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Clinical significance of miR-492 in peripheral blood of acute myocardial infarction

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Abstract. – OBJECTIVE: To detect the potential of microRNA-492 (miR-492) as a diagnostic biomarker of acute myocardial infarction (AMI) in the acute phase.

PATIENTS AND METHODS: A total of 100 AMI patients and 100 controls (non-AMI patients with chest pain) were retrospectively analyzed. Blood samples were collected at 0, 6, 12, and 24 h after admission, followed by detection of the serum miR-492 level. Serum levels of cTnI and creatine kinase-MB (CK-MB) in AMI patients were examined by enzyme-linked immunosorbent assay (ELISA). The potential relationship between miR-492 level with cTnI and CK-MB levels was analyzed by Pearson correlation test. Moreover, diagnostic value of miR-492 was assessed by depicting receiver operating characteristic (ROC) curves.

RESULTS: Serum level of miR-492 achieved the peak at 6 h after admission, which was time-dependently reduced at 12 h and 24 h. Serum levels of cTnI and CK-MB were higher in AMI patients than those of controls. However, miR-492 level achieved the peak before cTnI and CK-MB increased to the highest levels. MiR-492 level was positively correlated to cTnI and CK-MB levels. ROC curves verified the diagnostic value of miR-492 in AMI (AUC=0.8621, 95% CI=0.8129-0.9112, sensitivity=80%, specificity=75%).

CONCLUSIONS: Serum level of miR-492 remarkably increases in the acute phase of AMI, which may be used as an effective biomarker for diagnosing AMI.

Key Words:
Acute myocardial infarction (AMI), MiR-492, Biomarker.

Introduction

Acute myocardial infarction (AMI) is the pathological manifestation of acute and chronic ischemia. The presence of ST-segment elevation on the ECG suggests that the coronary arteries in the corresponding area have been occluded. MI without ST-segment elevation suggests that the coronary artery in the corresponding area is not completely occluded, but it may aggravate into ST-segment elevation MI in the future if active treatment lacks. Early detection of AMI contributes to disease control and treatment[1-3]. Currently, there are several AMI biomarkers, including cTnI, creatine kinase-MB (CK-MB), etc. In particular, cTnI is the preferred biomarker for AMI, which is started to increase at 2-4 h at post-AMI, achieves the peak at 10-24 h, and gradually recovers at 5-10 d[4]. Clinical specificity of CK-MB in determining AMI is relatively high. Peripheral blood level of CK-MB is dynamically changed in AMI patients[5]. Nevertheless, both of cTnI and CK-MB present limitations in the diagnosis of AMI[6].

A mature microRNA (miRNA) contains 19-25 nucleotides, which induces degradation or translation inhibition of target mRNA through complementary base pairing. MiRNAs exert tissue-specificity and cell-specificity and participate in post-transcriptional regulation[7]. Early diagnosis of AMI relies on sensitive and specific biomarkers, and miRNAs are promising candidates[8]. So far, about 20 miRNAs have been identified to be involved in AMI development[9].

MiRNA-492 locates on chromosome 12q22, which is found to be upregulated in many types of tumors and closely linked to tumor metastasis[10-12]. Diagnostic potential of miR-492 has been previously discovered[13]. The correlation between miRNA-492 and AMI has not been reported. Therefore, in this paper, we first detected the serum level of miR-492 in AMI patients, and its clinical significance in AMI was further analyzed.

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Patients and Methods

Patients

A total of 100 AMI patients and 100 controls (non-AMI patients with chest pain) in The Second Hospital, Cheeloo College of Medicine from April 2016 to December 2018 were included. Diagnostic criteria of AMI: increases in myocardial marker levels (CK-MB level doubled or cTnI level > 0.1 ng/mL); persistent chest pain > 30 min; pathological Q wave and ST-T change in ECG; imaging evidence of abnormal ventricular wall motion. Exclusion criteria: patients with history of heart failure, atrial fibrillation, cardiomyopathy and other cardiac diseases, malignant tumors, renal replacement therapy, surgery within months and skeletal muscle injury that may affect myogenic miRNAs. Coronary angiography was conducted in 100 controls to exclude coronary heart disease. This study was performed after the approval of the Hospital Ethic Committee and informed consent from subjects.

Detection of Serum cTnI and CK-MB

Blood samples (3 mL × 2) were collected from AMI patients at 0, 6, 12, and 24 h after admission. One sample was used for extraction of serum RNA, and the other was centrifuged at 1,000 r/min for 10 min. The supernatant was collected for determining cTnI and CK-MB levels using ELISA (Abnova, Taipei City, Taiwan).

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

TRIzol method (Invitrogen, Carlsbad, CA, USA) was applied for isolating RNAs from serum samples. Through reverse transcription of RNA, the extracted complementary deoxyribose nucleic acid (cDNA) was used for qRT-PCR detection by SYBR Green method (TaKaRa, Otsu, Shiga, Japan) at 95°C for 5 min, and 40 cycles at 95°C for 10 s and 60°C for 30 s, followed by 72°C for 5 min. U6 was used as the internal reference. The primer sequences were listed as follows: MiR-492-F: 5′-CTCAACTGGTGTCGTGGAGTCGGC AATTCAGTTGAGAAGAATCT-3′, and miR-492-R: 5′-ACACTCCAGCTGGGAGGACCTGCGGACAAG-3′; U6-F: 5′-CTCGCTTCGGCAGCACA-3′, and U6-R: 5′-AACGCTTCACGAATTTGCGT-3′.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analysis. Data were expressed as mean ± SD (standard deviation). The *t*-test and Chi-square test were used for analyzing measurement data and enumeration data, respectively. Pearson correlation test was applied for assessing the relationship between miR-492 level and serum levels of cTnI and CK-MB. Receiver operating characteristic (ROC) curves were depicted for assessing the diagnostic value of miR-492 in AMI. $p<0.05$ indicated the significant difference.

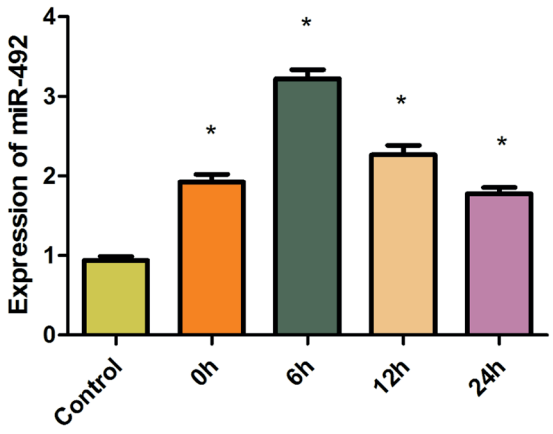

Figure 1. Serum level of miR-492 in AMI patients at 0, 6, 12, and 24 h. *$p<0.05$ compared to control group.

Results

Baseline Characteristics of Subjects

Clinical data of subjects were analyzed. The data showed higher levels of cTnI and CK-MB in AMI patients than controls. However, no significant differences in age, sex, SBP, DBP, TC, HDL-C, LDL-C, TG, Scr, history of smoking, hyperlipidemia, hypertension, and diabetes were found between AMI patients and controls (Table I).

Serum Level of MiR-492 in AMI Patients

Serum level of miR-492 could be detected in all subjects. Compared with controls, serum level of miR-492 in AMI patients at 0, 6, 12, and 24 h after admission was 1.82 ± 0.60, 2.92 ± 0.55, 2.04 ± 0.60 and 1.68±0.53, respectively (Figure 1). It is suggested that the serum level of miR-492 achieved the peak at 6 h following AMI, and gradually decreased since after.

Serum Level of cTnI in AMI Patients

Serum level of cTnI in AMI patients and subjects was detected by ELISA. Compared with

Table I. Baseline characteristics of subjects.

Variable	AMI group	Control group	$t/\chi 2$	p
Age	60.48±8.75	59.23±7.35	1.094	0.275
Sex (male/female)	57/43	54/46	0.182	0.776
SBP (mmHg)	134.21±16.27	128.73±21.49	2.033	0.043
DBP (mmHg)	80.35±9.07	81.32±8.89	0.764	0.446
TC (mmol/L)	5.14±1.16	4.89±1.07	1.584	0.115
HDL-C (mmol/L)	1.32±0.45	1.41±0.62	1.175	0.241
LDL-C (mmol/L)	2.08±1.15	2.47±1.98	1.703	0.09
TG (mmol/L)	1.97±1.24	2.16±1.87	0.847	0.398
Scr (μmol/L)	82.31±18.72	79.12±17.91	1.231	0.22
cTnI (ng/ml)	4.98±1.21	0.01±0.02	41.069	<0.001
CK-MB (IU/L)	187.66±23.87	6.98±1.43	75.558	<0.001
Smoking [n (%)]	65 (65%)	59 (%)	0.764	0.466
Hyperlipidemia [n (%)]	77 (77%)	71 (71%)	0.936	0.42
Hypertension [n (%)]	76 (76%)	70 (70%)	0.913	0.426
Diabetes mellitus [n (%)]	20 (20%)	23 (23%)	0.267	0.731

SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglyceride; Scr: serum creatinine; cTnI: cardiac troponin I: CK-MB: creatinine kinase isoenzyme.

controls, serum level of cTnI time-dependently increased in AMI patients and achieved the peak at 12 h following AMI. Its level was reduced at 24 h (Figure 2).

Serum Level of CK-MB in AMI Patients

Serum level of CK-MB was much higher in AMI patients compared with controls. It gradually increased to the peak at 12 h following AMI and started to reduce since after (Figure 3).

Correlation Between MiR-492 and Serum Levels of cTnI and CK-MB

The above data uncovered that miR-492 was upregulated in peripheral blood of AMI patients, and its peak time was prior to those of cTnI and CK-MB. Pearson correlation test was applied for assessing the relationship between miR-492 and the serum levels of cTnI and CK-MB in AMI patients. The data revealed that miR-492 level was positively correlated to cTnI level (r=0.876, p=0.019), and CK-MB level (r=0.713, p=0.036) (Table II).

Diagnostic Value of MiR-492 in AMI

ROC curves were depicted for assessing the diagnostic value of miR-492 in AMI. The results identified that AUC was 0.8621 (95% CI=0.8129-0.9112, p<0.005). Sensitivity, specificity, and

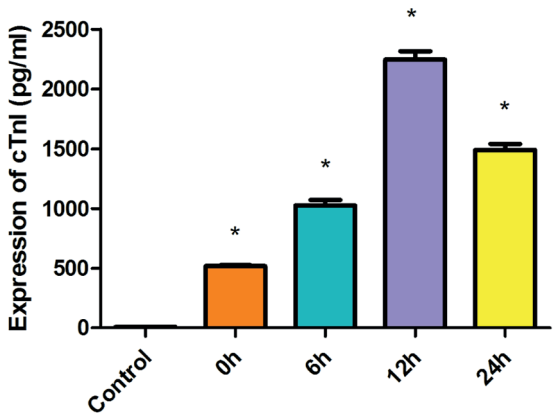

Figure 2. Serum level of cTnI in AMI patients at 0, 6, 12, and 24 h. *p<0.05 compared to control group.

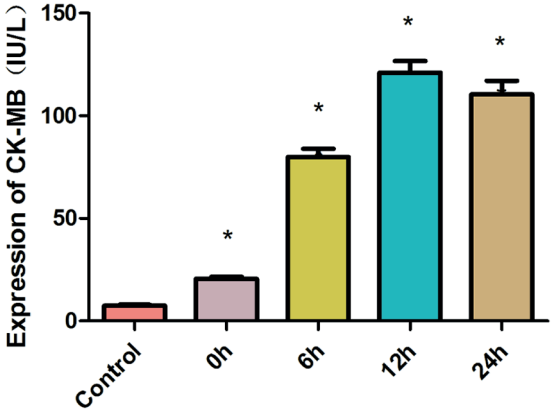

Figure 3. Serum level of CK-MB in AMI patients at 0, 6, 12, and 24 h. *p<0.05 compared to control group.

Table II. Correlation between miR-492 and serum levels of cTnI and CK-MB.

Variable	*p*-value	*p*
cTnI	0.876	0.019
CK-MB	0.713	0.036

cTnI: cardiac troponin I: CK-MB: creatinine kinase isoenzyme.

Youden index of miR-492 were 80%, 75% and 0.55%, respectively when the cut-off was 1.305, demonstrating the diagnostic value of miR-492 in AMI (Figure 4).

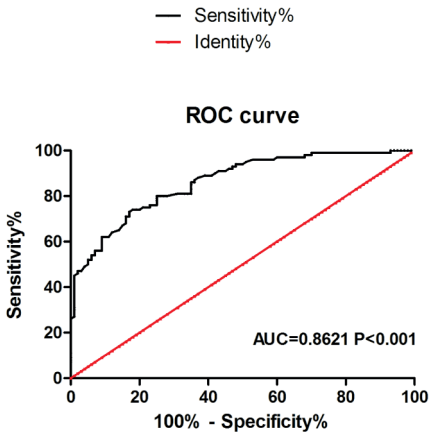

Figure 4. Diagnostic value of miR-492 in AMI. ROC curves verified the diagnostic value of miR-492 in AMI (AUC=0.8621, 95% CI=0.8129-0.9112, sensitivity=80%, specificity=75%).

Discussion

AMI is a severe cardiac event resulting in cardiac remodeling and chronic heart failure. Its disability and mortality are extremely high[3]. Timely and effective treatment of AMI is urgently required. MiRNAs have been well concerned due to their potentials as sensitive, effective, and specific biomarkers[14]. Over 1,000 miRNAs have been identified so far[15,16]. It is reported[17] that miRNAs are stable in serum, plasma, urine, and other body fluids against the degradation from RNA enzymes by forming stable complexes and microparticles with proteins. Advantages of miRNAs in disease diagnosis in the early stage have been highlighted[18,19]. Clinical evidences of abnormally expressed miRNAs in peripheral blood of AMI patients have emerged. Li et al[20] demonstrated that there are over 200 miRNAs expressed in myocardium. Cheng et al[21] reported that miR-1 level rapidly increases in AMI rats following the disease onset, which peaks at 6 h (200 folds higher than the baseline) and gradually reduces to the baseline at 3 d. Peripheral blood level of miR-1 is positively correlated to the infarct size of AMI rats. Increased plasma level of miR-208a is detectable at 1 h following AMI, which can last for 4 h. Compared with cTnI and CK-MB, miR-208a exerts a higher specificity and longer detection window[22]. MiR-499 is mainly distributed in myocardium. Plasma level of miR-499 is able to be detected at 1 h after chest pain onset of AMI patients, and persistently increases for 9 h[23].

Our findings uncovered increased serum level of miR-492 immediately after AMI onset, which peaked at 6 h and gradually decreased. Notably, the peak time of miR-492 was prior to those of cTnI and CK-MB, and moreover, miR-492 had longer detection window than cTnI and CK-MB. Furthermore, ROC curves illustrated the pronounced sensitivity and specificity of miR-492 in diagnosing AMI at admission. We believed that miR-492 was an effective diagnostic indicator for AMI. Further studies are required to explore miRNA functions in other cardiovascular diseases.

In this paper, the research on miR-492 was limited to experimental evidence. Clinical application of miR-492 in early diagnosis, prognosis evaluation, and target therapy still requires further explorations. Meanwhile, the possibility of popularizing miR-492-based gene detection in affected patients should be validated in *in vivo* experiments.

Conclusions

Serum level of miR-492 remarkably increases in the acute phase of AMI, which may be used as an effective biomarker for diagnosing AMI.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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