Association of MMP-9, ADMA, and sCD40L with ischemic stroke and correlations with stroke severity: A case-control study

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Research Article

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Abstract

Background

In the present study, we aimed to examine the correlation of serum levels of MMP-9, ADMA, and sCD40L with the occurrence and severity of acute ischemic stroke.

Methods

All routines analyses were performed using a Cobas platform. MMP-9, ADMA, and sCD40L were measured using ELISA kit.

Results

Compared with the controls, the stroke group showed higher MMP-9, higher sCD40L and higher ADMA. There were significant positive correlations between the NIHSS scores and MMP-9, sCD40L and ADMA.

Conclusions

Our study indicated that serum levels of MMP-9, sCD40L, and ADMA are associated with ischemic stroke and are correlated with the NIHSS scores at admission.

Background

A stroke is an acute neurological dysfunction episode from either ischemic infarction or a collection of blood within the brain or ventricular system with resultant focal injury of the central nervous system (CNS) [1]. The determination of stroke can be based on clinical evidence of cerebral, spinal cord, or retinal injury based on symptoms or can be made based on pathological, imaging, or other objective evidence of cerebral, spinal cord, or retinal focal injury in a defined vascular distribution [1]. Stroke is a serious public health problem. The estimated global stroke incidence is 2–3 per 1000 person-years, with older patients and patients with carotid artery stenosis or atrial fibrillation having the highest risk [2, 3]. With the westernization of lifestyle habits and the aging of the population, the incidence of cerebrovascular diseases is increasing every year [4–6], especially ischemic stroke, which accounts for 70%-80% of cerebrovascular diseases [1].

Previous studies on various stroke biomarkers have shown that many factors are associated with stroke, including natriuretic peptides, lipoprotein (a), copeptin, procalcitonin, vascular endothelial growth factor, mannose-binding lectin, adipocyte fatty acid-binding protein, inflammatory biomarkers, and cortisol [7–11]. The optimal biomarkers are still being sought since the available biomarkers display variable sensitivity, specificity, and accuracy.

Matrix metalloproteinase-9 (MMP-9) can degrade the extracellular matrix to weaken the fibrous cap of atherosclerosis plaques and damage the blood-brain barrier, thus causing brain edema and cerebral hemorrhage transformation [12, 13]. Thus, high MMP-9 levels appear to be associated with the occurrence and progression of ischemic stroke and prognosis [12, 13]. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of endothelial nitric oxide (NO) synthase (NOS) and is associated with endothelial damage and cardiovascular diseases [14–16]. Soluble CD40 ligand (sCD40L) is generated by the cleavage of CD40L. High levels of sCD40L can promote platelet activation and aggregation and play an important role in promoting thrombosis and inflammatory reaction [17]. Recent studies have shown a significant increase in plasma sCD40L in acute cerebral infarction patients [18, 19]. Still, the association of these non-classical factors with stroke remains controversial, and it is poorly known whether they are independent of each other.

Therefore, this study aimed to examine the correlation of serum levels of MMP-9, ADMA, and sCD40L with the occurrence and severity of acute ischemic stroke. The results could help determine the value of these biomarkers for the occurrence of stroke.

Methods

Study design and patients

This retrospective study included consecutive patients admitted to the Neurology and Emergency Departments of the Affiliated Hospital of Chengde Medical College from April 2015 to November 2015. This study was approved by the Ethics Committee of the Affiliated Hospital of Chengde Medical College and all methods were performed in accordance with the Helsinki Declaration and relevant guidelines. The requirement for informed consent was waived because of the retrospective nature of the study.

The inclusion criteria were 1) diagnosis of stroke in accordance with the Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke (2014) (i. acute onset; ii. focal neurological impairment; iii. imaging lesions or symptoms and signs lasting > 24 h; iv. non-vascular causes were excluded; v. cerebral hemorrhage was excluded by brain CT or MRI) and 2) acute ischemic stroke. The exclusion criteria were 1) cerebral hemorrhage, 2) history of previous stroke, 3) complicated with a serious injury to the heart, liver, kidney, or other organs, 4) chronic infectious diseases, 5) malignant tumors, 6) long-term use of immunosuppressant drugs or hormones before admission, or 7) severe mental disorders.

Control subjects were selected among the patients who visited the Affiliated Hospital of Chengde Medical College for routine health checkups during the same period. The exclusion criteria were 1) history of cerebrovascular disease, 2) complicated with serious damage to the heart, liver, kidney, or other organs, 3) chronic infectious diseases, 4) malignant tumors; 5) long-term use of immunosuppressants or hormones, or 6) severe mental disorders.

Data collection

Age, sex, smoking history, drinking history, history of hypertension, diabetes, and coronary heart disease, and general laboratory test results at admission (triglycerides, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)) were collected from the medical charts.

Laboratory

All laboratory measurements were made on fasting peripheral venous blood samples (5 ml) taken within 24 h of admission, using EDTA tubes. Within 30 min after collection, the plasma was centrifuged for 15 min (3000 rpm) in a high-speed centrifuge, and the plasma was separated. The samples were stored at -80°C. All routines analyses were performed using a Cobas platform (Roche Diagnostics, Basel, Switzerland). MMP-9, ADMA, and sCD40L were measured using ELISA kit (Mlbio, Shanghai, China).

Stroke assessment

All patients admitted for stroke during the study period underwent a routine National Institutes of Health Stroke Scale (NIHSS) scoring.

Statistical analysis

SPSS 26.0 (IBM, Armonk, NY, USA) was used for statistical analysis. The continuous data were tested for normal distribution using the Kolmogorov Smirnov test. The data conforming to the normal distribution are expressed as means $\pm$ standard deviations and were tested using the independent sample t-test. Data that did not conform to the normal distribution are presented using median (interquartile range) and were compared using the Mann-Whitney U-test. Categorical data are presented as n (%) and were tested using the chi-square test. The Spearman correlation analysis was used to analyze the correlations between the indicators. A multivariable logistic regression analysis was used to identify the factors associated with stroke. Two-sided P-values < 0.05 were considered statistically significant.

Results

Characteristics of the subjects

A total of 197 subjects who visited the hospital between April 2015 and November 2015 were included. Among them, 135 patients (93 males and 42 females; 59.0 ± 8.9 years of age) were admitted due to ischemic stroke, and 62 patients (36 males and 26 females; 57.9 ± 7.4 years of age) were admitted for a physical. Table 1 presents their characteristics. Hypertension (57.0% vs. 30.6%, $P = 0.001$) and smoking (40.7% vs. 21.0%, $P = 0.007$) were more frequent in the stroke group than in controls. Compared with the controls, the stroke group showed higher triglycerides (median, 1.41 vs. 1.29 mmol/L, $P = 0.036$), lower HDL-C (median, 0.91 vs. 1.06 mmol/L, $P = 0.001$), higher MMP-9 (median, 323.6 vs. 272.9 mg/L, $P < 0.001$), higher sCD40L (median, 588.2 vs. 458.0 mmol/L, $P < 0.001$), and higher ADMA (median, 15.87 vs. 13.86 mmol/L, $P < 0.001$).

Table 1
Patient characteristics

Variables	Control group (n = 62)	Stroke group (n = 135)	P
Age (years)	57.9 ± 7.4	59.0 ± 8.8	0.396
Female, n (%)	26 (41.9)	42 (31.1)	0.138
Hypertension, n (%)	19 (30.6)	77 (57.0)	0.001
Coronary heart disease, n (%)	3 (4.8)	10 (7.4)	0.500
Diabetes mellitus, n (%)	13 (21.0)	26 (19.3)	0.780
Smoking, n (%)	13 (21.0)	55 (40.7)	0.007
Drinking, n (%)	14 (22.6)	36 (26.7)	0.541
Triglycerides (mmol/L)	1.29 (0.82, 1.78)	1.41 (0.93, 2.22)	0.036
TC (mmol/L)	4.14 (3.82, 4.7)	4.21 (3.62, 5.01)	0.750
HDL-C (mmol/L)	1.06 (0.97, 1.25)	0.91 (0.79, 1.09)	0.001
LDL-C (mmol/L)	2.33 (2.04, 2.7)	2.18 (1.86, 2.67)	0.199
MMP-9 (mg/L)	272.9 (135.7, 368.3)	323.02 (258.6, 551.9)	< 0.001
sCD40L (mmol/L)	458.0 (367.2, 582.6)	588.2 (420.6, 1337.5)	< 0.001
ADMA (mmol/L)	13.86 (8.45, 16.95)	15.87 (12.50, 38.17)	< 0.001
TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; MMP-9: matrix metalloproteinase-9; sCD40L: soluble CD40 ligand; ADMA: asymmetric dimethylarginine.			

Factors associated with stroke

Table 2 shows that hypertension ($P = 0.001$), smoking ($P = 0.008$), triglycerides ($P = 0.010$), HDL-C ($P = 0.031$), MMP-9 ($P < 0.001$), sCD40L ($P < 0.001$), and ADMA ($P < 0.001$) were associated with stroke in the univariable analyses. In the multivariable analysis, hypertension (OR = 2.62, 95%CI: 1.27–5.40, $P = 0.009$), triglycerides (OR = 1.66, 95%CI: 1.08–2.57, $P = 0.02$), HDL-C (OR = 0.36, 95%CI: 0.13–0.98, $P = 0.046$), MMP-9 (OR = 2.30, 95%CI: 1.31–4.02, $P = 0.004$), sCD40L (OR = 2.36, 95%CI: 1.26–4.39, $P = 0.007$), and ADMA (OR = 2.02, 95%CI: 1.07–3.83, $P = 0.031$) were independently associated with stroke (Table 2).

Table 2
Univariable /multivariable regression analysis

Variable	Univariable		Multivariable	
	OR (95%CI)	P	OR (95%CI)	P
Hypertension	3.005 (1.587–5.689)	0.001	2.616 (1.268–5.397)	0.009
Smoking	2.591 (1.285–5.225)	0.008	-	-
TG	1.661 (1.128–2.448)	0.010	1.664 (1.077–2.572)	0.022
HDL-C	0.38 (0.158–0.917)	0.031	0.355 (0.128–0.984)	0.046
Ln (MMP-9)	2.737 (1.698–4.411)	0.000	2.295 (1.309–4.024)	0.004
Ln (sCD40L)	2.936 (1.706–5.055)	0.000	2.355 (1.263–4.391)	0.007
Ln (ADMA)	2.864 (1.669–4.915)	0.000	2.022 (1.067–3.833)	0.031
Regression analysis was performed using the natural logarithm transformation of MMP-9, SCD40L, and ADMA.				
TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; MMP-9: matrix metalloproteinase-9; sCD40L: soluble CD40 ligand; ADMA: asymmetric dimethylarginine.				

Correlation analysis between the NIHSS score on admission and various indicators

The correlation results between the NIHSS score on admission and various indicators were shown in Table 3. The NIHSS scores at admission were not correlated with age, sex, hypertension, coronary heart disease, diabetes mellitus, smoking, drinking, triglycerides, TC, HDL-C, LDL-C, MMP-9, sCD40L, and ADMA. On the other hand, there were significant positive correlations between the NIHSS scores and MMP-9 ($r = 0.23$, $P = 0.007$), sCD40L ($r = 0.37$, $P < 0.001$), and ADMA ($r = 0.26$, $P = 0.003$) (Table 3 and Fig. 1).

Table 3
Correlation analysis of the NIHSS score at admission and various indicators

Indicators	NIHSS	
	r	P
Female	0.048	0.579
Age	0.056	0.519
Diabetes mellitus	-0.043	0.621
Hypertension	-0.031	0.722
Coronary heart disease	-0.008	0.923
Smoking	0.089	0.305
Drinking	0.028	0.745
TG	-0.023	0.788
TCH	0.058	0.502
HDL-C	0.051	0.554
LDL-C	-0.071	0.414
MMP-9	0.231 ^{**}	0.007
sCD40	0.371 ^{**}	< 0.001
ADMA	0.257 ^{**}	0.003
Note: ^{**} P < 0.01.		
NIHSS; National Institutes of Health Stroke Scale; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; MMP-9: matrix metalloproteinase-9; sCD40L: soluble CD40 ligand; ADMA: asymmetric dimethylarginine.		

Discussion

MMP-9, ADMA, and sCD40L are possibly associated with stroke. This study aimed to examine the correlation of serum levels of MMP-9, ADMA, and sCD40L with the occurrence and severity of acute ischemic stroke. The results showed that the serum levels of MMP-9, sCD40L, and ADMA are associated with ischemic stroke and are correlated with the NIHSS scores at admission.

MMP-9 is involved in the remodeling of the atherosclerosis plaques by digesting the extracellular matrix that constitutes the fibrous cap, making it more prone to rupture, in which case a thrombus will form when the highly thrombotic necrotic core is exposed to the blood [20]. MMP-9 is also involved in the destabilization of the blood-brain barrier, brain edema, and cerebral hemorrhage transformation, which

can further deteriorate the condition of patients with stroke [12, 13, 21]. Thus, high MMP-9 levels appear to be associated with the occurrence and progression of ischemic stroke and prognosis [12, 13]. A meta-analysis of case-control studies revealed that the MMP-9 levels are directly correlated with stroke volume, stroke severity, and functional outcome [22]. Although stroke volume and functional outcomes were not assessed in the present study, MMP-9 was independently associated with stroke and positively correlated with the NIHSS scores.

NO is a potent vasodilator and antioxidant necessary to the normal functions of the vascular endothelium [23, 24]. Low levels of NO are associated with endothelial dysfunction, which is in itself a risk factor for the initiation and progression of atherosclerosis [25, 26]. NO is produced by the NOS enzymes, and the endothelial NOS (eNOS) is directly involved in normal endothelial dysfunction [27, 28]. Besides the numerous factors involved in the transcription levels of eNOS [27, 28], ADMA is an endogenous inhibitor of eNOS, and high levels of ADMA are associated with endothelial damage and cardiovascular diseases [14–16]. Chen et al. [29] showed that ADMA is both a marker and a mediator of stroke. Janes et al. [30] showed that ADMA levels are associated with cerebral small vessel disease in asymptomatic patients. A recent review showed that high ADMA levels were associated with carotid atherosclerosis, which is a risk factor for ischemic stroke [31]. Still, some studies suggest that ADMA levels are not associated with stroke [32–34]. In the present study, ADMA levels were independently associated with stroke and correlated with the NIHSS. The discrepancies among studies could be due to several factors, including sample size, selection of the patients and controls, and genetics.

sCD40L is involved in platelet activation and aggregation, which is involved in ischemic stroke pathogenesis when the plaque necrotic core is exposed [17, 35]. High levels of sCD40L are also involved in systemic inflammation, which is involved in pro-atherogenic mechanisms [17]. Plasma sCD40L levels are elevated in patients with acute ischemic stroke, but the causality relationship is unknown [18, 19]. A study revealed that sCD40L levels were associated with inflammatory markers levels but not with stroke itself [36]. Since the inflammatory markers could not be analyzed in the present study because they were inconsistently measured among the patients, the present study could not determine whether the independent association of sCD40L with stroke was a direct independent association or through inflammation. Still, a study showed that sCD40L levels were associated with stroke severity and outcomes [18], supporting the correlation between sCD40L and the NIHSS score.

Still, the association of these non-classical factors with stroke remains controversial, and it is poorly known whether they are independent of each other. Indeed, several studies examined different panels of biomarkers for their association with stroke, but few studies examined MMP-9, ADMA, and sCD40L within the same study. In the present study, all three markers were each independently associated with stroke. Future studies should examine whether predictive models could be built using these markers.

The NIHSS is a common neurological function score used to determine the prognosis of patients with cerebral infarction at admission [37–39]. The NIHSS can be used to objectively reflect the severity of cerebral infarction and determine patient prognosis [37–39]. Still, it has limitations [40] that could not be

controlled for in the present study. These limitations could explain, at least in part, why the correlations were weak (all $r < 0.4$). Another explanation could also be that MMP-9, sCD40L, and ADMA all contribute together to stroke. In addition, other factors could be involved but were not quantified in this study.

This study has limitations. It was a retrospective study limited to the data available in the charts. The sample size was small and did not allow deeper analyses such as receiver operating characteristics analyses and nomograms. Only the NIHSS score could be used to grade the stroke because it is the grading system used in all patients. Many factors that might be associated with stroke were not evaluated in this study.

Conclusions

In conclusion, the serum levels of MMP-9, sCD40L, and ADMA in patients with ischemic stroke are significantly higher than those in the control group, suggesting that the serum levels of MMP-9, sCD40L, and ADMA are correlated with the occurrence of acute ischemic stroke. In addition, MMP-9, sCD40L, and ADMA are correlated with the NIHSS score. Future studies should examine whether these markers could be used as predictors of the occurrence of acute ischemic stroke.

Abbreviations

CNS: Central nervous system

MMP-9: Matrix metalloproteinase-9

ADMA: Asymmetric dimethylarginine

NO: Nitric oxide

NOS: Nitric oxide synthase

sCD40L: Soluble CD40 ligand

TC: Total cholesterol

HDL-C: High-density lipoprotein cholesterol

LDL-C: Low-density lipoprotein cholesterol

NIHSS: National Institutes of Health Stroke Scale

eNOS: Endothelial NOS

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Affiliated Hospital of Chengde Medical College and all methods were performed in accordance with the Helsinki Declaration and relevant guidelines. The requirement for informed consent was waived because of the retrospective nature of the study.

Consent for publication

Not applicable.

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

The authors declare that they have no competing interests

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None.

Authors' contributions

ML carried out the studies, participated in collecting data, and drafted the manuscript. HW performed the statistical analysis and participated in its design. YG participated in acquisition, analysis, interpretation of data, and drafted the manuscript. All authors have read and approved the final manuscript.

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Figures

Figure 1

Correlation analysis of the National Institutes of Health Stroke Scale (NIHSS) scores with (A) matrix metalloproteinase-9 (MMP-9), (B) soluble CD40 ligand (sCD40L), and (C) asymmetric dimethylarginine (ADMA).