ORIGINAL

# Relationship between adhesion molecules with hs-CRP and changes therein after ARB (Valsartan) administration in patients with obstructive sleep apnea syndrome

Norihito Kageyama, Masahiro Nomura*, Yutaka Nakaya**, Tomonori Watanabe, and Susumu Ito

*Department of Digestive and Cardiovascular Medicine, Institute of Health Biosciences, The University of Tokushima Graduate School, *Faculty of Integrated Art and Sciences, Department of Human and Social Sciences, The University of Tokushima, and ** Department of Nutrition and Metabolism, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan*

**Abstract : It has been reported that a relationship exists between obstructive sleep apnea syndrome (OSAS) and cardiovascular and cerebrovascular diseases. To address this issue, we evaluated whether OSAS is associated with adhesion molecules and inflammatory signs, important indicators of atherosclerosis. Levels of high-sensitivity CRP (hs-CRP) and intercellular adhesion molecule-1 (ICAM-1) were measured in 30 patients with ischemic heart disease, confirmed by coronary arteriography (IHD group). Twenty healthy volunteers without sleep apnea were used as controls (Group N). Sleeping respiratory information was collected using a portable sleep polygraph, together on information about oronasal flow, tracheal sound, chest respiration, and percutaneous oxygen saturation ($SpO_2$) to obtain the apnea-hypopnea index (AHI). In the IHD group, 9 (30%) of the 30 patients showed evidence of OSAS [IHD(AHI≥40) group] and 21 did not [IHD(AHI<40) group]. The levels of hs-CRP and ICAM-1 were significantly higher in the IHD group than in the N group ($p<0.01$). Moreover, the levels of hs-CRP and ICAM-1 were significantly higher in the IHD(AHI≥40) group than in the IHD(AHI<40) group ($p<0.01$). However, after the administration of valsartan, angiotensin II receptor antagonists (ARB) to both IHD groups, the levels of hs-CRP and ICAM-1 decreased significantly in both groups. Moreover, a multivariate analysis revealed that the levels of hs-CRP and ICAM-1 were associated with the severity of sleep apnea.These findings suggest that, in OSAS the levels of hs-CRP and ICAM-1 are decreased and that the administration of ARB decreases the risk of atherosclerosis.**
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***Keywords*** **:** *OSAS, hs-CRP, adhesion molecule, valsartan, angiotensin II receptor antagonists (ARB), atherosclerosis*

## INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) has been reported to not only aggravate impaired glucose tolerance by inducing insulin resistance through nocturnal hypoxemia and enhanced sympathetic tonus, but also serves as an independent risk factor of cardiovascular disorders and hypertension (1). Wilcox *et al.* (2) proposed Syndrome Z as a pathological state with cumulative risk factors, and Reaven *et al.* proposed that, when sleep apnea is added to Syndrome X, this situation corresponds to the current metabolic syndrome. It has been reported that OSAS is a cause of

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Address correspondence and reprint requests to Masahiro Nomura, M.D., Ph.D., Faculty of Integrated Art and Sciences, The University of Tokushima, Minami-Jyosanjima-cho, Tokushima 770-8502, Japan and Fax : +81-88-656-6173.

an increase in the production of vascular adhesion molecules (intercellular adhesion molecule-1; ICAM-1, vascular cell adhesionmolecule 1; VCAM-1, and L-selectin), inflammatory cytokine(interleukin-8; IL-8), and monocyte chemoattractant protein-1, which serves as an important risk factor of cardiovascular disease, and nasal continuous positive airway pressure(CPAP) treatment decreases the level of vascular adhesion molecules and inflammatory cytokine in the treatment of OSAS.

Angiotensin II receptor antagonists (ARBs) have recently been suggested to inhibit the progression of vascular inflammation in arteriosclerotic lesions, and inhibit monocyte/macrophage infiltration by supressing an increase in vascular ICAM-1 expression (3, 4). However, only a few studies have focused on the issue of whether ARB improves vascular inflammation and the progression of atherosclerosis associated with OSAS. In the present study, we examined ARB-induced changes in the levels of adhesion molecules and inflammatory cytokine to investigate whether OSAS-associated risks, such as arteriosclerotic disease, can be reduced without the use of nasal CPAP, which is difficult in continuous treatment.

## METHODS

*1) Subjects*

The subjects were 30 patients with ischemic heart disease (IHD) who had undergone coronary angiography (16 men and 14 women with a mean age of 62.7±10.8 years) and were confirmed to have significant stenosis of the coronary artery. Patients with a medical history of myocardial infarction, heart failure, and renal failure (serum creatinine level>2.5 mg/dl), and patients with many branch lesions of the coronary artery, a left ventricular ejection fraction of less than 50%, and diabetic and hypertensive patients were excluded. For the diagnosis of IHD, patients with significant stenosis of the coronary artery by 50% or higher on coronary angiography were selected. Patients that had been treated with angiotensin II receptor antagonists, angiotensin converting enzyme inhibitors, statin, and autonomic modifiers were excluded from the study. The IHD group was divided into two groups; i.e., patients with OSAS [IHD (AHI≥40) group] and those without [IHD(AHI<40) group].

The control group was comprised of 20 healthy subjects without a sleep apnea attack (N group, 12 men and 8 women with a mean age of 60.4±8.6 years). These subjects without a past medical history of circulatory disorders who exhibited typical normal physical findings, a normal standard 12-lead electrocardiogram, and chest X-ray radiogram without a sleep apnea attack were investigated.

These studies were approved by the Tokushima University Ethics Committee (No. 133), and the patients gave written, informed consent prior to their participation.

*2) Sleep apnea test*

The sleep apnea test was performed 1 week before coronary arteriography. Respiratory information during sleep was collected using a portable sleep polygraph (Sleep Tester LT-200, Fukuda Denshi Co., Ltd., Tokyo, Japan). Oronasal airflow measured by the thermocouple method, tracheal sound by the microphone method, chest respiration by the air-bag method, and percutaneous arterial oxygen saturation by the 2-wavelength pulse waveform method were continuously recorded during night sleep.

A personal computer (Windows XP) and sleep apnea measurement software (SAS-100, Fukuda Densh Co., Ltd.) were used for the measurements. The apnea-hypopnea index (AHI), percutaneous arterial oxygen saturation, and the duration of apnea were measured. A respiratory volume of 1/4 or less was regarded as hypopnea, and the apnea-hypopnea threshold was set to 10 seconds or longer. The full-polygraph is fundamentally indispensable for diagnosis of OSAS, the diagnostic criterion for SAS is 5 times or more AHI per hour. But we adopted more than 40 times of AHI as criteria of sleep apnea in the present study because of a portable sleep polygraph.

*3) Measurement of high-sensitivity C reactive protein (hs-CRP), ICAM-1, and interleukin 6 (IL-6)*

Hs-CRP, ICAM-1, and IL-6 were measured one time at the start of this study in the N group, and twise before and 24 weeks after valsartan administration in the IHD group. We used a high-sensitivity CRP assay (N-Latex CRP II, Dade Behring, DE, USA), by which system the lowest detection limit is 0.05 mg/dl, for the measurement of hs-CRP. The concentrations of ICAM-1 and IL-6 in the serum were measured by enzyme-linked immunosorbent assey (ELISA) and chemiluminescnce enzyme immunoassay (CLEIA) methods, respectively.

*4) Administration of valsartan, an angiotensin II receptor antagonist (ARB)*

**After coronary arteriography, valsartan (80 - 160 mg/day) were orally administered to all patients in the IHD group. Hs-CRP, ICAM-1, and IL-6 were measured for 24 weeks afterward. Percutaneous coronary angioplasty and/or bypass surgery were performed 24 weeks after valsartan administration. Patients with severe stenosis and unstable angina who needed these treatments before 24 weeks were excluded from this study.**

*5) Statistical analysis*

**All values are expressed as the mean±standard deviation (SD), and statistical analyses were performed using StatView 5.0 (SAS Institute Inc. Cary, NC, USA). A comparison among each group was performed using ANOVA, while the correlation between the two groups was evaluated using a single regression analysis. In addition, the influence of the respective factors on AHI was evaluated by calculating the 95% confidence intervals in a logistic regression model using a multivariate analysis. P-values less than 0.05 were considered significant.**

## RESULTS

*1) OSAS in IHD group*

**Fig. 1 shows a portable sleep polygram of a patient with OSAS in whom significant stenosis was detected by coronary arteriography. Oronasal respiration was decreased, but no decrease the thoracic movement was noted, and percutaneous oxygen saturation was decreased. The AHI was 42.2, and, as a result, this patient was diagnosed with OSAS. The body mass index was 24.5, hs-CRP, ICAM-1 and IL-6 were 0.369 mg/dl, 661 ng/ml, and 7.71 pg/ml, respectively**

**Fig. 2 shows the frequency of complications with OSAS in the IHD group. OSAS was detected in 9 of the 30 patients (30%) in the IHD group.**

*2) Comparison of hs-CRP, ICAM-1 and IL-6 before and after valsartan administration between N and IHD groups*

**Figs. 3 shows comparisons of hs-CRP (panel a), ICAM-1 (panel b), and IL-6 (panel c) before and after valsartan administration between the N, IHD(AHI≥40), and IHD(AHI<40) groups. The hs-CRP, ICAM-1, and IL-6 levels before valsartan administration were significantly higher in the IHD group than in the N group (p<0.01). Moreover, hs-CRP, ICAM-1, and IL-6 were significantly higher in the IHD (AHI≥40) group than in the IHD (AHI<40) group (p<0.01) before valsartan administration.**

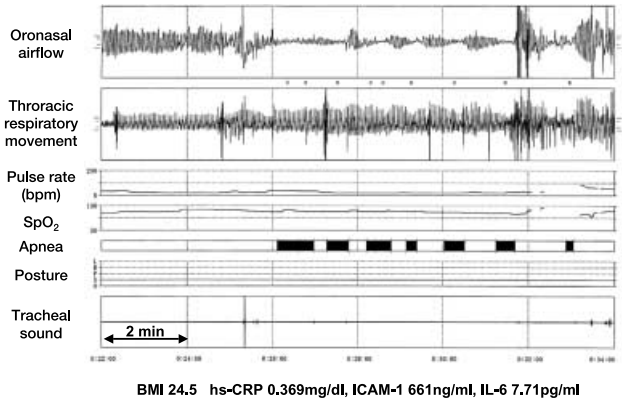


**Fig. 1. Portable sleep polygram of a patient with OSAS in whom significant stenosis was detected on coronary arteriography. SpO2, percutaneous arterial oxygen saturation; BMI, body mass index ; hs-CRP, high sensivity C reactive protain ; ICAM-1, intercellular adhesion molecule-1 ; IL-6, interleukin-6**

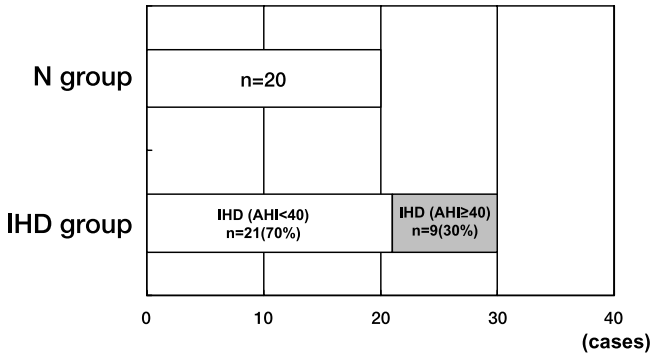


**Fig. 2. Frequency of complications with OSAS in the IHD group**

**After the valsartan administration, valsartan significantly decreased hs-CRP, ICAM-1, and IL-6 in the IHD group, especially the (AHI≥40) group. The significant differences among the IHD(AHI≥40), IHD(AHI<40), and N groups disappeared after the administration of valsartan.**

*3) Logistic multivariate analysis of humoral factors using AHI index*

**Table 1 shows a logistic multivariate analysis of the humoral factors (hs-CRP, ICAM-1, and IL-6) using AHI index. The hs-CRP (relative risk=1.642, p=0.038), ICAM-1(relative risk=1.560, p=0.042), and IL-6 (relative risk=1.301, p=0.048) values were individually independent risk factors for the severity of OSAS in the IHD group.**

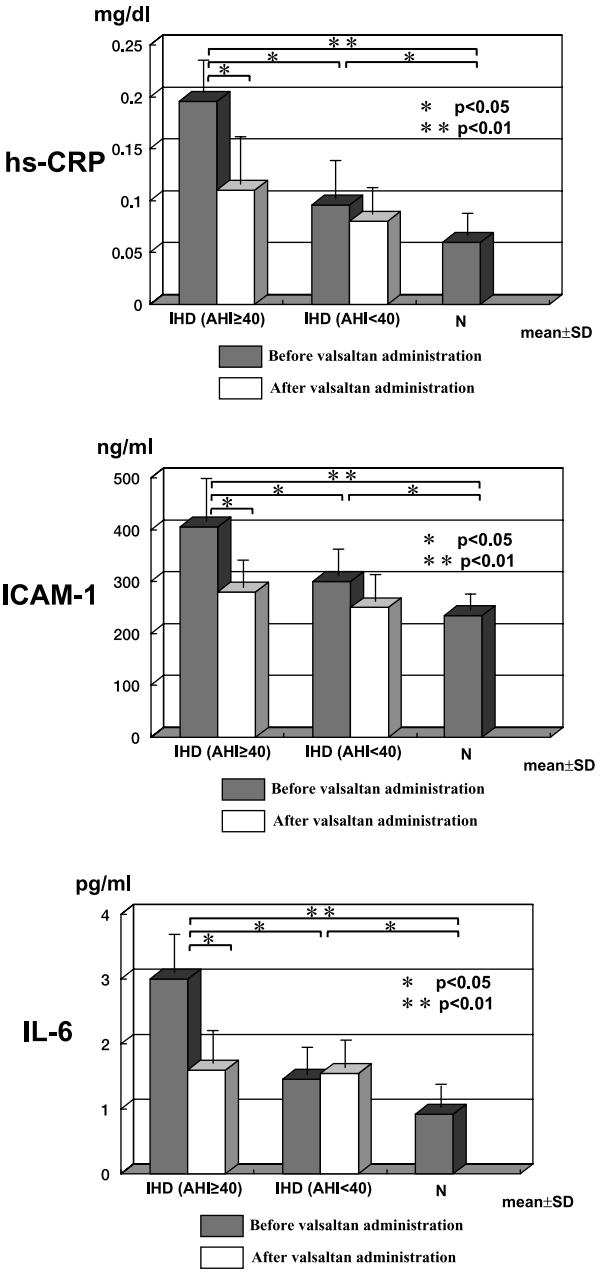


**Fig. 3.** Comparisons of hs-CRP (panel a), ICAM-1 (panel b), and IL-6 (panel c) before and after valsartan administration between the N, IHD(AHI≥40), and IHD(AHI<40) groups.
hs-CRP, high sensivity C reactive protain ; ICAM-1, intercellular adhesion molecule-1 ; IL-6, interleukin-6 ; IHD, ischemic heart disease ; AHI, apnea-hypopnea index.

**Table 1.** Logistic multivariate analysis of the humoral factors (hs-CRP, ICAM-1,and IL-6) using AHI index

| | Relative risk | 95% CI | *p* |
|---|---|---|---|
| hs-CRP | 1.642 | 0.836～2.851 | 0.038 |
| ICAM-1 | 1.560 | 0.798～2.248 | 0.042 |
| IL-6 | 1.301 | 0.628～1.630 | 0.063 |

hs-CRP, high sensivity C reactive protain ;
ICAM-1, intercellular adhesion molecule-1 ; IL-6, interleukin-6 ;
AHI index, apnea-hypopnea index.

## DISCUSSION

The levels of inflammatory marker (hs-CRP), the vascular adhesion molecule (ICAM-1), and the inflammatory cytokine (IL-6) were increased in OSAS patients with a coronary arterial lesion, suggesting that the risk of cardiovascular disorder is increased in OSAS patients compared to patients without OSAS. However, the findings indicate that valsartan, an angiotensin II receptor antagonist, decreased hs-CRP, ICAM-1, and IL-6 levels, suggesting that rennin-angiotensin pathways are involved in the development of atherosclerosis in OSAS. The results also suggest that angiotensin II receptor antagonists may decrease the risk of arteriosclerosis and cerebrovascular and cardiovascular in OSAS patients with a coronary arterial lesion (secondary prevention).

### *1) Relation between OSAS and arteriosclerosis*

A relation between OSAS and the progression of arteriosclerosis, especially an association with an increase in the risk of cardiovascular and cerebrovascular morbidity, has been reported (5). Intermittent hypoxemia in sleep apnea alters autonomic nervous activation, neuroendocrine function, releases potent proinflammatory mediators (TNF-a, interleukin-6), fibrinogen, plasminogen activator inhibitor, and reduces fibrinolytic activity (6-11). Moreover, leukocyte adhesion and accumulation on endothelial cells are common in patients with OSAS (9). Hein *et al.* also reported that hs-CRP, IL-6, IL-8, IL-10, TNF-a, ICAM-1, VCAM-1, L-Selectin, and insulin resistance are increased in OSAS patients (12). Consistent with previous reports, the levels of hs-CRP, ICAM-1 and IL-6 were also found to be significantly increased in OSAS patients with a coronary arterial lesion compared to patients with a coronary arterial lesion alone in this study.

Regarding the increases in these humoral factors, many of the factors are fat tissue-derived, physiologically active substances, and this is more likely due to obesity than OSAS (13, 14). However, the ICAM-1 gene is associated with a significant reduction in objective sleepiness in obese patients with OSAS (15). Moreover, obesity is a risk factor for high serum CRP levels in patients with sleep-disordered breathing (15).

Many reports on the severity of OSAS indicate the association of humoral factors with OSAS. Shamsuzzaman *et al.* reported that CRP levels, a marker of inflammation and cardiovascular risk, were independent of the severity of OSAS (16). In addition, the increased levels of adhesion mole-

cules were correlated with the AHI and oxygen desaturation index, but not with the severity of hypoxemia or mediators (17).

Yokoe *et al.* and Chin *et al.* reported that CRP and IL-6 are important risk factors of arteriosclerosis and coronary arterial disorders, and that these factors were significantly decreased by nasal CPAP treatment and were related to the prognosis of OSAS (18, 19). Harsch *et al.* also reported that nasal CPAP decreased IL-6 and ICAM-1 levels and increased insulin sensitivity (20). In the present study, an association between hs-CRP and ICAM-1 levels and the severity of OSAS was also noted in a multivariate analysis, suggesting that hs-CRP and ICAM-1 are also related to the severity of OSAS, and that this is not due to obesity alone.

*2) Effect of valsartan on OSAS*

The use of adhesion molecules as a prognostic marker of coronary arterial disease has been reported. Blankenberg *et al.*(21) reported that the blood levels of the vascular adhesion molecule (VCAM-1), the cell adhesion molecule (ICAM-1), and E-secretion were correlated with a risk of cardiovascular death, and the combination of these with the CRP level was useful for predicting survival (21). In another study (22), blood IL-6 levels were reported to be a strong independent predictive factor of high mortality in patients with unstable coronary arterial disease, and was useful for the determination for early invasive therapy indication.

Therefore, it is necessary to investigate the usefulness of therapeutic methods that inhibit these inflammatory markers in OSAS patients with coronary arterial lesions. Valsartan, an angiotensin II receptor antagonist, inhibits the production of inflammatory cytokines, MCP-1, IL-6, IL-1$\beta$, and TNF-$\alpha$, and inhibits the formation of new tunica intima and the migration of vascular smooth muscle cells, in addition to its hypotensive effect (23). Valsartan was also reported to prevent monocyte/macrophage infiltration and the excessive expression of the adhesion molecules, Plasminogen activator inhibitor-1 (PAI-1), and fibronectin (24). Moreove, valsartan has antioxidative action, and prevents atherosclerosis by inhibiting the oxidation of low-density lipoproteins (LDL) and decreases free radical levels (25, 26).

A number of reports of the usefulness of angiotensin II receptor antagonists for coronary arterial diseases have appeared, and such drugs are recommended for cases without contraindications. In the present study, valsartan improved inflammatory markers, adhesion molecules, and cytokines in patients with concomitant coronary arterial lesions and OSAS, suggesting that it might be useful for the secondary prevention of ischemic heart disease.

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