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Associations of matrix metalloproteinase-9 and monocyte chemoattractant protein-1 concentrations with carotid atherosclerosis, based on measurements of plaque and intima–media thickness

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ABSTRACT

Purpose: To examine associations of matrix metalloproteinase-9 (MMP-9) and monocyte chemoattractant protein-1 (MCP-1) concentrations with the severity of carotid atherosclerosis, based on measurements of carotid plaque and intima–media thickness (IMT).
Methods: This cross-sectional study included 116 stroke-free participants (45.7% males, 54.3% females; mean age, 64.73 ± 14.53 years). Serum MMP-9 and MCP-1 concentrations were measured, and plaque morphology, including total plaque score (PS), plaque stability, and IMT, was assessed ultrasonographically. Participants were grouped according to total PS (0, 1–2, ≥3), plaque stability (no plaque, stable, unstable) and IMT tertiles (<0.8 mm, 0.8–1 mm, >1 mm). Multinomial logistic regression models were used to assess the associations of MMP-9 and MCP-1 concentrations with plaque and IMT values after adjusting for vascular risk factors.
Results: MMP-9 quartiles (*vs.* quartile 1) were significantly associated with a greater prevalence of plaque instability [Q2: odds ratio (OR) = 5.13, 95% confidence interval (CI) = 1.01–24.9, $p = 0.042$; Q3: OR = 15.5, 95% CI = 3.1–78.1, $p = 0.001$; Q4: OR = 13.2, 95% CI = 2.7–64.97, $p = 0.001$] and high total PS (Q3: OR = 10.02, 95% CI = 1.5–65.33, $p = 0.016$; Q4: OR = 21.5, 95% CI = 3.5–132.1, $p = 0.001$). MCP-1 concentration was significantly associated with IMT (OR = 22.94, 95% CI = 2.14–245.66, $p = 0.01$).
Conclusions: Elevated serum MMP-9 concentration was independently associated with high total carotid artery PS, plaque instability, and large IMT value. MCP-1 concentration was independently associated with IMT, but not with plaque morphology.

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1. Introduction

Carotid atherosclerosis, detected noninvasively by high-resolution ultrasound imaging, has been used as a marker for the development of cardiovascular and cerebrovascular diseases. The severity of carotid atherosclerosis can be assessed using intima–media thickness (IMT), total plaque area (TPA), and plaque stability. Previous studies have shown that increased IMT [1–6] and plaque instability [6] predict future vascular events independently of conventional vascular risk factors. The results of a large-scale 10-year follow-up study suggested that TPA is a stronger predictor than IMT of first ischemic stroke [7].

Atherosclerosis is a chronic inflammatory process in the arterial wall involving matrix metalloproteinases (MMPs) and monocyte chemotactic protein-1 (MCP-1). It is characterized by extracellular matrix (ECM) remodeling, a complex process in which MMPs play an important role [8–13]. Increased MMP-9 expression has been found in carotid atherosclerotic plaques rendered unstable by carotid endarterectomy [14–16]. However, total blood MMP-9 levels have been reported variously to be positively [17] or negatively [18,19] correlated with carotid plaque stability or IMT. The initiation and progression of atherosclerosis depend on the recruitment of monocytes to sites of active inflammation; for example, MCP-1 initiates the development of atheroma. Elevated serum MCP-1

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concentrations have been documented in patients with cardiocerebrovascular disease [20,21], especially in those undergoing hemodialysis [22]. However, a large-scale study failed to prove that MCP-1 level was an independent predictor of ischemic stroke and cardiovascular disease [23]. To our knowledge, the relationship between MCP-1 and the severity of carotid atherosclerosis has not been reported. For this reason, and given the conflicting results regarding the relationship between MMP-9 and carotid atherosclerosis severity, we examined blood concentrations of MMP-9 and MCP-1 in relation to the severity of carotid atherosclerosis, assessed using the carotid plaque score (PS), IMT, and plaque stability.

2. Methods

2.1. Study participants

Study participants were recruited from among consecutive patients aged ≥30 years who were referred for carotid ultrasound examination at the Neurology Department of the Second Affiliated Hospital of Chongqing Medical University (China) in 2011–2012. Exclusion criteria were as follows: 1) histories of stroke, 2) histories of transient ischemic attack, 3) recent acute coronary syndrome, 4) cancer, and 5) inflammatory disease (e.g., rheumatoid arthritis and gingivitis). The institutional ethics committee approved this study and all participants provided informed consent.

2.2. Baseline clinical data

All participants underwent baseline clinical examination, including medical history documentation, physical examination, laboratory testing, and carotid ultrasound examination. Collected data included patients' age and blood pressure; histories of cigarette and alcohol use, hypertension, and/or diabetes mellitus; and measurement of total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, uric acid (UA), and C-reactive protein (CRP) levels in fasting blood samples. Blood samples were promptly centrifuged at 3000× *g* for 10 min at 4 °C. Aliquots of serum were stored in a central laboratory at −80 °C.

Hypertension was defined as the use of anti-hypertensive medication, average systolic blood pressure ≥140 mmHg, or average diastolic blood pressure ≥90 mmHg. Diabetes was defined as the use of anti-diabetic medication or fasting glucose level ≥7.0 mmol/L. Smokers were classified on the basis of a history of smoking >100 cigarettes, using the simplified definition of the United States Centers for Disease Control. Alcohol use was defined as alcohol intake ≥100 g/d for at least 5 years.

2.3. Carotid ultrasound examination

The carotid arteries were evaluated with high-resolution B-mode ultrasonography (model iE33; Philips Medical Systems, Eindhoven, The Netherlands). A reader blinded to all clinical information determined measurements at a central reading facility. TPA was indirectly represented using a previously reported PS method [19,24,25]. In brief, longitudinal images of the bilateral carotid arteries, including the proximal (>10 mm proximal to bulb bifurcation) and distal common carotid artery, bulb, and internal and external carotid arteries (10 segments), were acquired. The presence of plaque was scored according to the encroachment of localized echoic structures into the arterial lumen, representing at least 50% of the surrounding IMT value [26]. PSs were assigned as follows: 0, normal/no plaque; 1, one small plaque (<30% stenosis); 2, one medium plaque (30–49% stenosis) or multiple small plaques; 3, one large plaque (50–99% stenosis) or multiple plaques with more than one medium plaque; and 4, 100% occlusion [19,24,25]. PSs for the 10 segments were summed to obtain a total PS for each patient. The study participants were divided into three groups based on total PS (0, 1–2, ≥3) [19].

Carotid IMT was measured in three segments: the distal common carotid artery (1 cm proximal to the carotid bulb), the carotid artery bifurcation (1 cm proximal to the flow divider), and the proximal internal carotid artery (1 cm length). The mean of all IMT measurements from the right and left sides was calculated for each patient [27]. The participants were divided into three groups based on IMT tertiles (T1, <0.8 mm; T2, 0.8–1 mm; T3, >1 mm).

Plaque stability was classified as absent, stable, or unstable according to surface characteristics, echogenicity, and texture, as reported previously [6]. The absence of plaque was characterized by a smooth intimal surface with no focal thickening. Unstable plaque was defined as the presence of a markedly irregular or ulcerated surface or hypodense or heterogeneous plaques occupying >50% of the TPA. Stable plaque was defined as the presence of isoechoic, hyperdense, calcified, or homogeneous plaques or a slightly irregular surface. When more than one type of plaque was detected in an individual, plaque risk was determined using the more severe type.

2.4. Enzyme-linked immunosorbent assay

A researcher blinded to the clinical data measured serum MMP-9 and MCP-1 levels using commercially available enzyme-linked immunosorbent assay kits (4A Biotech Co., Beijing, China). Samples were handled in an identical and blinded fashion throughout the study. They were analyzed in duplicate and in random order to reduce systemic bias and interassay variation. Mean intra-assay coefficients of variation for the method were 9.5% for MMP-9 and 8.6% for MCP-1. The mean inter-assay coefficients of variation for the method were 11.3% for MMP-9 and 9.1% for MCP-1. MMP-9 and MCP-1 concentrations were examined as continuous variables and in quartiles.

2.5. Statistical analyses

Continuous data, expressed as means ± standard deviations or medians and interquartile ranges, were analyzed using a general linear model or a nonparametric test. Categorical data, expressed as numbers and percentages, were compared using the chi-squared test. Multinomial logistic regression models with no plaque as the reference were constructed to examine associations between MMP-9 (as a continuous variable and in quartiles) and plaque phenotypes after adjusting for age, UA level, and vascular risk factors (diabetes mellitus, hypertension, HDL and LDL cholesterol levels, smoking history, and alcohol use). All analyses were performed with SPSS software (ver. 11.5 for Windows; SPSS Inc., Chicago, IL, USA). Two-tailed p values <0.05 were considered to indicate statistical significance.

3. Results

3.1. Cohort characteristics

The study sample comprised 116 participants (53 men, 63 women) with a mean age of 64 ± 14.5 years. Baseline cohort characteristics according to total PS, plaque stability, and IMT tertile are shown in Tables 1–3. Total PS and plaque stability were significantly affected by age ($p = 0.008$ and $p = 0.003$, respectively) and hypertension ($p = 0.001$ and $p = 0.044$, respectively; Tables 1 and 2). IMT was significantly affected by hypertension ($p = 0.009$) and LDL cholesterol ($p = 0.014$) and MCP-1 ($p = 0.049$) levels

Table 1
Characteristics of study participants according to total carotid artery plaque score.

Variable	Total plaque score			p
	0 $n = 43$	1–2 $n = 37$	≥3 $n = 36$	
Age (years)[a]	60 ± 8.7	65 ± 16.7	70 ± 16	0.008
Males (%)	39.5	51.4	47.2	0.558
Alcohol use (%)	4.7	16.2	13.9	0.211
Hypertension (%)	34.9	43.2	75	0.001
Diabetes mellitus (%)	18.6	24.3	33.3	0.32
Cigarette smoking (%)	14	29.7	22.2	0.23
TC (mg/dL)[a]	189.9 ± 35.5	197.8 ± 47.3	201.1 ± 45.4	0.488
LDL cholesterol (mg/dL)[a]	105.3 ± 31.6	112.0 ± 32.7	119.9 ± 39.2	0.179
HDL cholesterol (mg/dL)[b]	48 (39–60)	45 (39–53)	44 (38–52)	0.397
TG (mg/dL)[b]	114 (72–188)	147 (84–186)	136 (90–179)	0.539
Uric acid (umol/L)[a]	266 ± 76.7	289 ± 104.9	329 ± 152.6	0.053
CRP (mg/L)[b]	1.6 (0.8–3.6)	1.1 (0.5–3.8)	2 (0.9–8.3)	0.289
MMP-9 (ng/mL)[b]	244 (78–440)	479 (308–680)	762 (427–1232)	<0.001
MCP-1 (pg/mL)[a]	64 ± 39	78 ± 53	83 ± 82	0.376

[a] Mean ± standard deviation.
[b] Median (interquartile range). TC: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TG: triglycerides; CRP: C-reactive protein; MMP: matrix metalloproteinase; MCP-1: monocyte chemoattractant protein-1.

(Table 3). In general linear models, UA was significantly associated with plaque instability ($p = 0.013$) and large IMT value ($p = 0.028$). The association of UA with plaque instability persisted after adjustment for age, hypertension, diabetes mellitus, and LDL cholesterol level [odds ratio (OR) = 8.36, 95% confidence interval (CI) = 1.46–47.90, $p = 0.017$], but that with IMT did not (OR = 1.01, 95% CI = 1.00–1.02, $p = 0.052$). We found no relationship between CRP level and the severity of carotid atherosclerosis.

3.2. Associations of MMP-9 with plaque and IMT

When MMP-9 was examined as a continuous variable, clear dose–response relationships were observed between increased MMP-9 level and plaque instability ($p = 0.010$; Table 2), high total PS ($p = 0.000$; Table 1), and increased IMT ($p = 0.000$; Table 3).

MMP-9 quartiles were ≤201.5 ng/mL (Q1), 201.6–427.9 ng/mL (Q2), 428.0–786.7 ng/mL (Q3), and ≥786.8 ng/mL (Q4). Table 4 shows the relationships between MMP-9 quartile and total PS, IMT, and plaque stability in multivariate adjusted models. MMP-9 quartiles (*vs.* quartile 1) were significantly associated with a greater prevalence of plaque instability (Q2: OR = 5.13, 95% CI = 1.01–24.9, $p = 0.042$; Q3: OR = 15.5, 95% CI = 3.1–78.1, $p = 0.001$; Q4: OR = 13.2, 95% CI = 2.7–64.97, $p = 0.001$) and high total PS (Q3: OR = 10.02, 95% CI = 1.5–65.33, $p = 0.016$; Q4: OR = 21.5, 95% CI = 3.5–132.1, $p = 0.001$). No significant association was observed between MMP-9 Q2 and total PS (OR = 5.15, 95% CI = 0.79–33.3, $p = 0.085$).

Because unstable plaque was observed more frequently in patients with more cardiovascular risk factors and regression analysis might not allow proper exclusion of the effects of these factors on serum markers, we performed a power analysis to ascertain the relationship between MMP-9 and unstable plaque. A receiver operating characteristic curve was used to determine a cutoff value of 510.15 ng/mL related to unstable plaque (area under the curve = 0.732). This cutoff value was then applied to the unstable and no plaque groups. We used the chi-squared test to determine the association between elevated MMP-9 and unstable plaque (OR = 8.179). The Power and Sample Size Calculation program (version 3.0) [28] provided a power of 0.997.

3.3. Associations of MCP-1 with plaque and IMT

In general linear models, high serum MCP-1 level was significantly associated with increased IMT ($p = 0.049$; Table 3), but not with plaque stability ($p = 0.399$; Table 2) or total PS ($p = 0.376$; Table 1). MCP-1 quartiles were ≤32.4 pg/mL (Q1), 32.5–62.5 pg/mL (Q2), 62.6–106.4 pg/mL (Q3), and ≥106.5 pg/mL (Q4). The association with IMT remained stable in a multinomial logistic regression model adjusted for covariates, including age, hypertension, diabetes mellitus, cigarette smoking, and LDL cholesterol and UA levels (OR = 22.94, 95% CI = 2.14–245.66, $p = 0.01$; Table 5).

Table 2
Characteristics of study participants according to plaque stability.

Variable	Plaque stability			p
	No plaque $n = 43$	Stable $n = 32$	Unstable $n = 41$	
Age (years)[a]	60 ± 8.7	71 ± 12.4	64 ± 18.6	0.003
Males (%)	39.5	43.8	53.7	0.432
Alcohol use (%)	4.7	12.5	17.1	0.184
Hypertension (%)	34.9	59.4	58.5	0.044
Diabetes mellitus (%)	18.6	34.4	24.4	0.294
Cigarette smoking (%)	14	31.3	22	0.204
TC (mg/dL)[a]	190 ± 36	203 ± 51	197 ± 43	0.437
LDL cholesterol (mg/dL)[a]	105 ± 32	113 ± 36	118 ± 36	0.245
HDL cholesterol (mg/dL)[b]	48 (39–60)	46 (41–58)	41 (38–49)	0.145
TG (mg/dL)[b]	115 (72–188)	127 (77–168)	151 (102–194)	0.273
Uric acid (umol/L)[a]	266 ± 76.7	275 ± 103.3	335 ± 145.4	0.013
CRP (mg/L)[b]	1.6 (0.8–3.6)	1.7 (0.6–5.6)	1.8 (0.7–3.3)	0.923
MMP-9 (ng/mL)[b]	244 (78–440)	449 (316–786)	658 (394–1138)	<0.001
MCP-1 (pg/mL)[a]	64 ± 39	81 ± 56	80 ± 78	0.399

[a] Mean ± standard deviation.
[b] Median (interquartile range). TC: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TG: triglycerides; CRP: C-reactive protein; MMP: matrix metalloproteinase; MCP-1: monocyte chemoattractant protein-1.

Table 3
Characteristics of study participants according to tertiles of carotid intima–media thickness.

Variable	Carotid IMT tertile			p
	T1 $n = 43$	T2 $n = 37$	T3 $n = 36$	
Age (years)[a]	58 ± 12.4	65 ± 15.0	68 ± 13.1	0.058
Males (%)	36.4	44.6	60	0.293
Alcohol use (%)	4.5	9.5	25	0.106
Hypertension (%)	36.4	45.9	80	0.009
Diabetes mellitus (%)	18.2	24.3	35	0.443
Cigarette smoking (%)	9.1	21.6	35	0.139
TC (mg/dL)[a]	179 ± 43	197 ± 36	208 ± 59	0.078
LDL cholesterol (mg/dL)[a]	97 ± 36	112 ± 30	128 ± 42	0.014
HDL cholesterol (mg/dL)[b]	45 (37–63)	46 (40–56)	42 (33–51)	0.153
TG (mg/dL)[b]	76 (59–140)	138 (81–191)	162 (122–206)	0.005
Uric acid (umol/L)[a]	245 ± 88.3	294 ± 89.6	339 ± 90.8	0.028
CRP (mg/L)[b]	1.8 (0.7–3.8)	1.6 (0.6–3.7)	2.3 (1.4–3.3)	0.340
MMP-9 (ng/mL)[b]	194 (70–375)	456 (261–757)	796 (426–1313)	<0.001
MCP-1 (pg/mL)[a]	46 ± 28	80 ± 60	84 ± 76	0.049

[a] Mean ± standard deviation.
[b] Median (interquartile range). IMT: intima–media thickness; TC: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TG: triglycerides; CRP: C-reactive protein; MMP: matrix metalloproteinase; MCP-1: monocyte chemoattractant protein-1.

Table 4
Multinomial logistic regression model of the associations of MMP-9 with total plaque score and plaque stability.

Outcome	MMP-9 quartile							
	Q1	Q2		Q3		Q4		
		Or (95% CI)	*p*	Or (95% CI)	*p*	Or (95% CI)	*p*
Total plaque score								
1–2 *vs.* 0	Reference	2.36 (0.66–8.49)	0.187	5.86 (1.52–22.55)	0.01	2.04 (0.47–8.79)	0.339
>3 *vs.* 0	Reference	5.15 (0.79–33.3)	0.085	10.02 (1.5–65.33)	0.016	21.5 (3.5–132.1)	0.001
Plaque stability								
Stable plaque *vs.* no plaque	Reference	1.68 (0.4–7.06)	0.476	2.37 (0.52–10.85)	0.264	2.34 (0.53–10.33)	0.263
Unstable plaque *vs.* no plaque	Reference	5.13 (1.01–24.9)	0.042	15.5 (3.1–78.1)	0.001	13.2 (2.7–64.97)	0.001

MMP: matrix metalloproteinase; Q: quartile; OR: adjusted odds ratio; CI: confidence interval.

4. Discussion

In this study, we sought to relate serum biomarkers to carotid atherosclerosis parameters predictive of vascular events [IMT, plaque instability, and TPA (indirectly represented by total PS)]. Our results demonstrate relationships between circulating biomarkers of ECM remodeling and the severity of carotid atherosclerosis, assessed using duplex ultrasound. First, we found significant associations between high MMP-9 level and plaque instability, high total PS, and increased IMT. The associations with plaque instability and high total PS remained significant after adjustment for traditional vascular risk factors. Second, high MCP-1 concentration was associated with increased IMT, but not with plaque instability or high total PS. Third, high UA level was associated with plaque instability. To our knowledge, this study is the first to demonstrate a dose–response relationship between serum MMP-9 level and carotid plaque stability.

Elevated MCP-1 is a predictor of cardio-cerebrovascular disease, perhaps due to its effect on atherosclerosis. A previous study found a positive correlation between MCP-1 level and coronary atherosclerosis, but this association was not independent of traditional vascular risk factors, especially age [29]. Thakore et al. [30] reported that blood inflammatory markers, including CRP, interleukin-6, soluble intercellular adhesion molecule-1, MCP-1, CD40 ligand, and P-selectin, assessed as a group, were significantly associated with internal carotid artery IMT ($p = 0.01$). However, in contrast to our results, MCP-1 showed no significant positive relationship with IMT when assessed individually [30]. These findings may reflect the distinct determinants of different phenotypes [26].

Cao et al. [6,31] noted that elevated CRP level was associated with increased risks of cardiovascular and cerebrovascular diseases and all-cause mortality in patients with atherosclerosis detectable with carotid ultrasound. Moreover, they confirmed that CRP level was closely associated with IMT [31], in agreement with Alizadeh Dehnavi et al. [32]. In contrast, we failed to demonstrate a relationship between CRP and atherosclerosis severity. The existence of such an association remains controversial. Equivalent or more severe atherosclerotic lesions were observed in transgenic CRP-deficient mice compared with controls [33], and transgenic rabbits with low and high CRP expression fed a high-cholesterol diet developed similar degrees of aortic atherosclerosis [34]. Moreover, Rozalski et al. [35] detected the highest CRP levels in patients with the most stable plaque. These data challenge the atherogenic effects of CRP and are consistent with the results of a large-scale genetic epidemiology study [36]. Thus, CRP may have no effect on the severity of atherosclerosis.

Our finding that high UA level was associated with plaque instability is in accord with those of previous studies [37,38]. The mechanisms by which UA reflects the risk of carotid atherosclerosis are incompletely understood. UA regulates critical pro-inflammatory pathways in vascular smooth-muscle cells [39,40], resulting in low-grade inflammation and insulin resistance in subjects with metabolic syndrome, as well as endothelial cell dysfunction and atherosclerosis.

Our study has some limitations. First, we measured total serum MMP-9 levels, not those of tissue inhibitors of metalloproteinases (TIMPs). MMP-9/TIMPs or active MMP-9 concentrations may be better indicators than total MMP-9 levels of ongoing vascular remodeling activities. Second, carotid IMT values can be biased by subjective measurement, and automated image-processing software can provide more objective values.

In conclusion, our results indicate that blood MMP-9 and MCP-1 levels are associated with carotid atherosclerosis. MMP-9 concentration showed a dose–response relationship with carotid plaque stability. Our findings also support a dose–response relationship between MCP-1 level and IMT. Moreover, we found that high UA level was associated with plaque instability. These serum biomarkers can be used to distinguish unstable from stable plaque,

Table 5
Multinomial logistic regression model of IMT associations with traditional vascular risk factors and MCP-1.

Variable	Carotid IMT tertile				
	T1	T2		T3	
		Or (95% CI)	*p*	Or (95% CI)	*p*
Age	Reference	1.07 (1.01–1.14)	0.03	1.04 (1.00–1.09)	0.03
Sex	Reference	0.54 (0.07–3.78)	0.53	0.83 (0.20–3.34)	0.79
Hypertension	Reference	1.60 (0.39–6.56)	0.52	6.08 (1.07–34.53)	0.04
Diabetes mellitus	Reference	0.59 (0.09–4.05)	0.59	0.53 (0.11–2.69)	0.45
Cigarette smoking	Reference	0.63 (0.05–7.79)	0.72	0.96 (0.12–7.60)	0.97
LDL cholesterol	Reference	4.68 (1.69–12.92)	0.003	2.65 (1.15–6.13)	0.022
Uric acid	Reference	1.01 (0.99–1.02)	0.096	1.01 (1.00–1.02)	0.052
MCP-1 (Q4 *vs.* Q1)	Reference	12.37 (0.80–191.94)	0.072	22.94 (2.14–245.66)	0.01

MCP-1: monocyte chemoattractant protein-1; IMT: intima–media thickness; T: tertile; OR: adjusted odds ratio; CI: confidence interval; LDL: low-density lipoprotein; Q: quartile.

reflect the extent of carotid atherosclerosis beyond carotid ultrasound findings, and can help to target vulnerable patients and monitor the beneficial effects of pharmacological agents.

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