

# Implication of peripheral blood miRNA-124 in predicting acute myocardial infarction

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**Abstract. – OBJECTIVE:** This study aimed to determine the expression of miR-124 in the patients with acute myocardial infarction (AMI) and elucidated the role of miR-124 on early diagnosis of AMI.

**PATIENTS AND METHODS:** A total of 90 AMI patients were recruited, along with 45 healthy individuals as the control group. Blood samples were collected at different time points (0 h at admission, 6 h, 12 h and 24 h of disease onset). Real-time PCR was used to test miRNA-124 level. ELISA was used to test serum troponin (cTnI) and creatine kinase-MB isoenzyme (CK-MB) levels. The correlation between miRNA-124, cTnI and CK-MB was analyzed. Receiver operating characteristic curve (ROC) was used to analyze sensitivity and specificity of AMI.

**RESULTS:** MiRNA-124 expression in experimental group was significantly elevated in peripheral blood of AMI patients. It can reach the peak at 6h after onset. AMI patients had significantly elevated cTnI and CK-MB expression level ( $p < 0.05$  compared to control group). The expression of miRNA-124 reached the peak earlier than cTnI and CK-MB. miRNA-124 was positively correlated with cTnI and CK-MB ( $p < 0.05$ ). The area under the curve of ROC of miRNA-124 was 0.86 (95% CI: 0.815-0.937), with 52% sensitivity and 91% specificity.

**CONCLUSIONS:** AMI patients presented a significantly elevated level of miRNA-124 in peripheral blood. Our data suggested that miR-124 contributed to an earlier detection than other diagnostic markers for AMI. Therefore, peripheral miRNA-124 can serve as a novel biological marker for early diagnosis of AMI.

Key Words:

MicroRNA-124, Acute myocardial infarction, cTnI, CK-MB, Early diagnosis.

## Introduction

Life styles, diet habit, population aging and social/psychological factor effects led to a growing

incidence of acute myocardial infarction (AMI) recently, making it one common severe disease in clinics<sup>1,2</sup>. AMI is one of the cardiovascular diseases with the highest incidence and severity, as nearly half of patients with cardiovascular disease died from AMI<sup>3</sup>. Currently, the incidence of AMI is rapidly increasing, as reaching 45-55 per 100,000 rate<sup>4,5</sup>. Under acute and persistent ischemia/hypoxia of coronary artery, sharply decrease or blockade of coronary artery blood supply leads to ischemia necrosis of myocardial tissues, resulting in the most severe type of coronary heart disease<sup>6,7</sup>. Clinical symptoms of AMI mainly presented as severe and consistent post-sternal pain. Severe manifestation even developed into cardiac arrhythmia, shock or heart failure, thus severely threatening life<sup>8</sup>. AMI, thus, poses a potential risk to patients' life. The timely prediction and diagnosis at early phase combined with prompt re-perfusion intervention could improve patient diagnosis and decreased mortality rate<sup>9,10</sup>. MicroRNA is a type of small double stranded RNA, composed of 19-25 nucleic acids sharing similar molecular biology features, and participates body biological functions. MiRNA possesses multiple functional mechanisms in the modulation of body growth/development and body's acclimation for the environment<sup>11</sup>. Under the regulation by physiological and developmental signals<sup>12</sup>, miRNA participates in various pathophysiological processes, including tumor, inflammation, cardiovascular disease and immune response. Therefore, unique miRNA expressional profiles may exist among different diseases<sup>13,14</sup>. Blood microRNA has stable expression, thus can be considered as one target for disease diagnosis<sup>15</sup>. Previous studies<sup>16,17</sup> showed the role of miRNA-124 in cardiovascular diseases including myocardial remodeling. The expression of miRNA-124 in AMI and its significance, however, has not been fully illustrated.

## Patients and Methods

### Patients

A total of 90 AMI patients who received percutaneous coronary intervention (PCI) in the Affiliated Hospital of Qingdao University from January 2015 to January 2016 were enrolled. There were 48 males and 42 females, aging between 26 and 58 years old (average age =  $35.2 \pm 5.6$  years). Inclusive criteria: all research objects fitted the diagnostic criteria of AMI in Diagnosis and Treatment Guideline for Acute Myocardial Infarction stipulated by Chinese Medicine Academy, including typical chest pain for longer than 30 min, novel signs of myocardial ischemia on ECG (ST-segment change, left bundle branch block, pathology Q wave, dynamics of troponin (cTnI) and creatine kinase-MB isoenzyme (CK-MB), continuous chest pain for more than 30 min, no relief after nitrate drugs, within 12 h of chest pain onset, and primary onset of AMI. Exclusive criteria: previous history of myocardial infarction; received PCI treatment; complicated with myocardial disease, pericardial disease, infectious pericarditis; having acute heart failure when admission; with infectious disease, malignant tumor, severe diabetes mellitus, liver/kidney disease, pulmonary fibrosis, bone metabolic disorder, systemic immune disease and complication of malignant tumor; complicated with cardiac shock. 45 healthy individuals admitted for body examination were recruited as control group. No one has the history of heart disease, vascular disease, normal chest ray, ECG, liver/kidney function, biochemical index or other major disease. There were 25 males and 20 females, aging between 27 and 60 years (average age =  $36.2 \pm 5.2$  years). This study has been approved by the Ethical Committee of the Affiliated Hospital of Qingdao University. All participants signed informed consents of this study.

### Major Reagents and Equipment

Trizol reagent was purchased from Sigma-Aldrich (St. Louis, MO, USA). RNA extraction kit, RT-PCR primer, reverse transcription (RT) kit, Real-time PCR kit were purchased from Axygen (Thermo Fisher Scientific, Waltham, MA, USA). cTnI ELISA kit was purchased from BD (San Jose, CA, USA). ABI7900 HT Real-time PCR was purchased from ABI (Thermo Fisher Scientific, Waltham, MA, USA). NanoDrop2000 RNA analyzer was purchased from Thermo (Thermo Fisher Scientific, Waltham, MA, USA). Labsy-

stem Version 1.3.1 microplate reader was purchased from Bio-Rad (Hercules, CA, USA).

### Sample Collection

The time for occurrence of typical AMI symptom (admission time) was set as T0 in blood collection. All-inclusive patients were within 6 h of disease onset. Those individuals longer than 6 h were excluded from this study. Using T0 as the starting point, blood collection was performed based on pre-scheduled time interval. At different time points of AMI onset (0 h at admission, 6 h, 12 h and 24 h of onset), 10 ml blood samples were collected from patients. 5 ml whole blood was frozen at  $-80^{\circ}\text{C}$  for extracting total RNA. Other 5 ml whole blood was centrifuged at 2000 g for 10 min. The supernatant was saved at  $-20^{\circ}\text{C}$  for further use.

### Real-time PCR

Real-time PCR was used to test miRNA-124 expression in peripheral blood. 1 ml Trizol was added into peripheral blood and was mixed on ice until clear and no precipitation in the mixture. The solution was then centrifuged at 12000 g for 10 min. The supernatant was saved and added with chloroform for 2 min vortex and 5 min incubation. The mixture was then centrifuged at 12000 g for 15 min at  $4^{\circ}\text{C}$ . The supernatant was saved and added to equal volume of isopropanol for inverted mixture and  $4^{\circ}\text{C}$  incubation for 1.5 h. After centrifugation at 12000 g for 10 min at  $4^{\circ}\text{C}$ , the supernatant was removed. 75% ethanol was added to rinse white precipitations for 2-3 times. Centrifugation was performed at 7500 g for 10 min at  $4^{\circ}\text{C}$ . The supernatant was discarded for adding DEPC water to resolve RNA. The analyzer was used to measure RNA purity and concentration. RT kit was used to synthesize cDNA using purified RNA as the template. Primer Premier 6.0 (Palo Alto, CA, USA) was employed to design PCR primers, which were synthesized by Invitrogen (Shanghai, China) as shown in Table I. Real-time PCR was performed to test target gene. Reaction conditions were:  $95^{\circ}\text{C}$  pre-denature for 10 min, followed by 35 cycles each containing  $95^{\circ}\text{C}$  10 s,  $58^{\circ}\text{C}$  45 s and  $72^{\circ}\text{C}$  35 s. Solution conditions were:  $60^{\circ}\text{C}$  60 s and  $95^{\circ}\text{C}$  15 s. Each sample was tested in triplicates for data collection to determine amplification and melting curve. CT stands for initial cycle number. CT values of all samples and standards were collected to plot the standard curve.  $\Delta\text{Ct}$  was calculated as sample CT minus standard CT value. Using GAPDH as the reference,  $2^{-\Delta\text{Ct}}$  method was used for semi-quantitative analysis.

**Table I.** Primer sequence.

Target gene	Forward primer 5'-3'	Reverse primer 5'-3'
GAPDH	ACCAGGTATCTGCTGGTTG	TAACCATGATGTCAGCGTGGT
miRNA124	CTTAGTCTCTGTATGACGT	TCACCCAGCTTGAGTCGA

**Table II.** General clinical information of AMI patients.

	AMI group	Control group
n	90	45
Age (years)	35.2±5.6	36.2±5.2
Sex (M/F)	48/42	25/20
HR (/min)	86.3±10.2	82.5±9.8
SBP (mmHg)	135.5±15.6	131.2±11.4
DBP (mmHg)	82.1±5.3	81.8±6.2
Smoking (N (%))	15 (16.7%)	9 (20%)

### ELISA

ELISA was used to test serum cTnI expression in all research objects. Peripheral blood samples were centrifuged to extract the supernatant. The experimental procedure was performed according to ELISA kit. Microplate reader was used to quantifying absorbance value (A) of all wells. A value of standards and respective concentrations were used to plot standard curve, on which sample concentration was calculated.

### Patient Information Record

Age, sex, smoking history, heart rate (HR), blood pressure, general conditions and CK-MB were recorded on all patients and control people.

### Statistical Analysis

SPSS19.0 software (IBM, Armonk, NY, USA) was used for data analysis. Measurement data were presented as mean ± standard deviation. The comparison of means among multiple groups was done by one-way analysis of variance (ANOVA). LSD and S-N-K tests were provided as equal variances were assumed while Tamhane's T2 test was used as equal variances, were not assumed. Pearson correlation analysis was used for correlation analysis. ROC analysis was used to elucidate diagnostic value of miRNA-124 on AMI. The statistical significance was defined when  $p < 0.05$ .

## Results

### General Information of Patients

Age, sex, smoking, HR, systolic blood pressure (SBP) and diastolic blood pressure (DBP) of all

patients were collected. Results showed that no statistically significant difference of general information was found between AMI and control group ( $p > 0.05$ , Table II).

### MiRNA-124 Expression in AMI Patients

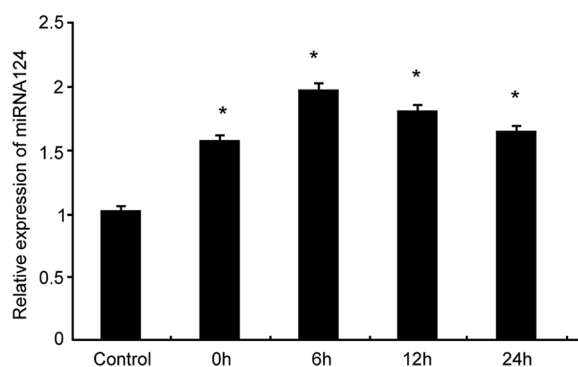
Real-time PCR was used to test peripheral miRNA-124 expression in AMI patients' healthy individuals at different time points. The results showed significantly higher level of miRNA-124 in peripheral blood of AMI patients than those in healthy control group ( $p < 0.05$ ). The expression of miRNA-124 increased since AMI patients received admission, and reached the peak at 6 h (Figure 1).

### Serum cTnI Expression in AMI Patients

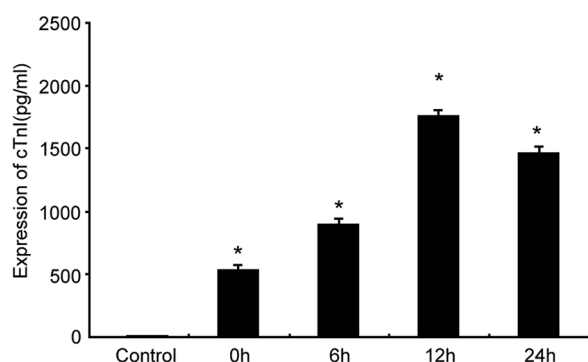
ELISA was used to detect serum expression of cTnI in AMI patients and healthy individuals at different time points. Results showed significantly higher serum cTnI expression in AMI patients compared to that in healthy control group ( $p < 0.05$ ). cTnI level in AMI patients started to increase since admission, and reached the peak at 12 h after onset (Figure 2).

### Serum CK-MB Expression in AMI Patients

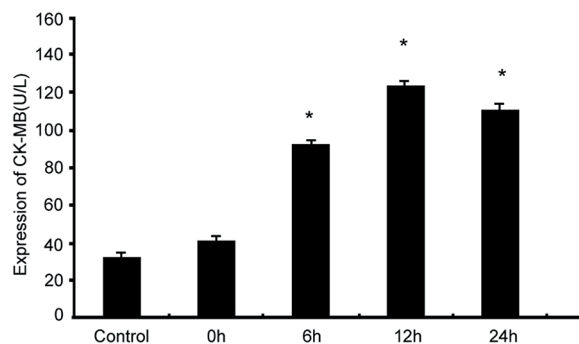
Results by ELISA detection showed significantly higher serum CK-MB expression in AMI patients compared to that in healthy control group ( $p < 0.05$ ). In a similar fashion, CK-MB level rose at 6 h after disease onset as those of cTnI, and rea-



**Figure 1.** MiRNA-124 expression in AMI patients. \*,  $p < 0.05$  compared to control group.



**Figure 2.** Serum cTnI expression in AMI patients. \*,  $p < 0.05$  compared to control group.



**Figure 3.** Serum CK-MB expression in AMI patients. \*,  $p < 0.05$  compared to control group.

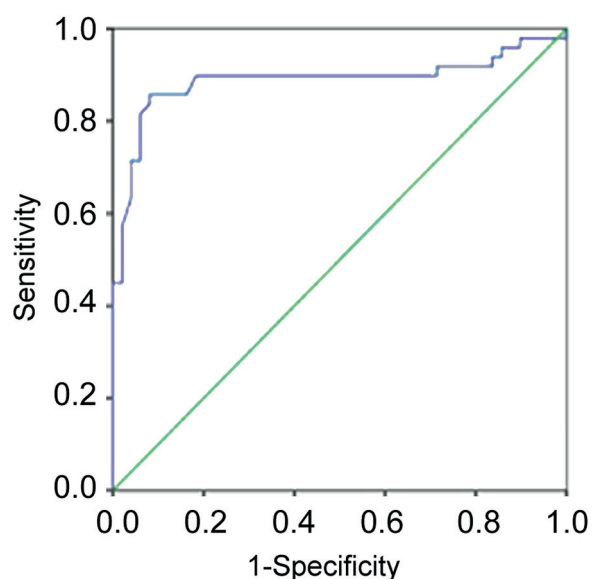
ched the peak at 12 h after onset (Figure 3). These results demonstrated that elevated expression of miRNA-124 in peripheral blood of AMI patients, and the time of peak value was ahead of that of cTnI and CK-MB.

#### Correlation Analysis Between miRNA-124, cTnI and CK-MB

Correlation analysis was performed on miRNA-124 in AMI patients along with common test indexes including cTnI and CK-MB. Results showed positive correlation between miRNA-124 and cTnI or CK-MB ( $p < 0.05$ , Table III).

#### Significance of Peripheral miRNA-124 in early Prediction of AMI

ROC analysis was employed to elucidate the significance of peripheral miRNA-124 in AMI



**Figure 4.** ROC curve of miRNA-124.

patients in early diagnosis of the disease. Results showed the area-under-curve of miRNA-124 was 0.86 (95% CI, 0.815-0.937) with 52% sensitivity and 91% specificity (Figure 4).

### Discussion

AMI is one common disorder of cardiovascular disease, and it is one important reason causing higher mortality due to cardiovascular disorder. AMI is characterized as acute disease onset with insidious onset and atypical symptom. Moreover, characteristics presented changes of ECG include novel Q wave, elevated ST-segment and dynamic progression of ST-T, all of which are insignificant, thus causing difficulty in early diagnosis of AMI<sup>18</sup>. Therefore, timely detection with high specificity and sensitivity, are of utmost necessity for early prediction of AMI. The timely evaluation of AMI disease condition provides to the patients an early and effective treatment, and it guarantees a cardiac re-perfusion, thus playing an important role in suppressing patient mortality<sup>19</sup>. Common biochemical markers for AMI diagnosis include CK-MB, cTnI, cTnT and myoglobin. CK-MB, cTnT and cTnI are important diagnostic markers for AMI<sup>20</sup>, yet the clinical implication in early diagnosis of AMI is unsatisfactory. Particularly, CK-MB and cTnI have lower sensitivity in early phase. Both indexes normal-

**Table III.** Correlation analysis between miRNA-124, cTnI and CK-MB.

	cTnI	CK-MB
R value	0.786	0.827
p-value	<0.05	<0.05

ly increased at 3-6 h after disease onset, whilst CK-MB and cTnI returned to normal levels at 3-4 d and 11-14 d after onset<sup>21</sup>. Myoglobin cannot differentiate between skeletal muscle and myocardial injury, thus lacking specificity with low practical value<sup>22</sup>. The previous study found that miRNA could participate in physiological and pathological processes of cardiovascular system via regulating myocardial cell growth, apoptosis and reconstruction of extracellular matrix<sup>23</sup>. Moreover, it has been demonstrated that microRNA in cardiovascular disease could inhibit target gene expression, thus modulating myocardial cell growth/apoptosis, participating in occurrence and progression of cardiac physiology and pathology including myocardial thickness, injury, remodeling and heart failure<sup>24</sup>. Specific expression of miRNA in peripheral blood can reflect disease occurrence, progression and severity. MiRNA performs high stability as RNAase cannot easily degrade miRNA, making it tolerable for repeated freeze-thawing cycle and long-term storage, maintaining stable quantity and quality in plasma or serum, thus becoming a potential serum molecular marker of disease<sup>15,25</sup>. More researches<sup>26</sup> have reported about miRNA participation in AMI pathogenesis. MiRNA-124, on the other hand, plays a role in cardiovascular disease including myocardial remodeling<sup>16,17</sup>. This study investigated the expression of miRNA-124 in peripheral blood from AMI patients at different onset time points. Our data showed significantly elevated miRNA-124 expression in AMI patients compared to that in normal control group. MiRNA-124 reached the peak level at 6 h after AMI onset. Moreover, miRNA-124 is positively correlated with cTnI and CK-MB, but reached its peak level earlier than cTnI or CK-MB. ROC analysis confirmed high specificity of miRNA-124.

## Conclusions

AMI patients had significantly elevated miRNA-124 in peripheral blood, showing the earlier phase of peak level than other diagnostic markers for AMI, suggesting favorable detection timeliness. Therefore, peripheral miRNA-124 contributed as one novel biological marker for early diagnosis of AMI.

## Conflict of interest

The authors declare no conflicts of interest.

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