those with low-density lipoprotein cholesterol (LDL-c) >140 mg/dL and/or triglycerides >150 mg/dL. The patients were classified as diabetics by their use of insulin and/or oral hypoglycemic agents or by a glycosylated hemoglobin A1c (HbA1c) >6.5%. Subjects who had taken an anticoagulant or anti-thrombotic medications were excluded from the study. The study followed the institutional guidelines of the University of Kanazawa, and informed consent was obtained from all patients according to the Declaration of Helsinki.

Polysomnography
PSG monitoring was performed on patients with possible OSAS using the Healthdyne Technologies’ Alice 3 or Alice 4 system. An AHI of >5 events/hour
was considered diagnostic of OSAS. An AHI of 5 events/hour or <15 events/hour indicated mild OSAS, and an AHI of ≥15 events/hour and <30 events/hour indicated moderate OSAS, and an AHI of ≥30 events/hour indicated severe OSAS. In general, many reports are classified into 2 groups based on the severity of AHI for a range of criteria. In this study, we classified the patients in our study into 2 groups (mild to moderate vs. severe).

**Measurements of Plasma PDMP, PAI-1 and SF**

Blood samples were collected after performing PSG. For the PDMP assay, blood samples were collected from a peripheral vein into Vacutainers containing EDTA-ACD (Nipro Co. Ltd., Japan), with a 21-gauge needle to minimize platelet activation, between AM 6:00 and AM 7:00 after the completion of PSG recording. The samples were gently mixed by turning the tube upside-down once or twice, and centrifuged at 8,000 g for 5 minutes at room temperature. Immediately after centrifugation, we collected 200 μL of the upper-layer supernatant from 2 mL samples to avoid contamination of the platelets, and stored the samples at −20°C until analysis. For ELISA of PDMPs we used an ELISA kit (Jimro Co. Ltd., Japan). The plates were washed 3 times with 350 μL/well of wash buffer, then 50 μL pretreatment liquid was added to each well of 96-well microtiter plates. A total of 50 μL of samples or standards was added to each well and incubated for 3 h at 20-30°C on a plate shaker. After plates were washed 3 times with 350 μL/well of wash buffer, 100 μL peroxidase-conjugated avidin was added to each well and incubated for 1h at 20-30°C on a plate shaker. Each well was washed 3 times with 350 μL/well of wash buffer, and then incubated with 100 μL peroxidase substrate solution for 20 min at 20-30°C. After this incubation, 100 μL stop solution was added to each well, and the absorbance was measured with an EIA reader at 450 nm.

For the PAI-1 antigen and SF assays, blood samples were collected using vacuum tubes containing 3.8% trisodium citrate between AM 6:00 and AM 7:00 after the completion of PSG recording. Samples were centrifuged at 2,000 g for 15 min. Plasma was separated and stored at −80°C until analysis. The plasma levels of PAI-1 antigen and SF were determined by latex immunoassay using a latex reagent (Mitsubishi Chemical Medience, Japan).

**CPAP Treatment**

We recommended that 13 patients with moderate to severe OSAS should receive CPAP treatment. Of these patients, 7 agreed to undergo CPAP treatment, and 2 used a mouthpiece during follow-up. More than 1 month after CPAP had been started, PSG was performed again while the patient received...
CPAP (mean: 6.0 ± 1.4 months). Blood samples were collected as described previously.

**Statistical Analysis**

All data were analyzed using the statistical software package jmp6.0 for Windows. Differences between three groups were investigated by the Kruskal-Wallis test, and differences between pre-CPAP and after CPAP treatment were assessed using the Mann-Whitney $U$ test. The correlations were analyzed with Spearman's rank correlation. All data are expressed as the means ± SD, and $p < 0.05$ was considered significant.

**Results**

**Characteristics of the Subjects**

The characteristics of the study subjects are shown in Table 1. There were significant differences in age, body weight, BMI, high-density lipoprotein cholesterol (HDL-c), triglycerides and blood glucose between control subjects and patients with OSAS. In contrast, there were no significant differences in body weight, BMI, HDL-c, LDL-c, triglycerides and blood glucose between subjects with mild to moderate OSAS and severe OSAS. The prevalence of hypertension, diabetes mellitus and hyperlipidemia did not differ significantly between subjects with mild to moderate OSAS and severe OSAS (mild to moderate OSAS: 36.4%, severe OSAS: 37.5%). The AHI and percentage of time with $\text{SpO}_2$ less than 90% were significantly higher in patients with severe OSAS than in those with mild to moderate OSAS ($p < 0.05$), and the lowest $\text{SpO}_2$ was significantly lower in patients with severe OSAS than in those with mild to moderate OSAS ($p < 0.05$).

**Plasma Levels of PDMPs, PAI-1 and SF**

Fig. 1 shows the differences in the plasma levels of PDMPs, PAI-1 and SF in patients with OSAS and controls. The plasma levels of PDMPs were significantly higher in patients with severe OSAS (15.8 ± 10.4 U/mL) than control subjects (10.8 ± 7.1 U/mL) and patients with mild to moderate OSAS (9.2 ± 3.5 U/mL) ($p < 0.05$, respectively). All patients with OSAS had significantly higher levels of PAI-1 (mild to moderate OSAS: 19.9 ± 10.8 ng/mL, severe OSAS: 26.1 ± 8.5 ng/mL) than control subjects (7.5 ± 4.5 ng/mL) ($p < 0.05$, respectively); however, no significant differences were observed between patients with severe
Correlation between plasma levels of PDMPs and the apnea hypopnea index (AHI) in OSAS patients.

* Spearman’s rank correlation.

OSAS and patients with mild to moderate OSAS. The plasma levels of SF were also not significantly different between all patients with OSAS (mild to moderate OSAS; 3.8 ± 2.2 μg/mL, severe OSAS; 4.0 ± 1.3 μg/mL) and control subjects (4.0 ± 2.3 μg/mL).

Correlation between Plasma PDMPs, PAI-1, SF, PSG Variables and Metabolic Variables in Patients with OSAS

There was a correlation between the plasma levels of PDMPs and the AHI in patients with OSAS (r = 0.39, p < 0.05) (Fig. 2). None of the other variables, including the lowest SpO2, percentage of time with SpO2 less than 90%, and BMI were correlated with the plasma levels of PDMPs.

The plasma levels of PAI-1 and SF were not significantly correlated with sleep parameters such as the AHI, lowest SpO2, and percentage of time with SpO2 less than 90%. In contrast, the level of PAI-1 was significantly correlated with BMI (r = 0.69, p < 0.01).

Changes in the Levels of PDMPs, PAI-1 and SF After CPAP Treatment

In seven patients with OSAS, the BMI, HDL-c, LDL-c, triglycerides and blood glucose did not change, and no new cardiovascular events were detected during treatment with CPAP (6.0 ± 1.4 months). Treatment with CPAP significantly decreased the AHI (36.7 ± 16.5 events/h to 5.2 ± 4.6 events/h, p < 0.05), increased the lowest SpO2 (76.6 ± 11.5% to 93.0 ± 2.3%, p < 0.05) and decreased the percentage of time with an SpO2 less than 90% (10.0 ± 12.9% to 0.0 ± 0.0%, p < 0.05) (Table 2). Furthermore, CPAP significantly decreased the plasma levels of PDMPs (11.9 ± 5.6 U/mL to 6.7 ± 3.2 U/mL, p < 0.05) (Fig. 3). In contrast, the plasma levels of PAI-1 and SF did not significantly change after treatment with CPAP (Table 2).

Discussion

We found that the plasma levels of PDMPs were significantly higher in patients with severe OSAS than control subjects and patients with mild to moderate OSAS. In addition, treatment with CPAP in patients with moderate to severe OSAS decreased the plasma levels of PDMPs. These results revealed that apnea-related hypoxia induces platelet activation in OSAS patients. On the other hand, there were no significant differences in body weight, BMI, HDL-c, LDL-c, triglycerides, or blood glucose between patients with mild to moderate OSAS and severe OSAS.

In patients with OSAS, cyclical alterations of arterial oxygen saturation are observed, with oxygen desaturation developing in response to apnea, followed by the resumption of oxygen saturation during hyperventilation. In addition, the present study found that the AHI correlated with the plasma level of PDMPs; therefore, these results suggest that repeated apnea-related hypoxia may also contribute to the plasma level of PDMPs in patients with severe OSAS. On the other hand, the percentage of time with a SpO2 less than 90% was significantly higher, and the lowest SpO2 was lower, in patients with severe OSAS than in those with mild to moderate OSAS; however, the lowest SpO2 and the percentage of time with SpO2
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PAI-1; however, the reduction was not significant in our study. In the near future, we plan to perform studies using a larger number of cases to determine whether our findings can be confirmed.

SF is a well-known indicator of thrombogenic conditions such as DIC. The plasma levels of SF were not significantly different between all patients with OSAS and control subjects in our study. Treatment with CPAP in patients with moderate to severe OSAS did not significantly decrease the plasma level of SF. It is likely that the plasma levels of SF were not different in this study because SF appears in the bloodstream during the extremely early stage of blood coagulation. Other markers of coagulation activation, such as prothrombin fragment 1 + 2 (F1 + 2) and TAT, may be needed to more accurately monitor coagulation in these patients.

In conclusion, the present study demonstrated that plasma levels of PDMPs and PAI-1 are elevated in OSAS patients. Moreover, treatment with CPAP decreased the plasma level of PDMPs. These findings suggest that patients with OSAS may have an increased thrombotic tendency brought about by elevated plasma levels of PDMPs and PAI-1. The increased risk of cardiovascular diseases in patients with OSAS might result from several causes, such as elevation of inflammatory cytokines and blood pressure, and can be predicted based on the elevated plasma levels of PDMPs and PAI-1 observed in this study. In fact, elevation of the plasma level of PDMPs might be part of the mechanism underlying the increased cardiovascular risk in patients with OSAS; therefore, treatment with CPAP might be useful for decreasing the risk of cardiovascular diseases in OSAS patients.

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References


![Fig. 3. Changes in the plasma level of PDMPs in seven patients with OSAS after CPAP.](image-url)
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