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# Association between Plasma Monocyte Chemoattractant Protein-1 Levels and the Extent of Atherosclerotic Peripheral Artery Disease

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Peripheral artery disease occurs at advanced ages and accounts for substantial cardiovascular morbidity and mortality. Monocyte chemoattractant protein-1 (MCP-1), a member of the cysteine-cysteine family of chemokines, is one of the cytokines involved in the pathogenesis of atherosclerosis and is also known as cysteine-cysteine chemokine ligand 2 (CCL2). The aim of the current study was to investigate the association between the extent of atherosclerotic peripheral artery disease (PAD) and the increase in MCP-1 level. Eighty consecutive patients who had undergone peripheral angiography for suspected PAD were included. Of these patients, 48 (60%) had hypertension, 23 (28.8%) had type 2 diabetes mellitus, 39 (48.8%) had a family history of coronary artery disease, 23 (28.8%) were cigarette smokers, and 42 (52.5%) had hypercholesterolemia. Angiography revealed that the peripheral arteries of the lower extremity were normal in 41 (51.3%) patients, whereas 39 (48.7%) patients had varying degrees of PAD. The patients were queried regarding age, gender, and atherosclerotic risk factors. The plasma MCP-1 levels were significantly lower in the patients without PAD than those in the patients with PAD (172.27 ± 38.05 pg/mL vs. 200.87 ± 39.31 pg/mL, $p = 0.001$). Moreover, as the severity of PAD increases, MCP-1 levels also increase. Thus, the plasma MCP-1 level can be used in the diagnosis of PAD and in determining the extent of atherosclerotic PAD of the lower extremities, as in determining the extent of coronary artery disease.

**Keywords:** atherosclerosis; monocyte chemoattractant protein-1; peripheral angiography; peripheral artery disease; risk factors
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Peripheral artery disease (PAD) is an atherosclerotic disease that occurs at advanced ages and leads to substantial cardiovascular morbidity and mortality. The clinical manifestations of PAD are variable, ranging from asymptomatic disease to intermittent claudication (i.e. pain in the calf or leg, aggravated by walking or running, but relieved and resolved by rest) and critical leg or foot ischemia that requires extremity amputation (Viles-Gonzales et al. 2004). Atherosclerotic risk factors have an important place in the development and progression of PAD. Atherosclerosis is a long-term disease that starts early in childhood, progresses asymptomatically during adulthood, and finally presents with clinical manifestations. Inflammation plays an important role in the initiation and development of atherosclerosis. Several growth factors, cytokines, and adhesion molecules are responsible for this inflammation (Shammas 2007).

Monocyte chemoattractant protein-1 (MCP-1), also known as cysteine-cysteine (CC) chemokine ligand 2 (CCL2), is a member of the CC family of chemokines, and is one of the cytokines involved in the pathogenesis of atherosclerosis. MCP-1 is expressed by monocytes, macrophages, smooth muscle cells, and endothelial cells in atherosclerotic plaques (Nelken et al. 1991). MCP-1 binds to the CC chemokine receptor-2 (CCR2) and promotes the migration and accumulation of monocytes in the vascular wall (Krishnaswamy et al. 1999). In animal models, it has been noted that atherosclerosis progresses faster with increased MCP-1 expression (Namiki et al. 2002) and is decreased with MCP-1 (Gu et al. 1998) or CCR2 gene deletion (Boring et al. 1998). MCP-1 enhances the superoxide production of monocytes (Aukrust et al. 2001) by means of stimulating tissue factors (Schecter et al. 1997), and consequently converts the stable atherosclerotic plaque into an unstable one. Elevated serum MCP-1 levels have been shown to be associated with gene polymorphisms and myocardial infarction (McDermott et al. 2005). An association between the elevated MCP-1 levels with the extent of atherosclerosis is likely. MCP-1 is thought to be a biomarker that can be used in the follow-up of the prognosis of PAD.

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In the current study, we investigated whether or not there was an increase in MCP-1 levels associated with the extent of atherosclerotic involvement in the peripheral arteries of the lower extremity.

## Materials and Methods

*Study population*

Eighty consecutive patients who had undergone peripheral angiography for suspected PAD were included in the study. Patients with renal failure, decompensated heart failure, acute coronary syndrome, valvular heart disease, cardiomyopathy, and Buerger's disease were excluded from the study. Informed consent was obtained from all patients before the initiation of the study. The study was performed in accordance with the principles outlined in the Declaration of Helsinki and approved by the local Ethics Committee.

The study population was divided into the following 2 groups according to peripheral angiography results: Patients without PAD consisted of 41 patients (20 males and 21 females) with normal peripheral arteries and a mean age of 56.1 ± 10.8 years; and patients with PAD consisted of 39 patients (32 males and 7 females) and a mean age of 64.0 ± 9.3 years. The patients were questioned with respect to age, gender, and atherosclerotic risk factors.

*Peripheral angiography*

Peripheral angiography was performed using a standard Judkins technique with a Philips Allura Xper FD10 device (Philips Medical Systems, Best, the Netherlands). Patients with a luminal stenosis of > 50% in at least 1 peripheral artery segment were considered to have severe PAD.

*MCP-1 levels and other biochemical analyses*

After a 12-hour fast, venous blood samples of the patients were collected, and then the separated serum samples were stored for routine biochemical analyses, including serum glucose, urea, creatinine, total cholesterol, triglycerides, high-density lipoprotein (HDL)-cholesterol, and low-density lipoprotein (LDL)-cholesterol measurements. The patients' venous blood samples were also collected into ethylenediaminetetraacetic acid (EDTA)-containing tubes for the measurement of MCP-1 levels. The separated plasma samples were frozen and stored at −80ºC for a period of 3 months. MCP-1 levels were measured by a human MCP-1 enzyme-linked immunosorbent assay (ELISA) kit (Bender MedSystems, Burlingame, CA, USA) using an automated enzyme immunoassay analyzer (Triturus®; Grifols, Miami, FL, USA).

*Statistical analysis*

Statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) version15.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics for numerical variables were presented as the mean, standard deviation, and minimum and maximum values, whereas the frequency and percentage distributions were utilized to describe categorical variables. Categorical variables were compared using the independent samples *t*-test, one-way analysis of variance (ANOVA) for MCP-1 levels and the extent of PAD. In the comparison of subgroups, $p < 0.013$ was considered statistically significant with Bonferroni correction. The association between PAD and the risk factors was analyzed using logistic regression analysis. A $p$ value $< 0.05$ was considered statistically significant.

## Results

The mean age of the 80 patients was 59.9 ± 10.8 years of whom 65% were male. Moreover, of the patients, 48 (60%) had hypertension, 23 (28.8%) had diabetes mellitus, 39 (48.8%) had a family history of coronary artery disease, 23 (28.8%) were cigarette smokers, and 42 (52.5%) had hypercholesterolemia. The general characteristics and MCP-1 levels of the patients are presented in Table 1. The MCP-1 levels were lower in the patients without PAD than those in the patients with PAD (172.27 ± 38.05 pg/mL vs. 200.87 ± 39.31 pg/mL, $p$= 0.001). The peripheral arteries of the lower extremities were normal in 41 (51.3%) of the study population, whereas extensive atherosclerosis with severe stenosis, involving all segments was noted in 12 (15%) of the study population. Patient groups according to the peripheral angiography results are presented in Table 2.

MCP-1 levels according to gender of the patients with and without PAD are presented in Table 3. In patients with-

Table 1. Characteristics of the study population.

| | Total ($n$ = 80) | Patients | | $p$ |
|---|---|---|---|---|
| | | Without PAD ($n$ = 41) | With PAD ($n$ = 39) | |
| Age, years | 59.9 ± 10.8 | 56.1 ± 10.8 | 64.0 ± 9.3 | **0.001** |
| Gender | | | | |
| Female | 28 (35.0) | 21 (51.2) | 7 (17.9) | **0.002** |
| Male | 52 (65.0) | 20 (48.8) | 32 (82.1) | |
| Hypertension | 48 (60.0) | 22 (53.7) | 20 (66.7) | 0.235 |
| Diabetes mellitus | 23 (28.8) | 8 (19.5) | 15 (38.5) | 0.061 |
| Family history of CAD | 39 (48.8) | 21 (51.2) | 18 (46.2) | 0.650 |
| Cigarette smoking | 23 (28.8) | 8 (19.5) | 15 (38.5) | 0.061 |
| Hypercholesterolemia | 42 (52.5) | 20 (48.8) | 22 (56.4) | 0.495 |
| MCP-1 levels, pg/mL | 186.21 ± 41.03 | 172.27 ± 38.05 | 200.87 ± 39.31 | **0.001** |

Data are presented as mean±standard deviation or n (%), where appropriate.
CAD, Coronary artery disease; MCP-1, monocyte chemoattractant protein-1; PAD, Peripheral artery disease.

out PAD, the mean MCP-1 levels were significantly higher in males compared to females ($p = 0.027$). In patients with PAD, the mean MCP-1 levels were higher in males than females; however, it was not statistically significant ($p = 0.616$). MCP-1 levels increased with the extent of PAD (Table 4). A significant difference was found between the groups determined based on extent of PAD in terms of MCP-1 levels ($p = 0.019$). In paired comparisons, there was a significant difference between the normal peripheral artery group (P0) and extensive PAD group (P3) in terms of MCP-1 levels (172.27 ± 38.05 pg/mL vs. 205.06 ± 39.11 pg/mL, $p = 0.012$).

In order to determine the risk factors for PAD, variables included in the logistic regression analysis were gender (male), age, high cholesterol, smoking, hypertension, diabetes mellitus, family history of coronary artery disease, and MCP-1 level. In the model developed by including gender (male), age and MCP-1 (Model 1), male gender and age were found to be significant risk factors ($p = 0.003$ and $p = 0.001$, respectively; Table 5). The same analysis was performed in another model (Model 2) including MCP-1 level of ≥ 238 pg/mL, male gender and age were found to be significant risk factors ($p = 0.001$ and $p = 0.001$ respectively; Table 5).

## Discussion

The prevalence of PAD among males > 60 years of age is 10%. As PAD can be asymptomatic or can present with atypical symptoms, the true prevalence of PAD is assumed to be higher. The risk factors for PAD are similar to those for coronary artery disease and cerebrovascular diseases; however, diabetes mellitus and smoking are strongly associated with PAD. Other risk factors include age, hypertension, hyperhomocysteinemia, and hypercholesterolemia (Criqui 2001). When PAD is asymptomatic, it can be identified by a pulse examination of the lower extremities and the ankle-brachial index (ABI) measurement on physical examination. An ABI < 0.9 is considered abnormal and indicates PAD (Gardner and Afag 2008). When there is no appropriate care, ischemia requiring amputation can occur in patients with PAD, with an influence on morbidity and mortality. Concomitant coronary artery disease has been reported in 10%-30% of the patients with PAD (Karnegis et al. 1992; Atmer et al. 1995; Criqui et al. 1997).

Inflammation plays an important role in clinical forms of atherosclerosis, such as PAD, coronary artery disease, and carotid artery disease. In patients with atherosclerosis,

Table 2. Patient groups according to the results of peripheral angiography.

| | Results of peripheral angiography | *N* (%) |
|---|---|---|
| P0 | Normal lower-extremity peripheral arteries | 41 (51.3) |
| P1 | Non-critical stenosis and plaques in the peripheral arteries | 23 (28.8) |
| P2 | Single critical stenosis in a proximal artery (single stenosis ≥ 70% in one of the segments of the common iliac and popliteal arteries) | 4 (5.0) |
| P3 | Multiple severe stenoses in all segments (graft restenosis, diffuse stenoses, as well as severe stenosis, and total occlusion in ≥ 1 segment) | 12 (15.0) |

Table 3. MCP-1 levels (pg/mL) according to gender.

| Gender | Patients without PAD ($n = 41$) | Patients with PAD ($n = 39$) |
|---|---|---|
| | Mean ± S.D. | Mean ± S.D. |
| Female | 159.62 ± 39.336 | 194.00 ± 47.784 |
| Male | 185.55 ± 32.508 | 202.38 ± 37.934 |
| *p* | 0.027 | 0.616 |

MCP-1, monocyte chemoattractant protein-1; PAD, Peripheral artery disease; SD, Standard Deviation.

Table 4. Association between the MCP-1 levels and the extent of peripheral artery disease detected on peripheral angiography.

| Results of peripheral angiography | MCP-1 levels Mean ± S.D. | *n* |
|---|---|---|
| P0: Normal peripheral arteries | 172.27 ± 38.05 | 41 |
| P1: Non-critical plaques in the peripheral arteries | 197.96 ± 40.05 | 23 |
| P2: Single severe stenosis in a single segment of a peripheral artery | 198.00 ± 38.90 | 4 |
| P3: Extensive severe stenosis and total occlusion, involving multiple segments of the peripheral arteries | 205.06 ± 39.11 | 12 |

MCP-1, monocyte chemoattractant protein-1; SD, Standard Deviation.

Table 5. Logistic regression analysis of risk factors.

| | *p* | OR | %95 CI | |
|---|---|---|---|---|
| Model 1 | | | | |
| Gender (Male) | 0.003 | 7.376 | 1.993 | 27.296 |
| Age | 0.001 | 1.110 | 1.045 | 1.180 |
| MCP-1 | 0.060 | 1.014 | 0.999 | 1.028 |
| Model 2 | | | | |
| Gender (Male) | 0.001 | 8.517 | 2.354 | 30.810 |
| Age | 0.001 | 1.116 | 1.048 | 1.188 |
| MCP-1 ≥ 238 pg/mL | 0.057 | 5.252 | 0.950 | 29.047 |

oxidized LDL, cell adhesion molecules, chemokines, and chemokine receptors play an important role in the accumulation of mononuclear cells (monocytes and macrophages) in the arterial intima (Mackay 2001). MCP-1, which is one of the chemokines that plays a role in inflammation, contributes to the development of atherosclerotic plaques and progression of atherosclerosis by promoting the migration and accumulation of monocytes in the arterial intima. In the current literature, it has been reported that the plasma concentration of MCP-1 increases in association with atherosclerosis (Charo and Taubman 2004). Studies have also indicated that MCP-1 concentrations in blood samples obtained from the coronary arteries during coronary angiography are associated with the extent of coronary atherosclerosis and unstable angina (Serrano-Martínez et al. 2003). In the Orbofiban in Patients with Unstable Coronary Syndromes-Thrombolysis in Myocardial Infarction (OPUS-TIMI) 16 study, MCP-1 levels > 75th percentile (238 pg/mL) were found to be associated with an increased risk for death and myocardial infarction (de Lemos et al. 2003).

In studies using mouse models, it has been shown that MCP-1 plays a role in atherogenesis. An increase in MCP-1 levels, macrophage accumulation in atherosclerotic plaques, and an increase in atherosclerotic lesions have been reported after lipid deposition in the arterial intima (Osada et al. 2000). In contrast, less lipid deposition and macrophage infiltration have been noted in the aortas of genetically MCP-1-deficient mice (Gu et al. 1998). Similarly, atherosclerotic damage has been reported to decrease in CC chemokine receptor CCR2-deficient mice (Dawson et al. 1999).

Large scale studies have revealed that increased plasma concentrations of MCP-1 are associated with several cardiovascular risk factors, such as advanced age, hypertension, diabetes mellitus, hypercholesterolemia, cigarette smoking, a family history of premature coronary artery disease, low creatinine clearance, and high C-reactive protein levels (Deo et al. 2004). Similar to traditional risk factors, elevated MCP-1 levels in patients with acute coronary syndromes have been reported to be an independent risk factor for myocardial infarction and death (de Lemos et al. 2007).

Clinical studies have predominantly investigated increased MCP-1 expression in patients with acute coronary syndromes, atherosclerotic risk factors, typical ischemic heart disease, and myocarditis. In a small scale study, a relationship was established between PAD and MCP-1 levels (Matsui et al. 2003). Such a relationship has also been noted in studies comparing MCP-1 levels associated with the clinical symptomatology of PAD. Monocytes and chemokines play a key role in the atherosclerosis and inflammation in the peripheral artery wall. Moreover, a decrease has been reported in circulating MCP-1 levels with parenteral administration of prostaglandin E1 to the patients with PAD; thus, MCP-1 is considered to be an important marker for atherosclerotic inflammation (Petrkova et al. 2004). In their study, Petrkova et al. (2004) demonstrated elevated levels of MCP-1 in the circulation of patients with PAD and also reported that MCP-1 levels was more elevated in the serum of patients with earlier disease stages and that this might have been associated with the presence of diabetes. Hoogeveen et al. (2005) reported that the mean plasma MCP-1 levels were significantly higher in PAD cases and obtained a significant association between MCP-1 level and PAD by logistic regression analyses, independent of traditional CHD risk factors.

In the current study, we found that the plasma level of MCP-1 increased in patients with PAD by a similar mechanism to that involved in the inflammatory phase of coronary artery disease. Furthermore, the MCP-1 levels were higher in the advanced age group and in males. No direct association existed between the MCP-1 levels and other risk factors in the present study. The lack of a significant association between the MCP-1 levels and other risk factors was attributed to the fact that no intervention or change was made to the antihypertensive, antihyperlipidemic, or antiaggregant treatment regimens that patients received. Thus, therapeutic differences among patients might have led to a change in the risk factors, as well as a lack of a significant association between the MCP-1 levels and risk factors, such as hypertension, diabetes mellitus, and hyperlipidemia.

The MCP-1 levels were found to be significantly higher in the group with PAD as compared with the group without PAD. There was a significant difference between the group with PAD and the group without PAD in terms of age and gender. The rate of male patients in the group with

PAD was higher, and the mean age of the patients with PAD was also higher. There was no difference between these two groups in terms of the presence of the risk factors for PAD, such as hypertension, diabetes mellitus, family history of CAD, smoking, and hypercholesterolemia, and thus, it was possible to compare the groups in terms of MCP-1 levels.

Moreover, within the group with PAD, the amount of increase in plasma MCP-1 levels was greater in the subgroup with critical atherosclerotic stenosis as compared with the subgroup without critical atherosclerotic stenosis. The amount of increase in plasma MCP-1 levels was greater in the group with total occlusion and multiple segment involvement as compared with the other group. Considering the fact that atherosclerosis is a chronic inflammation that persists lifelong, an increase in the MCP-1 level is likely to be detected in proportion with the severity of atherosclerosis and inflammation in the peripheral arteries. In the current study, the increase in MCP-1 levels in patients with extensive atherosclerotic PAD and severe obstruction, involving all segments was greater as compared with the other groups. Extensive atherosclerosis leads to ischemia and consequently amputation of the extremities. Thus, implementation of a biomarker, which can be used in the early diagnosis and treatment of PAD and which can be suggestive about the extent of the disease, is important in clinical use. As a conclusion, in the current study, increased MCP-1 levels are associated with PAD and with more extensive disease. We propose that the plasma MCP-1 level is a prognostic biomarker for the extent of the disease in patients with PAD, as in other clinical forms of atherosclerosis.

## Conflict of Interest

All authors declare no conflict of interest.

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