Original Article

High-sensitivity C-reactive Protein, Lipoprotein(a) and Homocysteine are Risk Factors for Coronary Artery Disease in Japanese Patients with Peripheral Arterial Disease

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***Aim*: The goal of the study was to investigate the relationships between coronary artery disease (CAD) and risk factors, including the serum levels of high-sensitivity C-reactive protein (hs-CRP), lipoprotein(a) (Lp(a)) and homocysteine, in Japanese patients with peripheral arterial disease (PAD).**
***Methods*: Coronary angiography was performed in 451 patients with PAD, among whom the prevalence and clinical characteristics of CAD were analyzed. A multiple logistic analysis was used to evaluate the relationships between CAD and the risk factors. The relationships between the severity of coronary arterial lesions and the risk factors were evaluated using multiple regression analysis.**
***Results*: The prevalence of CAD (≥70% luminal diameter narrowing or a history of CAD) and coronary artery stenosis (≥50%) was 55.9% and 74.1%, respectively, and the rate of CAD (≥70%) with single-, double- and triple-vessel disease was 25.9%, 13.5% and 10.6%, respectively. The prevalence of diabetes was higher among the patients with CAD than among those without. The serum levels of hs-CRP, Lp(a), and homocysteine were higher in the patients with CAD, whereas the estimated glomerular filtration rates and HDL-cholesterol levels were lower in these patients. According to the multiple logistic analysis, CAD was related to diabetes (hazard ratio [HR]: 2.253; 95% confidence interval [CI]: 1.137-4.464, $p=0.020$), hs-CRP (HR: 1.721; 95% CI: 1.030-2.875, $p=0.038$), Lp(a) (HR: 1.015; 95% CI: 1.001-1.029, $p=0.041$) and homocysteine (HR: 1.084; 95% CI: 1.012-1.162, $p=0.021$). Furthermore, diabetes and the D-dimer and LDL-cholesterol levels exhibited significant relationships with the number of stenotic coronary lesions in the stepwise multiple regression analysis ($p<0.05$).**
***Conclusions*: Diabetes, hs-CRP, Lp(a), homocysteine and lipid abnormalities are critical risk factors for CAD in Japanese patients with PAD.**

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Key words: Coronary artery disease, High-sensitivity C-reactive protein, Homocysteine, Lipoprotein(a), Peripheral arterial disease

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Introduction

Coronary artery disease (CAD) is closely associated with peripheral arterial disease (PAD) and a major cause of death in patients with PAD[1-3]. Patients with PAD also frequently develop diabetes or hyperlipidemia and may have extensive and severe systemic

atherosclerosis, which is responsible for the mortality due to CAD[1, 2]. The Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC-II) reports that half of patients with PAD have concomitant CAD[1].

Chronic inflammation is an important risk factor for both CAD and PAD[4-6], and there is a significant relationship between the level of high-sensitivity C-reactive protein (hs-CRP) and the risk of CAD[7]. The serum level of hs-CRP is also an independent risk factor for cardiovascular disease, regardless of the LDL-cholesterol level[7-9]. Lipoprotein(a) (Lp(a)) has a high degree of homology with plasminogen and is also a risk factor for cardiovascular disease[10, 11]. Lp(a) is involved in the adhesion of inflammatory cells and migration and uptake of macrophage foam cells into the arterial wall[12], and the Lp(a) level is positively associated with CAD events[13]. Homocysteine is an intermediate metabolite of methionine that contributes to cardiovascular disease via mechanisms such as endothelial dysfunction, increased lipid permeability and vascular inflammation[14, 15]. Hyperhomocysteinemia is an independent risk factor for PAD and CAD[16, 17]. Hence, the combination of high homocysteine and Lp(a) levels synergistically increases the likelihood of developing CAD[15].

The roles of the serum hs-CRP, Lp(a) and homocysteine levels as potential risk factors for CAD are not fully understood in Japanese patients with PAD. Therefore, the purpose of the present study was to examine the risk factors and prevalence of CAD in this population.

Patients and Methods

Patients

The study cohort consisted of 451 consecutive patients referred to the Cardiovascular Hospital of Central Japan (Kitakanto Cardiovascular Hospital) between January 1st, 2009 and December 31st, 2013. Written consent to participate in this study was obtained from all patients, and the study protocol was approved by our institutional ethics committee before the initiation of the study. All patients had an ankle brachial pressure index (ABI) of <0.90 at their first visit and were diagnosed with PAD based on clinical symptoms and iliac or femoropopliteal artery stenosis of $\geq 70\%$ on angiography. The clinical stages of PAD and critical limb ischemia (CLI) and the Fontaine stage were classified according to the TASC-II criteria[1]. ABI was determined in all subjects using the ABI-form (Colin, Tokyo, Japan), which simultaneously measures the bilateral arm and ankle blood pressure according to an oscillometric method.

Risk Factors

Blood was collected during fasting in the morning in order to determine the levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, albumin, glucose, glycated hemoglobin A1c (HbA1c), creatinine, uric acid, hs-CRP, Lp(a), remnant-like particle cholesterol, homocysteine, D-dimer, fibrinogen, thrombin-antithrombin complex (TAT), plasmin-$\alpha 2$ plasmin inhibitor complex (PIC) and brain natriuretic peptide. The plasma levels of total cholesterol, triglycerides, HDL cholesterol, creatinine, glucose and uric acid were measured using a standard autoanalyzer in the clinical laboratory at the Cardiovascular Hospital of Central Japan (Hitachi 7180 automatic analyzer; Hitachi High-Tech Fielding Co, Tokyo, Japan). The LDL cholesterol level was calculated according to the Friedewald formula. If the triglyceride level was greater than 400 mg/dL, the level of LDL cholesterol was determined using a direct assay. The serum level of hs-CRP was measured using a latex enhanced immunonephelometric assay on a Dade Behring BN II nephelometer. The serum concentrations of homocysteine and HbA1c were measured using high-performance liquid chromatography. The serum Lp(a) level was measured using a turbidimetric immunoassay and the remnant-like particle cholesterol level was measured according to the immunoaffinity method. The plasma level of brain natriuretic peptide was measured using a fluorescence enzyme immunoassay. The D-dimer and PIC levels were measured using the latex coagulating method. The TAT level was measured according to an enzyme immunoassay. All assays were performed at the Health Science Research Institute, Inc. (Saitama, Japan).

Diabetes mellitus, hypertension and cerebral infarction were examined as risk factors for arteriosclerosis. Diabetes was defined as a fasting plasma glucose level of >126 mg/dL for at least two measurements or the need for antidiabetic therapy[18]. Hypertension was defined as a blood pressure of $\geq 140/90$ mmHg recorded at least twice or the use of antihypertensive agents. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation for creatinine, as modified by the Japanese Society of Nephrology: eGFR (mL/min/1.73 m^2) $= 194 \times (Scr)^{-1.094} \times (Age)^{-0287}$ ($\times 0.739$ if female)[19]. Cerebral infarction was considered positive based on the presence of a relevant medical history and/or any lesions on brain computed tomography. Smoking was defined as smoking an average of ≥ 5

cigarettes/day. Alcohol intake was defined as an average consumption of >10 g/day of absolute alcohol for >1 year within the past five years.

Assessment of PAD and CAD

Peripheral lesions were evaluated using digital subtraction angiography. Left and right coronary angiography (CAG) and left ventriculography were performed before abdominal and peripheral angiography. CAG was recorded at 25 frames/second. The luminal diameter (LD) was measured at end-diastole via quantitative coronary angiography using the automatic software program provided by the manufacturer (Philips Med, Best, The Netherlands). In cases involving stenotic coronary arteries, changes in the LD of stenosis were analyzed separately from the changes in the adjacent reference segments. The prevalence of CAD was defined as coronary artery stenosis with ≥70% LD narrowing or a history of treatment for CAD, defined as revascularization via percutaneous coronary intervention or coronary artery bypass surgery and/or the use of medical treatment after CAG.

Statistical Analysis

Continuous variables are expressed as medians (interquartile range) and were compared using the Wilcoxon test. Categorical variables are expressed as numbers (%) and were compared using the chi-square test. Odds ratios (ORs) and confidence intervals (CIs) were calculated for individual risk factors in a univariate logistic analysis. Factors with a p value of <0.05 in the univariate analysis were evaluated in a multivariate logistic analysis to detect predictors of CAD. In the multiple regression analysis, simple Pearson correlations were calculated for all risk factors, including medical treatment, the number of affected coronary arteries and the Lp(a), homocysteine and hs-CRP levels. Factors with a p value of <0.05 in the correlation analysis were evaluated using a stepwise forward multiple regression analysis to examine the relationships between individual risk factors and the number of affected coronary arteries, Lp(a), homocysteine and hs-CRP. The SPSS v. 17.0 (SPSS Inc., Chicago, IL) software program was used for all calculations, and a p value of <0.05 was considered to be significant.

Results

Patient Characteristics

The subjects included 451 patients with PAD who underwent CAG. The median age was 72 (65 to 77) years. The clinical characteristics, including comorbidities and risk factors, are summarized in **Table 1**. The prevalence of diabetes was higher among the patients with CAD than among those without. The mean age, ABI, body mass index and prevalence of CLI did not differ significantly between the two groups. The levels of hs-CRP, Lp(a) and homocysteine were higher in the patients with CAD, whereas the eGFR and HDL cholesterol values were lower in these patients. In contrast, the levels of brain natriuretic peptide and fibrinogen were not significantly different between the two groups. Meanwhile, there were no significant differences in medical treatment between the two groups, although the frequency of statin or antiplatelet therapy exhibited a tendency to be higher in the patients with CAD.

Prevalence of CAD

The results of CAG are summarized in **Fig. 1**. The prevalence of coronary artery stenosis with ≥70% LD narrowing was 50.1%, while that of coronary artery stenosis with ≥70% LD narrowing or a history of treatment for CAD was 55.9%. The prevalence of coronary artery stenosis with ≥50% LD narrowing was 74.1%. The rate of coronary artery stenosis (≥70%) with single-, double- and triple-vessel disease, including left main artery disease, was 25.9%, 13.5% and 10.6%, respectively. The prevalence of CAD was 57.4%, 56.2%, 34.6% and 57.8% in Fontaine stages I, II, III and IV, respectively, with no significant differences between the stages ($p=0.165$).

Risk Factors for CAD

According to the univariate logistic analysis, CAD was significantly related to diabetes, eGFR, hs-CRP, HDL cholesterol, triglyceride, Lp(a) and homocysteine. In the multiple logistic analysis, CAD was found to be significantly associated with diabetes, hs-CRP, Lp(a) and homocysteine and showed a tendency toward an association with triglycerides and a low HDL cholesterol level (**Table 2**). The number of stenotic coronary arteries demonstrated simple correlations with diabetes, smoking, eGFR, HDL-cholesterol, LDL-cholesterol and D-dimer ($p<0.05$). In the stepwise multiple regression analysis, diabetes, D-dimer and LDL-cholesterol displayed significant positive relationships with the number of stenotic coronary arteries (**Table 3**). The sum of the three coronary artery stenotic rates in each segment was also significantly related to LDL-cholesterol, diabetes and D-dimer in the stepwise multiple regression analysis.

Lp(a) had simple correlations with LDL-cholesterol, fibrinogen and body mass index (BMI) ($p<0.05$). The stepwise forward multiple regression analysis of the relationship of these factors with Lp(a)

Table 1. Baseline clinical characteristics and risk factors in the patients with peripheral arterial disease with or without coronary artery disease

Risk factor	All patients $n=451$	CAD (+) $n=252$ (55.9%)	CAD (−) $n=199$ (44.1%)	p-value
Age (year)	72 (65-77)	71 (64-77)	72 (66-78)	0.115
Gender (male)	381 (84.4%)	214 (84.9%)	167 (83.9%)	0.771
ABI	0.68 (0.52-0.81)	0.70 (0.52-0.81)	0.65 (0.50-0.80)	0.354
BMI (kg/m^2)	22.4 (20.1-24.5)	22.5 (20.3-24.6)	22.1 (20.3-24.4)	0.143
CLI	71 (15.7%)	35 (13.9%)	36 (18.1%)	0.224
History of CAD	95 (21.1%)	95 (37.7%)	0 (0%)	<0.001
CAD (current or former)	252 (55.9%)	252 (100%)	0 (0%)	<0.001
Risk factors				
Hypertension	272 (60.3%)	151 (59.9%)	121 (60.8%)	0.849
Diabetes mellitus	172 (38.1%)	113 (44.8%)	59 (29.6%)	<0.001
Cerebral infarction	78 (17.3%)	46 (18.3%)	32 (16.1%)	0.545
Smoking	347 (76.9%)	191 (75.8%)	156 (78.4%)	0.515
Hemodialysis	44 (9.8)	29 (11.5%)	15 (7.5%)	0.158
Laboratory data				
Albumin (g/dL)	4.0 (3.7-4.2)	4.0 (3.8-4.2)	4.0 (3.7-4.2)	0.605
eGFR (mL/min/1.73 m^2)	55.8 (43.2-67.1)	51.2 (39.0-64.5)	57.6 (47.6-69.3)	<0.001
hs-CRP (mg/dL)	0.19 (0.09-0.48)	0.20 (0.09-0.54)	0.17 (0.08-0.42)	0.045
Total-C (mg/dL)	185 (160-214)	184 (160-216)	185 (160-213)	0.818
LDL-C (mg/dL)	111 (89-137)	109 (88-139)	112 (91-135)	0.627
HDL-C (mg/dL)	45 (38-54)	44 (36-52)	47 (39-56)	0.001
RLP-C (mg/dL)	5.5 (3.6-8.3)	5.5 (3.5-8.3)	5.6 (3.7-8.4)	0.708
Triglyceride (mg/dL)	121 (81-169)	121 (81-169)	119 (81-160)	0.082
Lipoprotein(a) (mg/dL)	20.1 (10.0-34.3)	22.0 (12.0-37.8)	19.0 (9.3-31.0)	0.007
Homocysteine (nmol/mL)	12.5 (10.0-16.7)	13.4 (10.8-18.3)	12.0 (9.5-15.5)	0.004
D-dimer (μg/dL)	0.9 (0.5-1.9)	0.9 (0.5-1.9)	0.9 (0.5-2.0)	0.479
Fibrinogen (mg/dL)	312 (255-384)	314 (256-382)	311 (253-384)	0.635
TAT (ng/mL)	3.2 (2.0-6.0)	3.3 (2.1-6.6)	3.2 (2.0-5.5)	0.245
PIC (μg/mL)	1.0 (0.7-1.3)	1.0 (0.7-1.3)	1.0 (0.7-1.3)	0.864
BNP (pg/mL)	48.5 (20.1-154.3)	50.5 (21.9-168.2)	46.5 (19.9-147.8)	0.152
Drugs				
Statin	117 (25.9%)	74 (29.4%)	43 (21.6%)	0.062
Aspirin	256 (56.8%)	152 (60.3%)	104 (52.3%)	0.086
Clopidogrel	87 (19.3%)	56 (22.2%)	31 (15.6%)	0.076
Ticlopidine	63 (14.0%)	40 (15.9%)	23 (11.6%)	0.282
Cilostazol	147 (32.6%)	79 (31.3%)	68 (34.2%)	0.526
Beraprost	159 (35.3%)	81 (32.1%)	78 (39.2%)	0.120
Sarpogrelate	36 (8.0%)	23 (9.1%)	13 (6.5%)	0.313
ACE inhibitor	63 (14.0%)	32 (12.7%)	31 (15.6%)	0.381
ARB	126 (27.9%)	63 (25.0%)	63 (31.7%)	0.118
Ca antagonist	239 (53.0%)	133 (52.8%)	106 (53.3%)	0.918

Continuous variables are expressed as median (interquartile range) and were compared using the Wilcoxon test. Categorical variables are expressed as numbers (%) and were compared using the chi-square test.

ABI: ankle brachial pressure index, BMI: body mass index, CLI: critical limb ischemia, eGFR: estimated glomerular filtration rate, hs-CRP: high-sensitivity C-reactive protein, Total-C: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, RLP-C: remnant-like particle cholesterol, TAT: thrombin-antithrombin complex, PIC: plasmin-α2 plasmin inhibitor complex, BNP: brain natriuretic peptide, ACE: angiotensin-converting enzyme, ARB: angiotensin receptor blocker

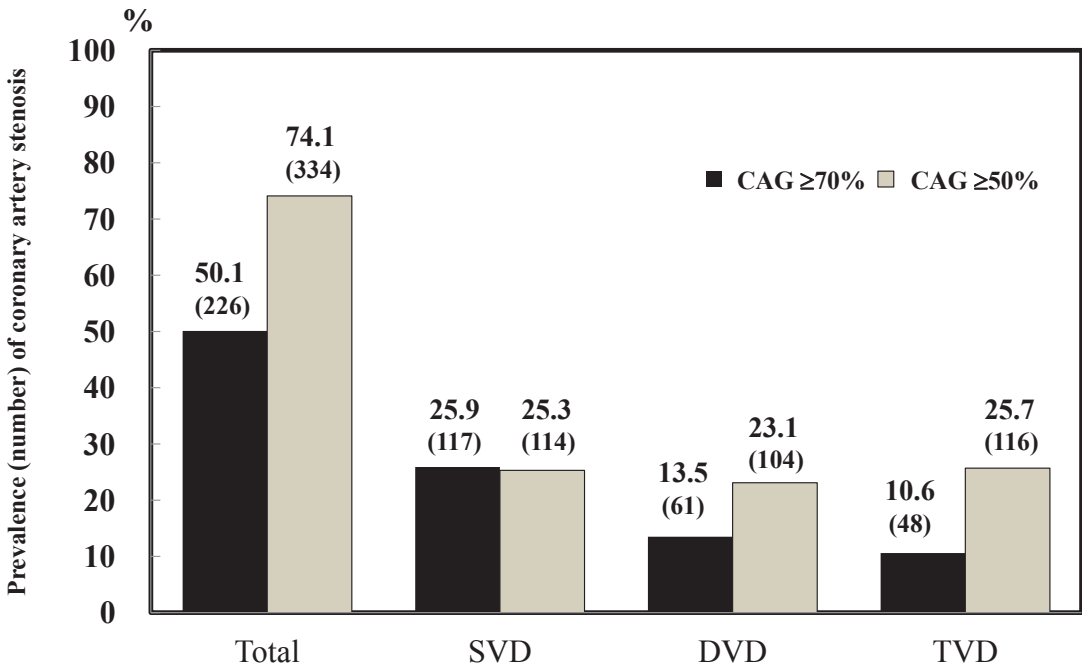

Fig. 1. Prevalence (number) of coronary artery stenosis with a luminal diameter (LD) of ≥70% narrowing and ≥50% LD narrowing.

SVD: single-vessel disease, DVD: double-vessel disease, TVD: triple-vessel disease

revealed significant positive correlations with LDL-cholesterol and fibrinogen and a significant negative correlation with BMI (**Table 4A**). The homocysteine level exhibited simple positive correlations with diabetes, HbA1c and brain natriuretic peptide and negative correlations with age, HDL-cholesterol and eGFR ($p<0.05$). The stepwise forward multiple regression analysis of the relationship of homocysteine with these factors showed significant negative correlations with eGFR and age (**Table 4B**). The hs-CRP level displayed simple positive correlations with CLI, fibrinogen and D-dimer ($p<0.05$). In the stepwise forward multiple regression analysis, fibrinogen and D-dimer were found to have significant positive relationships with hs-CRP (**Table 4C**).

The clinical characteristics of the patients who did and did not receive statin therapy are summarized in **Table 5**. The patients treated with statins were younger and more frequently had diabetes and a history of CAD compared to those who did not receive statins, whereas CLI and current or former CAD were less frequent in this group. Meanwhile, BMI, eGFR and the serum levels of albumin, total cholesterol, RLP cholesterol and triglycerides were higher in the patients treated with statins, whereas the serum levels of homocysteine, D-dimer, TAT and PIC were lower in these patients. The prevalence of aspirin, clopidogrel, ticlopidine and angiotensin receptor blocker use was higher in the patients treated with statins.

Discussion

In this study, the rate of a history of CAD was 21.1%, while the prevalence of CAD and coronary artery stenosis (≥50%) was 55.9% and 74.1%, respectively, on CAG. TASC-II reports that half of patients with PAD have concomitant CAD[1)], and the baseline data in the REduction of Atherothrombosis for Continued Health (REACH) registry indicate that 25.5% and 29.7% of PAD patients have a history of acute myocardial infarction (AMI) and stable angina, respectively, worldwide[20)]. Among the Japanese population in the REACH registry, 27.8% of the PAD patients had a history of CAD[21)], and Hirose *et al.* found a prevalence of concomitant CAD of 55% in Japanese patients with PAD using pharmacologic stress single-photon emission computed tomography[22)]. The present data confirm the high prevalence of asymptomatic CAD in patients with PAD. This finding is of importance because asymptomatic CAD is a precursor to symptomatic angina and/or AMI. Moreover, the prevalence of CAD did not differ among the Fontaine stages in this study, which indicates a poor prognosis for patients with PAD[1, 2)].

Table 2. Relationships between coronary artery disease and risk factors in the multiple logistic analyses with univariate and multivariate analyses

Risk factor	Univariate analysis			Multivariate analysis		
	OR	95%CI	*p*-value	OR	95%CI	*p*-value
Age (per 1 year)	0.988	0.966-1.012	0.326			
Gender (male vs. female)	1.052	0.600-1.846	0.859			
ABI (per 1 mmHg/mmHg)	1.078	0.451-2.578	0.865			
BMI (per 1 kg/m^2)	1.046	0.985-1.110	0.143			
CLI (CLI vs. others)	0.724	0.417-1.259	0.253			
Risk factors						
Hypertension (+ vs. −)	0.874	0.571-1.338	0.535			
Diabetes mellitus (+ vs. −)	2.324	1.477-3.656	<0.001	2.253	1.137-4.464	0.020
Cerebral infarction (+ vs. −)	1.289	0.733-2.269	0.378			
Smoking (+ vs. −)	0.726	0.436-1.207	0.217			
Hemodialysis (+ vs. −)	1.506	0.854-2.654	0.157			
Laboratory data						
Albumin (per 1 g/dL)	1.249	0.751-2.080	0.392			
eGFR (per 1 mL/min/1.73 m^2)	0.987	0.978-0.996	0.005	0.995	0.983-1.006	0.348
hs-CRP (per 1 mg/dL)	1.677	1.028-2.737	0.037	1.721	1.030-2.875	0.038
Total-C (per 1 mg/dL)	1.004	0.999-1.009	0.118			
LDL-C (per 1 mg/dL)	1.004	0.997-1.012	0.223			
HDL-C (per 1 mg/dL)	0.975	0.961-0.990	0.001	0.983	0.965-1.002	0.078
RLP-C (per 1 mg/dL)	1.023	0.987-1.060	0.212			
Triglyceride (per 1 mg/dL)	1.003	1.000-1.006	0.003	1.003	1.003-1.007	0.056
Lipoprotein(a) (per 1 mg/dL)	1.011	1.000-1.021	0.042	1.015	1.001-1.029	0.041
Homocysteine (per 1 nmol/mL)	1.044	1.004-1.086	0.030	1.084	1.012-1.162	0.021
D-dimer (per 1 μg/dL)	1.032	0.914-1.166	0.607			
Fibrinogen (per 1 mg/dL)	1.000	0.998-1.003	0.654			
TAT (per 1 ng/mL)	1.003	0.981-1.027	0.769			
PIC (per 1 μg/mL)	0.842	0.612-1.158	0.291			
BNP (per 1 pg/mL)	1.000	1.000-1.000	0.631			
Drugs						
Statin (+ vs. −)	1.605	0.824-2.967	0.130			
Aspirin (+ vs. −)	1.416	0.803-2.734	0.168			
Clopidogrel (+ vs. −)	1.359	0.736-2.855	0.151			
Ticlopidine (+ vs. −)	1.521	0.839-2.989	0.226			
Cilostazol (+ vs. −)	0.875	0.315-1.237	0.442			
Beraprost (+ vs. −)	0.751	0.301-1.102	0.103			
Sarpogrelate (+ vs. −)	0.662	0.354-1.236	0.195			
ACE inhibitor (+ vs. −)	0.780	0.488-1.247	0.299			
ARB (+ vs. −)	0.662	0.362-1.134	0.135			
Ca antagonist (+ vs. −)	1.019	0.734-1.415	0.910			

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for individual risk factors in the univariate logistic analysis. Factors with a p value of <0.05 in the univariate analysis were evaluated in a multivariate logistic analysis to detect predictors of CAD.

vs.: versus, ABI: ankle brachial pressure index, BMI: body mass index, CLI: critical limb ischemia, eGFR: estimated glomerular filtration rate, hs-CRP: high-sensitivity C-reactive protein, Total-C: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, RLP-C: remnant-like particle cholesterol, TAT: thrombin-antithrombin complex, PIC: plasmin-α2 plasmin inhibitor complex, BNP: brain natriuretic peptide, ACE: angiotensin-converting enzyme, ARB: angiotensin receptor blocker

Table 3. Correlations between the number of affected coronary arteries and risk factors in the stepwise forward multiple regression analysis

Risk factor	β	95% CI	p-value
Diabetes mellitus	0.240	0.156 to 0.777	0.004
D-dimer	0.223	0.026 to 0.166	0.007
LDL cholesterol	0.206	0.001 to o.010	0.014

$R^2 = 0.115$, F for change in $R^2 = 6.476$, $p = 0.001$
Factors with a p value of < 0.05 in the Pearson correlation analysis: diabetes mellitus, smoking, estimated glomerular filtration rate, high-density lipoprotein cholesterol, LDL cholesterol, homocysteine, D-dimer.
β: standardized coefficient, CI: confidence interval, LDL: low-density lipoprotein

Table 4A. Correlations between lipoprotein(a) and risk factors in the stepwise forward multiple regression analysis

Risk factor	β	95% CI	p-value
LDL cholesterol	0.167	0.001 to 0.010	< 0.001
Fibrinogen	0.165	0.047 to 0.163	0.001
Body mass index	-0.124	-1.152 to -0.217	0.009

$R^2 = 0.068$, F for change in $R^2 = 6.882$, $p < 0.001$
Factors with a p value of < 0.05 in the Pearson correlation analysis: LDL-cholesterol, fibrinogen, body mass index.
β: standardized coefficient, CI: confidence interval, LDL: low-density lipoprotein

Table 4B. Correlations between homocysteine and risk factors in the stepwise forward multiple regression analysis

Risk factor	β	95% CI	p-value
eGFR	-0.434	-0.253 to -0.164	< 0.001
Age	-0.173	-0.356 to -0.108	< 0.001

$R^2 = 0.218$, F for change in $R^2 = 31.4$, $p < 0.001$
Factors with a p value of < 0.05 in the Pearson correlation analysis: diabetes, glycated hemoglobin A1c, brain natriuretic peptide, age, high-density lipoprotein cholesterol, eGFR.
β: standardized coefficient, CI: confidence interval, eGFR: estimated glomerular filtration rate

Table 4C. Correlations between the high-sensitivity C-reactive protein levels and risk factors in the stepwise forward multiple regression analysis

Risk factor	β	95% CI	p-value
Fibrinogen	0.381	0.004 to 0.006	< 0.001
D-dimer	0.189	0.061 to 0.192	< 0.001

$R^2 = 0.185$, F for change in $R^2 = 14.551$, $p < 0.001$
Factors with a p value of < 0.05 in the Pearson correlation analysis: critical limb ischemia, fibrinogen, D-dimer.
β: standardized coefficient, CI: confidence interval

Our results provide the first evidence that hs-CRP, Lp(a) and homocysteine are independent risk factors for concomitant CAD in Japanese patients with PAD. Chronic inflammation is an important risk factor for CAD and PAD[4-6], and elevated hs-CRP levels are associated with limb amputation and all-cause mortality after endovascular treatment in hemodialysis patients with PAD[23]. Moreover, Seo *et al*. found a significant relationship between the level of hs-CRP and the risk of CAD[7], and the serum level of hs-CRP is an independent risk factor for cardiovascular disease, regardless of the LDL-cholesterol level[7-9]. Our results confirm these findings in that an elevated hs-CRP level was found to be associated with an increased risk of CAD in Japanese patients with PAD.

We also demonstrated that CAD is similarly associated with diabetes, Lp(a) and homocysteine and exhibits a tendency toward an association with triglycerides and a low HDL cholesterol level. Lp(a) possesses a high degree of homology with plasminogen and is a known risk factor for cardiovascular disease[10, 11]. In addition, Lp(a) is involved in inflammatory cell adhesion and migration as well as the uptake of macrophage foam cells into the arterial wall and inactivation of tissue-factor pathway inhibitors[12]. Moreover, the Lp(a) levels are positively associated with CAD events, with a stepwise increase in the risk of AMI in association with an increasing level of Lp(a) without evidence of a threshold effect in the Copenhagen City Heart Study[13]. The Lp(a) level is also a reported risk factor for CAD in Japanese patients with type II diabetes[24]. In the current study, the Lp(a) level demonstrated significant positive correlations with LDL-cholesterol and fibrinogen and significant negative correlations with BMI. Fibrinogen is a precursor of fibrin formation and activates platelet aggregation with increasing plasma viscosity[25]; it is also an acute-phase reactant that is increased in an inflammatory state[25]. In addition, the fibrinogen level is particularly associated with the severity and potential for the further development of PAD[26] and functions as an independent risk factor for CAD[25].

Hyperhomocysteinemia is an independent risk factor for atherosclerosis and a stronger risk factor for PAD than for CAD[16, 17], with an association with death or severe vascular events in patients with PAD[27]. We previously found that a high plasma homocysteine level is also a risk factor for CLI as well as PAD[17]. Furthermore, Masuda *et al*.[28] showed that diabetic subjects have higher homocysteine levels and that homocysteine has significant relationships with eGFR,

Table 5. Clinical characteristics and risk factors in the patients with peripheral arterial disease who did or did not receive treatment with statins

Risk factor	Statins (+) $n=117$ (25.9%)	Statins (−) $n=334$ (74.1%)	p-value
Age (year)	71 (64-76)	73 (66-77)	0.005
Gender (male)	105 (89.7%)	276 (82.6%)	0.086
ABI	0.70 (0.54-0.80)	0.67 (0.51-0.80)	0.040
BMI (kg/m^2)	22.4 (20.2-24.5)	22.1 (19.7-24.2)	<0.001
CLI	10 (8.5%)	61 (18.3%)	0.013
History of CAD	33 (28.2%)	62 (18.6%)	0.023
CAD (current or former)	54 (46.1%)	198 (59.3%)	0.014
Risk factors			
Hypertension	73 (62.3%)	199 (59.6%)	0.593
Diabetes mellitus	54 (46.2%)	118 (35.3%)	0.038
Cerebral infarction	22 (18.8%)	56 (16.8%)	0.616
Smoking	89 (76.1%)	258 (77.2%)	0.795
Hemodialysis	10 (8.5%)	34 (10.2%)	0.609
Laboratory data			
Albumin (g/dL)	4.1 (3.8-4.2)	4.0 (3.7-4.2)	0.002
eGFR (mL/min/1.73 m^2)	59.0 (44.7-70.0)	54.2 (42.3-656)	0.014
hs-CRP (mg/dL)	0.17 (0.08-0.40)	0.20 (0.09-0.50)	0.189
Total-C (mg/dL)	196 (163-228)	182 (158-209)	<0.001
LDL-C (mg/dL)	113 (88-144)	110 (89-134)	0.298
HDL-C (mg/dL)	45 (38-54)	45 (37-54)	0.758
RLP-C (mg/dL)	6.1 (4.0-8.9)	5.4 (3.5-7.9)	0.010
Triglyceride (mg/dL)	148 (104-196)	112 (77-156)	<0.001
Lipoprotein(a) (mg/dL)	21.0 (10.0-35.1)	20.0 (11.0-33.8)	0.808
Homocysteine (nmol/mL)	12.1 (9.1-15.5)	12.7 (10.3-17.3)	0.049
D-dimer (μg/dL)	0.6 (0.5-1.3)	1.1 (0.6-2.0)	<0.001
Fibrinogen (mg/dL)	299 (251-379)	315 (257-384)	0.328
TAT (ng/mL)	2.8 (2.0-4.4)	3.4 (2.1-6.7)	0.003
PIC (μg/mL)	0.9 (0.7-1.1)	1.0 (0.8-1.3)	0.001
BNP (pg/mL)	52.6 (23.0-169.1)	44.5 (19.2-1457)	0.143
Drugs			
Aspirin	78 (66.7%)	178 (53.3%)	0.012
Clopidogrel	33 (28.2%)	54 (16.2%)	0.005
Ticlopidine	26 (22.2%)	37 (11.1%)	0.003
Cilostazol	31 (26.5%)	116 (34.7%)	0.102
Beraprost	36 (30.8%)	123 (36.8%)	0.238
Sarpogrelate	10 (8.5%)	26 (7.8%)	0.793
ACE inhibitor	13 (11.1%)	50 (15.0%)	0.300
ARB	45 (38.5%)	81 (24.3%)	0.003
Ca antagonist	59 (50.4%)	180 (53.9%)	0.518

Continuous variables are expressed as medians (interquartile range) and were compared using the Wilcoxon test. Categorical variables are expressed as numbers (%) and were compared using the chi-square test.

ABI: ankle brachial pressure index, BMI: body mass index, CLI: critical limb ischemia, eGFR: estimated glomerular filtration rate, hs-CRP: high-sensitivity C-reactive protein, Total-C: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, RLP-C: remnant-like particle cholesterol, TAT: thrombin-antithrombin complex, PIC: plasmin-α2 plasmin inhibitor complex, BNP: brain natriuretic peptide, ACE: angiotensin-converting enzyme, ARB: angiotensin receptor blocker

fasting glucose, triglycerides and HbA1c. In this study, the homocysteine level displayed significant negative relationships with eGFR and age in the patients with PAD. The presence of homocysteine in amounts as low as 8 μmol/L increases the affinity between Lp(a) and plasmin-treated fibrin by 20-fold[29], and the combination of high homocysteine and Lp(a) levels synergistically increases the likelihood of development of CAD[15]. Consistent with these findings, the results of the current study showed that high plasma homocysteine and Lp(a) levels may be risk factors for CAD in Japanese patients with PAD.

The present findings showed that diabetes, D-dimer and LDL-cholesterol each have a significant relationship with the number of stenotic coronary arteries and that diabetes and lipid abnormalities are important classical risk factors for the development of CAD and systemic vascular disease[30-33]. D-dimer is an end product of fibrinolysis that promotes the inflammatory cascade by activating neutrophils and monocytes, thus inducing the secretion of inflammatory cytokines and promoting the hepatic synthesis of acute-phase proteins[34]. In the current study, the hs-CRP level exhibited significant positive relationships with D-dimer and fibrinogen. Moreover, the plasma D-dimer levels are strongly and independently associated with the presence of CAD in patients with stable angina pectoris[35]. Hence, the high D-dimer and fibrinogen levels observed in patients with PAD may be linked to the levels of hs-CRP and Lp(a) in these subjects with CAD.

In this study, there were no significant differences in medical treatment between the two groups, although the prevalence of statin and antiplatelet therapy showed a tendency to be higher among the patients with CAD. This inverse tendency may be due to the patients with CAD including those with a history of CAD. Anti-inflammatory effects are expected in patients with diabetes or PAD treated with statins or aspirin[1, 36]. In the present study, the patients treated with statins were younger and more frequently had diabetes and a history of CAD than the patients who did not receive statins. In addition, the serum levels of homocysteine, D-dimer, TAT and PIC were lower in the patients treated with statins; however, these patients more frequently received antiplatelet therapy. Hence, the direct effects of statins or antiplatelet agents in the study were unclear.

The limitations of this study include the relatively small sample size and use of a single facility. We also did not employ population-based data and had no data for vitamin B_6 and B_{12} or folic acid, all of which may affect the homocysteine level. Therefore, further studies are needed to determine the exact prevalence and risk factors for CAD in Japanese patients with PAD.

Conclusion

The prevalence of CAD is markedly higher in patients with PAD. Diabetes, hs-CRP, Lp(a), homocysteine and lipid abnormalities are critical risk factors for CAD in Japanese patients with PAD, indicating the roles of chronic inflammation, coagulation and fibrinolysis in the onset of CAD in these patients.

Conflicts of Interest

None.

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