

# Association between monocyte chemoattractant protein-1 and blood pressure in smokers

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## Abstract

**Objective:** The expression level of monocyte chemoattractant protein-1 (MCP-1) is increased in atherosclerotic regions, inducing monocyte migration to the blood vessel wall. Although the serum MCP-1 concentration is higher in patients with than without cardiovascular disease, the precise correlations between the serum MCP-1 concentration and factors associated with smoking and atherosclerosis are unknown.

**Methods:** The serum MCP-1 concentration was measured using an enzyme-linked immunosorbent assay in 207 consecutive smokers who visited our smoking cessation clinic.

**Results:** Sex-adjusted analysis of smokers revealed that the MCP-1 concentration was positively correlated with age ( $\beta = 0.311$ ), smoking duration ( $\beta = 0.342$ ), systolic blood pressure ( $\beta = 0.225$ ), and diastolic blood pressure ( $\beta = 0.137$ ) but not with the body mass index. Multivariate regression analysis showed that smoking duration and systolic blood pressure were independent determinants of the MCP-1 concentration.

**Conclusions:** The MCP-1 concentration was positively correlated with blood pressure among smokers. Long-term smokers with high blood pressure may be more susceptible to plaque rupture at atherosclerotic lesion sites.

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## Introduction

Tobacco smoke contains >200 toxins, including nicotine, carbon monoxide (CO), and oxygen free radicals.<sup>1</sup> Nicotine acts directly on the vessel walls, activates the sympathetic nervous system, and induces vasoconstriction, whereas CO and oxygen free radicals induce oxidative stress in the vessel walls and cause vascular inflammation and arteriosclerosis. The risk of cardiovascular events, such as cerebral and myocardial infarction, increases with the duration of tobacco use and the number of cigarettes smoked per day.<sup>2</sup>

Monocyte chemoattractant protein-1 (MCP-1), also known as C-C motif chemokine 2, is an important chemotactic factor for monocytes and macrophages.<sup>3</sup> Inflammation of the vessel walls in response to oxidative stress results in synthesis of chemokines, including MCP-1, by endothelial cells, smooth muscle cells, and macrophages. MCP-1 induces monocyte migration to vascular walls and activates monocytes during atherosclerosis development.<sup>4-7</sup> Previous studies have shown that MCP-1 increases the risk of cardiovascular disease<sup>8</sup> and contributes to the development of coronary artery diseases such as atherosclerosis.<sup>9,10</sup> MCP-1 plays a vital role in endothelial dysfunction<sup>7</sup> and the instability and subsequent rupture of atheromatous plaques,<sup>11</sup> resulting in myocardial<sup>12</sup> and cerebral infarction.<sup>13,14</sup> Hence, the MCP-1-dependent pathway, which is activated during atherosclerosis development, represents an important therapeutic target.<sup>15,16</sup>

MCP-1 is involved in the pathogenesis of metabolic syndrome and is associated with various metabolic parameters, such as obesity,<sup>7,17,18</sup> diabetes,<sup>17-19</sup> and essential

hypertension.<sup>20,21</sup> The quantitative evaluation of MCP-1 is a diagnostic and prognostic marker of atherosclerotic disease.<sup>15,22</sup> With regard to inflammation caused by tobacco smoke, *in vitro* studies have demonstrated that nicotine promotes MCP-1 production by neutrophils and fibroblasts<sup>23</sup> and causes inflammation in cancerous tissues.<sup>24</sup> Moreover, the MCP-1 concentration in mice is reportedly increased by passive smoke.<sup>25</sup> However, the role of MCP-1 in smoking-induced atherosclerosis in humans is unknown. Therefore, this study was performed to determine the correlation between the serum MCP-1 concentration and smoking- and atherosclerosis-related factors.

## Materials and methods

### Patients

This prospective study included consecutive smokers who consulted the Smoking Cessation Clinic, Health Evaluation Center, National Hospital Organization, Kyoto Medical Center (Kyoto, Japan) from April 2007 to March 2010. During the same period, a retrospective study of nonsmokers (no smoking for >1 year) who consulted the outpatient clinic of the Department of Cardiology, National Hospital Organization, Kyoto Medical Center was also performed. The exclusion criteria in both studies were as follows: concomitant acute coronary syndrome, infection or pyrexia, recent (within the last 3 months) myocardial infarction or stroke, renal transplantation, a serum creatinine concentration of  $\geq 3$  mg/dL, hepatic failure [defined as chronic hepatic disease (i.e., cirrhosis)] or biochemical evidence of significant hepatic derangement (e.g.,

bilirubin concentration of >3 times the upper limit of normal in association with an aspartate aminotransferase/alanine aminotransferase/alkaline phosphatase concentration of >3 times the upper limit of normal), and active inflammatory disease.

Written informed consent was obtained from all participants. No patient was coerced into participating in this study. The study data were anonymized with no personal identifiers. The Ethical Review Board of the National Hospital Organization, Kyoto Medical Center approved the study protocol.

### Data collection

The body mass index (BMI) was calculated as the weight in kilograms divided by the height squared in meters. The waist circumference was measured at the level of the umbilicus with the patient in the supine position. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in a sitting position after resting for >5 min using an automatic electronic sphygmomanometer (BP-103iII; Nippon Colin, Komaki, Japan).<sup>26</sup> A regular-sized cuff appropriate for the Japanese population (arm length: 17–32 cm) was used as recommended. At each visit, a nurse measured the expiratory CO concentration with an EC50 Micro Smokerlyzer (Bedfont Scientific, Ltd., Kent, UK), which electronically measures the end-tidal CO concentration with a reported precision of >98%.<sup>27</sup> At the initial consultation, nicotine dependence was assessed using the Fagerström Test for Nicotine Dependence, a global standard test to assess physical dependence on nicotine.<sup>28–30</sup> The score ranges from 0 to 10, with higher scores indicating more severe nicotine dependence. The number of cigarettes smoked per day was determined by questioning the smoker as follows: “On average, in the past month, how many cigarettes did you smoke per day?” The Brinkman index was calculated as the daily number of

cigarettes multiplied by the number of smoking years. The Zung Self-Rating Depression Scale, a self-reported questionnaire, was used to assess the severity of depression, with a higher score indicating a more severe depressive state. This questionnaire is a useful tool with which to track changes in depression levels over time in research studies or during the post-treatment clinical course.<sup>31,32</sup> At each visit, a member of the study staff reviewed the questionnaires completed by the patients. If any omissions or errors were found, the patients were requested to complete the questionnaire again.<sup>33,34</sup>

### Blood sampling

Blood testing was performed to assess the biochemical and hematological profiles of the patients. Blood samples were collected from the antecubital vein 2 to 3 h after a meal to determine the estimated glomerular filtration rate, hemoglobin A1c (HbA1c) concentration, high-density lipoprotein-cholesterol (HDL-C) concentration, low-density lipoprotein-cholesterol (LDL-C) concentration, and high-sensitivity C-reactive protein concentration. The blood samples were immediately centrifuged at 3,000 rpm for 10 min at 4°C. The plasma HbA1c and serum HDL-C and LDL-C concentrations were measured using an automatic analyzer (LABOSPECT 008; Hitachi High-Technologies Co., Ltd., Tokyo, Japan) with enzyme-based reagents (Kyowa Medex Co., Ltd., Tokyo, Japan).<sup>35</sup> The serum MCP-1 concentration was measured using a specific sandwich enzyme-linked immunosorbent assay (Ikagaku Co., Ltd., Kyoto, Japan). The serum MCP-1 concentration was quantified with a sandwich enzyme immunoassay (human MCP-1, Quantikine; R&D Systems GmbH, Wiesbaden, Germany) according to the manufacturer’s protocol.<sup>36</sup> The maximum storage time of the aliquots before analysis

was 24 months without intermittent thawing. After completion of the study, sequential samples from each patient were run concurrently. The average serum recovery rate was 103%, with a sensitivity of 5.0 pg/mL and intra-assay and interassay variability of 4.8% and 5.8%, respectively. To detect any systematic drift in the MCP-1 protein concentration during storage, a subset of 35 pairs was measured twice: at the time of blood collection and 24 months later.<sup>36</sup>

### Statistical analysis

All statistical analyses were performed by a professional statistician using the Statistical Package for the Social Sciences (SPSS) Statistics 17.0 (SPSS Inc., Chicago, IL, USA). Normality of the data was assessed using the Shapiro–Wilk test. The MCP-1 values were logarithmically transformed for statistical analysis. Correlations between the serum MCP-1 concentration and smoking- and atherosclerosis-related factors were examined according to a sex-adjusted correlation analysis. Factors influencing the serum MCP-1 concentration were analyzed using a sex-adjusted multivariate analysis.

The power of the multiple regression analysis of smokers was calculated using a post-hoc statistical power analysis. At that time, the power was calculated using the sample size, number of independent values, and actual obtained  $R^2$  values. For the multiple regression analysis of smokers, the  $R^2$  value was 0.206. Three independent variables (sex, SBP, and smoking duration) were assessed using a sample size of 207; therefore, the power was calculated as 0.999, confirming a large value. The type I error probability ( $\alpha$ ) was 0.05.

### Results

Various parameters were evaluated in 207 consecutive smokers (age range, 25–81 years) who visited our smoking cessation

**Table 1.** Clinical characteristics of smokers.

	Smokers (n = 207)
Age (years)	59 ± 13
Male/female	155/52
BMI (kg/m <sup>2</sup> )	22.8 [21.0, 25.2]
Waist circumference (cm)	86 ± 11
SBP (mmHg)	129 ± 19
DBP (mmHg)	74 ± 12
eGFR (mL/min/1.73 m <sup>2</sup> )	78 [66, 93]
HbA1c (%)	5.9 [5.6, 6.7]
HDL-C (mg/dL)	55 ± 17
LDL-C (mg/dL)	112 [90, 130]
MCP-1 (pg/mL)	360 [294, 416]
hsCRP (mg/dL)	0.3 [0.1, 1.0]
Daily cigarette consumption (n)	20 [20, 30]
Duration of smoking (years)	38 ± 12
Brinkman index (pack-years)	880 [600, 1181]
CO (ppm)	16.0 [10.0, 25.8]
FTND score	7.2 ± 1.9
SDS score	37.3 ± 10.0

Data are presented as mean ± standard deviation or median [interquartile range].

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MCP-1, monocyte chemoattractant protein-1; hsCRP, high-sensitivity C-reactive protein; CO, carbon monoxide; FTND, Fagerström Test for Nicotine Dependence; SDS, Zung Self-Rating Depression Scale.

clinic. Among the smokers, 77 (37.2%) received antihypertensive agents, 38 (18.4%) received statins, and 43 (20.8%) received medications for diabetes mellitus.

The clinical characteristics of the smokers are shown in Table 1. The results of the clinical correlation analysis of the log-transformed serum MCP-1 concentrations in smokers are shown in Table 2. The log-transformed serum MCP-1 concentrations in smokers showed no correlation with the BMI, metabolic parameters (e.g., HbA1c, HDL-C, and LDL-C), number of cigarettes

**Table 2.** Sex-adjusted analysis on correlation between serum MCP-1 concentration and clinical parameters in smokers.

	Univariate		Multivariate	
	$\beta$ value	$p$ value	$\beta$ value	$p$ value
Age (years)	0.311	<0.001	–	–
BMI (kg/m <sup>2</sup> )	–0.037	0.581	–	–
Waist circumference (cm)	0.050	0.528	–	–
SBP (mmHg)	0.225	0.001	0.167	0.028
DBP (mmHg)	0.137	0.045	–	–
HbA1c (%)	–0.005	0.940	–	–
eGFR (mL/min/1.73 m <sup>2</sup> )	–0.244	0.001	–	–
HDL-C (mg/dL)	0.065	0.386	–	–
LDL-C (mg/dL)	–0.002	0.976	–	–
hsCRP (mg/L)	–0.008	0.921	–	–
Daily tobacco consumption (n)	–0.065	0.344	–	–
Duration of smoking (years)	0.342	<0.001	0.307	<0.001
Brinkman index (pack-years)	0.053	0.459	–	–
CO (ppm)	–0.131	0.056	–	–
FTND score	–0.119	0.080	–	–
SDS score	–0.092	0.194	–	–

$\beta$  value: correlation coefficient,  $R^2=0.206$

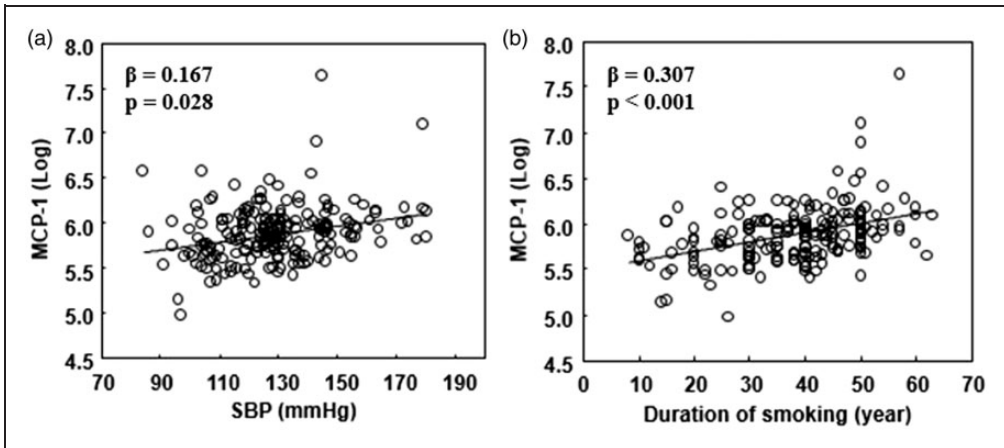
MCP-1, monocyte chemoattractant protein-1; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; CO, carbon monoxide; FTND, Fagerström Test for Nicotine Dependence; SDS, Zung Self-Rating Depression Scale.

per day, Brinkman index (pack-years), expiratory CO concentration, Zung Self-Rating Depression Scale score, or Fagerström Test for Nicotine Dependence score. However, the MCP-1 concentration was positively correlated with age ( $\beta=0.311$ ,  $p<0.001$ ), SBP ( $\beta=0.225$ ,  $p=0.001$ ), DBP ( $\beta=0.137$ ,  $p=0.045$ ), estimated glomerular filtration rate ( $\beta=-0.244$ ,  $p=0.001$ ), and smoking duration ( $\beta=0.342$ ,  $p<0.001$ ). In smokers, there was no significant correlation between the waist circumference and MCP-1 concentration. Waist circumference data were excluded from the multivariate analysis because of the significant correlation with the BMI and missing data of many patients. The sex-adjusted multivariate regression analysis revealed that SBP ( $\beta=0.167$ ,  $p=0.028$ ) (Figure 1(a)) and smoking duration ( $\beta=0.307$ ,  $p<0.001$ ) (Figure 1(b)) were

independent determinants of the MCP-1 concentration.

Various parameters were evaluated in 80 nonsmokers (age range, 26–80 years) who were recruited from the outpatient clinic of the Department of Cardiology. Among nonsmokers, 43 (53.8%) received antihypertensive agents, 22 (27.5%) received statins, and 2 (2.5%) received medications for diabetes mellitus.

The clinical characteristics of the nonsmokers are shown in Supplemental Table 1. In nonsmokers, we performed a sex-adjusted correlation analysis of the log-transformed serum MCP-1 concentration with age, BMI, blood pressure, and metabolic parameters (e.g., HbA1c, HDL-C, and LDL-C) (Supplemental Table 2). The serum MCP-1 concentration in nonsmokers showed a significant positive correlation with age, similar



**Figure 1.** Correlation of log-transformed serum monocyte chemoattractant protein-1 (MCP-1) concentration with systolic blood pressure (SBP) and smoking duration

Correlation between log-transformed serum MCP-1 concentration and (a) SBP ( $\beta = 0.167$ ,  $p = 0.028$ ) and (b) smoking duration ( $\beta = 0.307$ ,  $p < 0.001$ ).

to that observed in smokers. In contrast with the serum MCP-1 concentrations in smokers, those in nonsmokers were not correlated with SBP or DBP. The sex-adjusted multivariate regression analysis revealed that age ( $\beta = 0.418$ ,  $p = 0.001$ ) and BMI ( $\beta = 0.285$ ,  $p = 0.029$ ) were independent determinants of the MCP-1 concentration among nonsmokers.

The MCP-1 concentration was compared between hypertensive patients (SBP of  $\geq 140$  mmHg or DBP of  $\geq 90$  mmHg) and normotensive patients (SBP of  $< 140$  mmHg and DBP of  $< 90$  mmHg). As shown in Figure 2, the MCP-1 concentrations were significantly higher in hypertensive smokers ( $n = 59$ ) than in normotensive smokers ( $n = 148$ ) ( $p < 0.005$ ) (Figure 2(a)). However, there was no significant difference in the MCP-1 concentrations between hypertensive ( $n = 11$ ) and normotensive ( $n = 69$ ) nonsmokers (Figure 2(b)).

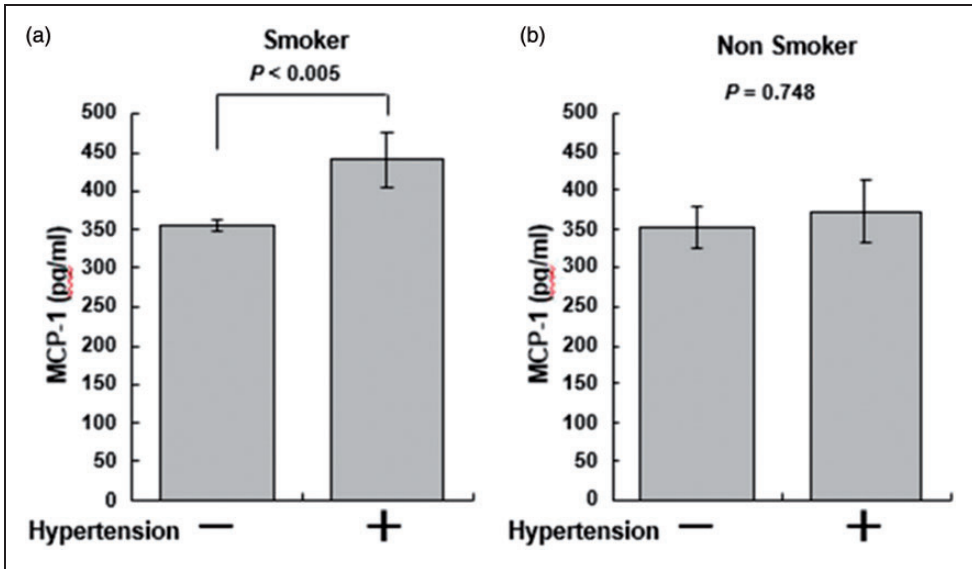
## Discussion

The results of the present study demonstrated that the MCP-1 concentration

increased with age in both smokers and nonsmokers. Arterial aging is the main contributor to the increased risk of morbidity from cardiovascular diseases, mainly because of the presence of chronic and low-grade inflammation of the vessels,<sup>37</sup> indicating that the MCP-1 concentration increases because of aging-related chronic inflammation.

The serum MCP-1 concentration was significantly and positively correlated with the SBP and DBP among smokers. The multivariate analysis showed that the SBP and smoking duration were determinants of the MCP-1 concentration. These findings imply that an increase in the serum MCP-1 concentration is closely associated with the presence of hypertension and a long-term history of smoking. Consistent with previous reports,<sup>21,38</sup> the MCP-1 concentration was significantly higher in hypertensive than normotensive smokers. However, there was no significant difference in the serum MCP-1 concentration between hypertensive and normotensive nonsmokers. The finding that the association between the MCP-1 concentration and blood pressure is





**Figure 2.** Comparison of serum monocyte chemoattractant protein-1 (MCP-1) concentration between hypertensive and normotensive smokers and nonsmokers

Comparison of serum MCP-1 concentration between hypertensive [systolic blood pressure (SBP) of  $\geq 140$  mmHg or diastolic blood pressure (DBP) of  $\geq 90$  mmHg] and normotensive patients (SBP of  $< 140$  mmHg and DBP of  $< 90$  mmHg). (a) The MCP-1 concentration was significantly higher in hypertensive smokers ( $n = 59$ ) than in normotensive smokers ( $n = 141$ ) ( $p < 0.005$ ). (b) Among nonsmokers, however, there was no significant difference in the MCP-1 concentration between hypertensive ( $n = 11$ ) and normotensive ( $n = 69$ ) patients ( $p = 0.748$ ).

dependent on the smoking status raises the possibility that MCP-1 contributes to the decreased vascular elasticity and increased vascular resistance caused by long-term smoking.

Obesity is a state of chronic inflammation that induces the release of fatty acids and inflammatory cytokines. In present study, the MCP-1 concentration was significantly correlated with the BMI among nonsmokers, which is in agreement with previous reports showing that the MCP-1 concentration increases because of obesity.<sup>7,17,18</sup> In contrast, the MCP-1 concentration was not associated with the BMI among smokers. *In vitro* studies have shown that nicotine promotes MCP-1 production<sup>23,24</sup> and that the MCP-1 concentration in mice increases due to tobacco smoke.<sup>25</sup> Additionally, the

MCP-1 concentration increases because of obesity.<sup>7,17,18</sup> Therefore, the MCP-1 concentration in smokers may reflect the effects of both body weight and smoking status. Because MCP-1 is a marker closely related to arteriosclerosis, the MCP-1 concentration in smokers can serve as a cardiovascular marker that comprehensively reflects both the body weight and smoking status.

This study had some limitations. With respect to the backgrounds of the patients (207 smokers and 80 nonsmokers), the nonsmokers were relatively older than the smokers. Hence, further studies are warranted to compare the traits between smokers and nonsmokers that impact the MCP-1 concentration. In addition, we investigated the association between the serum MCP-1 concentration

and smoking- and atherosclerosis-related factors using a cross-sectional design. Additional studies are required to clarify the cause-and-effect correlation between the MCP-1 concentration and the smoking-induced decreased blood vessel elasticity and increased vascular resistance.

## Conclusions

The serum MCP-1 concentration was positively correlated with the smoking duration and blood pressure in smokers. These findings suggest that MCP-1 contributes to the decrease in vascular elasticity and increase in vascular resistance caused by long-term smoking. MCP-1 plays a central role in inflammation of the vessel walls. Hence, long-term smokers with high blood pressure may be more susceptible to plaque rupture at atherosclerotic lesion sites.

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## Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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