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Correlation between acute myocardial infarction complicated with cerebral infarction and expression levels of MMP-2 and MMP-9

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Abstract. – OBJECTIVE: To investigate the correlation between the acute myocardial infarction (AMI) complicated with cerebral infarction (CI) and expression levels of matrix metalloproteinase-2 (MMP-2) and MMP-9.

PATIENTS AND METHODS: A total of 50 AMI patients treated in our hospital were enrolled, including 23 AMI patients complicated with CI in the AMI-CI group, and 27 patients with AMI alone in the AMI group. Venous blood was collected from each patient after admission. The serum levels of MMP-2 and MMP-9 were detected via enzyme-linked immunosorbent assay (ELISA), and their mRNA expressions were measured via quantitative Polymerase Chain Reaction (qPCR). The cerebral computed tomography (CT) scan was performed for calculating the CI size. The neurological function was evaluated using the neurological deficit score. The correlations between the levels of MMP-2 and MMP-9 with CI size and neurological deficit score were investigated using Pearson correlation analysis.

RESULTS: The expression levels of MMP-2 and MMP-9 in the AMI-CI group were significantly higher than those in the AMI group with significant differences ($p<0.05$). The mRNA expressions of MMP-2 and MMP-9 in AMI-CI group were also significantly higher than those in the AMI group, and the differences between the two groups were statistically significant ($p<0.05$). Larger CI size was observed in the AMI-CI group than that in the AMI group, showing a statistically significant difference between the two groups ($p<0.05$). The neurological deficit score in the AMI-CI group was significantly higher than that in the AMI group, with a statistically significant difference between the two groups ($p<0.05$). The expression levels of MMP-2 and MMP-9 were positively correlated with the CI size and neurological deficit score in AMI patients complicated with CI.

CONCLUSIONS: Disease severity of AMI complicated with CI is positively correlated with the expression levels of MMP-2 and MMP-9. Higher expression levels of MMP-2 and MMP-9 are expected to indicate a higher risk of AMI complicated with CI.

Key Words:
Acute myocardial infarction, Cerebral infarction, Metalloproteinase.

Introduction

Acute myocardial infarction (AMI) and cerebral infarction (CI) are common cardiovascular and cerebrovascular diseases that severely threaten human lives. With the lifestyle change, life pace acceleration and aging population, the incidence and mortality rates of AMI complicated with CI have increased year by year[1,2]. It is estimated that the mortality rate of cardiovascular and cerebrovascular diseases ranked third in human death. Both AMI and CI will easily lead to serious complication and sequelae, and pose a great burden on patient's family and society[3,4].

As the in-depth researches on AMI and CI show, matrix metalloproteinases (MMPs) are thought to be vital in the pathological processes of AMI and CI. Among them, MMP-2 and MMP-9 are important members of the MMP family. Studies have demonstrated that MMP-2 and MMP-9 are abundantly expressed in atherosclerotic plaques. The extracellular matrix leads to rupture and shedding of atherosclerotic plaques by its specific degradation effect, resulting in tissue and organ infarction[5,6]. Therefore, we believed that the expression levels of MMP-2 and MMP-9 are closely related to the occurrence of AMI and CI.

However, it is still unclear whether AMI complicated with CI is correlated with abnormally

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high expression levels of MMP-2 and MMP-9. This work aims to investigate the correlation between AMI complicated with CI and expression levels of MMP-2 and MMP-9, to further clarify its pathological cause.

Patients and Methods

General Data

This study was approved by the Ethics Committee of Hanzhong People's Hospital. Signed informed consents were obtained from all participants before the study. A total of 50 AMI patients treated in our hospital from October 2017 to March 2018 were enrolled. Among them, 23 AMI patients complicated with CI were assigned to the AMI-CI group, and the remaining 27 patients with AMI alone were enrolled in the AMI group. There were 13 males and 10 females aged 63.61±14.54 years in the AMI-CI group, whereas 15 males and 12 females aged 61.98±15.66 years were in the AMI group. No significant differences in gender and age of enrolled patients were found between the two groups ($p>0.05$).

Diagnostic criteria for AMI were as follows: (1) Symptoms of chest pain lasted for more than 30 minutes; (2) Electrocardiograph (ECG) showed the elevation ≥0.1 mV in two or more contiguous leads; (3) Laboratory tests showed that creatine kinase-MB (CK-MB) level increased more than twice. Diagnostic criteria of CI were based on the criteria proposed at the 4th National Cerebrovascular Conference. CI was confirmed by brain CT, and the infarct size was calculated.

Experimental Reagents and Instruments

MMP-9 enzyme-linked immunosorbent assay (ELISA) kits (Abcam, Cambridge, MA, USA); MMP-2 ELISA kits (Abcam, Cambridge, MA, USA); AceQ quantitative Polymerase Chain Reaction (qPCR) SYBR Green Master Mix kits; HiScript II Q RT SuperMix for qPCR (+ gDNA wiper) kits (TaKaRa, Otsu, Shiga, Japan); optical microscope (Leica DMI 4000B/DFC 425C, München, Germany); and fluorescence quantitative PCR instrument (ABI 7500, Vernon, CA, USA).

Study Methods

Enrolled patients underwent all relevant tests after admission. Venous blood was extracted and centrifuged for harvesting serum samples, and was subjected for determination of serum levels of MMP-2 and MMP-9 using enzyme-linked immunosorbent assay (ELISA) kits. The mRNA levels of MMP-2 and MMP-9 in serum were measured *via* qPCR. Cerebral CT scan was performed, and the infarct size was calculated. The neurological deficit score was recorded to evaluate the neurological function of patients.

qPCR Detection

Total RNA stored at -20°C was extracted using the RNA kits, and reversely transcribed into complementary Deoxyribose Nucleic Acid (cDNA) using the reverse transcription kits. 20 μL reaction system was prepared for reaction at 51°C for 2 min, pre-denaturation at 96°C for 10 min, denaturation at 96°C for 10 s and annealing at 60°C for 30 s, for 40 cycles in total. The relative mRNA levels of MMP-2 and MMP-9 were calculated with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal reference. The primer sequences were detailed in Table I.

Statistical Analysis

The statistical analysis was performed using Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA). Data were expressed as mean ± standard deviation. The *t*-test was used for comparing data with normal distribution and homogeneity of variance. Pearson correlation analysis was used for the correlation analysis. $p<0.05$ indicated the difference was statistically significant.

Results

Detection of Expression Levels of MMP-2 and MMP-9 by ELISA

ELISA kit was used to detect the expression levels of MMP-2 and MMP-9. As shown in Figure 1, the expression levels of MMP-2 and MMP-9 in the AMI-CI group were significantly higher than those in the AMI group ($p<0.05$).

Detection of mRNA Levels of MMP-2 and MMP-9 by qPCR

The mRNA levels of MMP-2 and MMP-9 were determined by qPCR. As shown in Figure 2, the mRNA levels of MMP-2 and MMP-9 in the AMI-CI group were significantly higher than those in the AMI group ($p<0.05$).

Infarction Size

As shown in Figure 3, the CI size was (4.39±0.48) cm^2 in the AMI-CI group, and (0.89±0.05) cm^2 in

Table I. List of primer sequences.

Name	Primer sequences
MMP-2	Forward: 5'-AGGTCTCCTCTGGCTCTG-3' Reverse: 5'-AGGTCTCCTCTGGCTCTG-3'
MMP-9	Forward: 5'-GACAAAGCGCTCCCC-3' Reverse: 5'-CAGTGCGTGTCGTGGAG-3'
GAPDH	Forward: 5'-ACGGCAAGTTCAACGGCACAG-3' Reverse: 5'-GAAGACGCCAGTAGACTCCACGAC-3'

the AMI group. It is revealed that the CI size in the AMI-CI group was significantly larger than that in the AMI group, and the difference was statistically significant (p<0.05).

Neurological Deficit Score

As shown in Figure 4, the neurological deficit score was 11.21±2.29 points in the AMI-CI group, and 3.88±0.21 points in the AMI group. The results revealed that the neurological deficit score in the AMI-CI group was significantly higher than that in the AMI group, and the difference was statistically significant (p<0.05).

Correlation Analysis

There was a positive correlation between the expression level of MMP-2 and CI size (r=0.821) (Figure 5). A positive correlation was also found between the expression level of MMP-2 and the neurological deficit score (r=0.798) (Figure 6). Besides, a positive correlation was found between the expression level of MMP-9 with CI size (r=0.711) (Figure 7), and the neurological deficit score (r=0.689) (Figure 8).

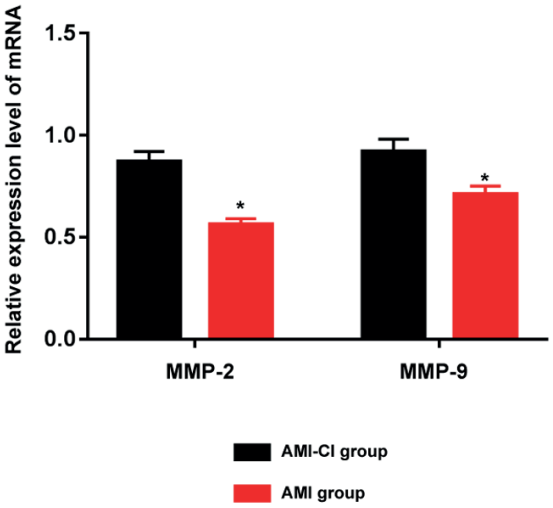

Figure 2. Detection of mRNA expression levels of MMP-2 and MMP-9 by qPCR. Note: *p<0.05 *vs.* AMI-CI group.

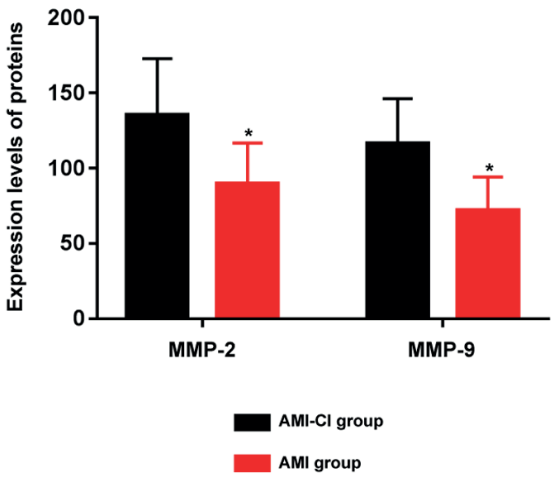

Figure 1. Detection of expression levels of MMP-2 and MMP-9 by ELISA. Note: *p<0.05 *vs.* AMI-CI group.

Discussion

With the further deepening of studies on atherosclerotic plaques, the vital role of extracellular matrix in the formation, rupture and shedding of atherosclerotic plaques has been increasing recognized[7]. Currently, the role of extracellular matrix in myocardial infarction and CI has been emphasized. Large molecular substances are secreted by various tissues and cells *in vivo*. In particular, fibroblast, epithelial and interstitial cells are considered to be the major components of the extracellular matrix, which mainly accumulate and distribute on the cell surface and intercellular substance. A network-like substance is formed to exert physiological effects and thought to be an external environment for protecting cell survival[8,9]. It has been shown that synthesis

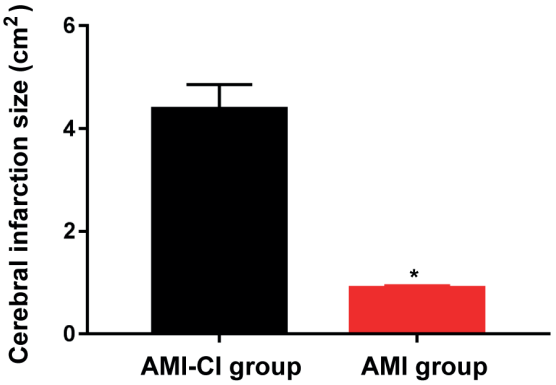

Figure 3. Cerebral infarction size. Note: *p<0.05 *vs.* AMI-CI group.

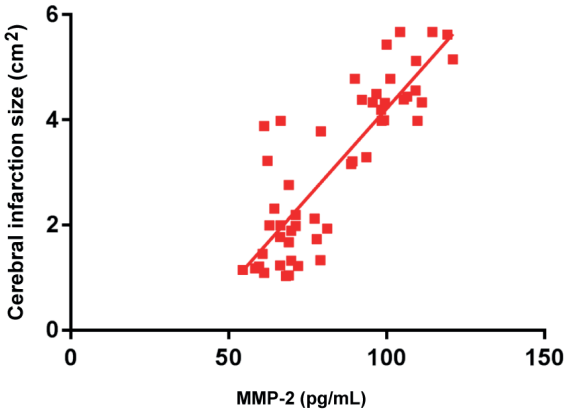

Figure 5. Correlation of MMP-2 with cerebral infarction size.

reduction and accumulative degradation of extracellular matrix weaken plaque fibrous cap, impair plaque stability and even cause a rupture, thereby resulting in massive aggregation and adhesion of platelets at the site of plaque rupture. Eventually, thrombus and vessel occlusion contribute to being the important pathologic factors of AMI[10,11]. Meanwhile, left ventricular dilatation and heart failure at post-AMI are also thought to be closely related to excessive degradation of extracellular matrix. Post-AMI ventricular remodeling is considered to be an important pathological process, resulting in tissue remodeling after excessive degradation of extracellular matrix[12]. The extracellular matrix plays a crucial pathological role in both AMI and CI. The MMPs are well-known zinc-dependent proteases that are differentially distributed in human organs and tissues. MMP-2 and MMP-9, important members of the MMP family, are a class of gelatinases[13,14]. The study indicated that MMP-2 and MMP-9, as gelatinases, exert a great degrading effect on modified fiber and a better decomposing effect on type-IV collagen in matrix[15,16]. Once MMP-2 and MMP-9 are activated after injury, their increased expression levels will lead to complete decomposition of the extracellular matrix. As a consequence, they play a crucial role in tissue infarction and post-infarction tissues remodeling[17,18]. Therefore, MMP-2 and MMP-9 are closely related to the incidence and prognosis of AMI and CI, the most common cardiovascular and cerebrovascular infarct diseases affecting human health and life quality. They are regarded to be important killers in human life[19,20]. This work demonstrated that the serum levels of MMP-2 and MMP-9 abnormally increased in AMI patients complicated with CI and patients with AMI alone. Upregulated expression levels of

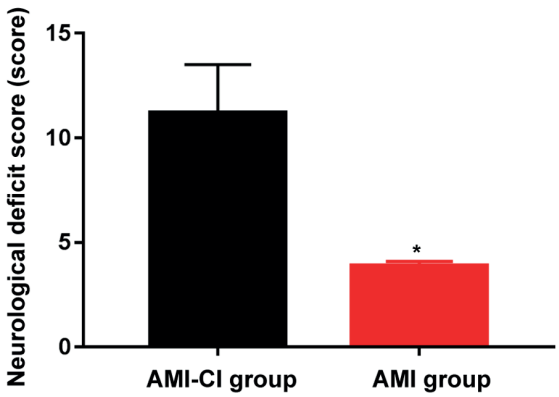

Figure 4. Neurological deficit score Note: *p<0.05 *vs.* AMI-CI group.

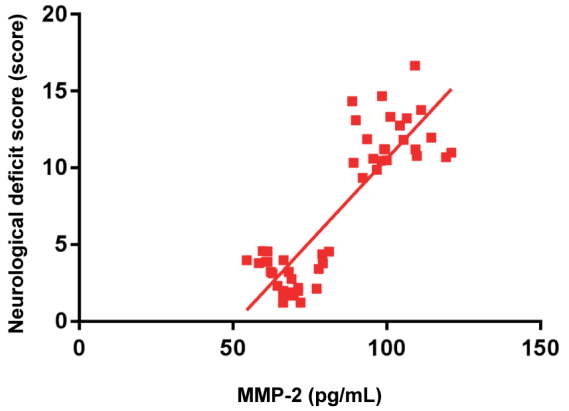

Figure 6. Correlation of MMP-2 with neurological deficit score.

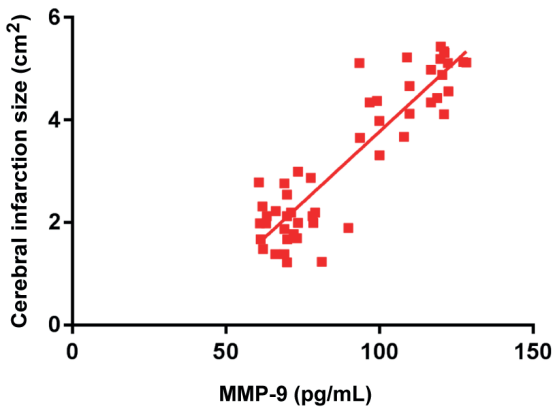

Figure 7. Correlation of MMP-9 with cerebral infarction size.

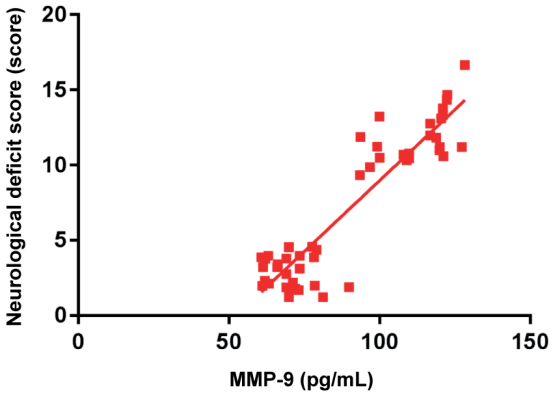

Figure 8. Correlation of MMP-9 with neurological deficit score.

MMP-2 and MMP-9 after injury played important pathological roles in accelerating the deposition of extracellular matrix. The expression levels of MMP-2 and MMP-9 were far higher in AMI patients complicated with CI, indicating more severe infarction and extracellular matrix degradation *in vivo*. Moreover, the correlation analysis further confirmed that the expression levels of MMP-2 and MMP-9 were positively correlated with the CI size and neurological deficit score.

Conclusions

We demonstrated that MMP-2 and MMP-9 are positively correlated with the disease severity of AMI complicated with CI. The higher expression levels of MMP-2 and MMP-9 indicate a higher risk of AMI complicated with CI.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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