

Original Article

Diagnostic value of serum miR-122 in acute myocardial infarction

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Abstract: As one common disease that severely affects public health, myocardial infarction has been shown to be related with alternation of serum levels of various microRNAs (miR) including miR-21, miR-34a and miR-328. These molecules can thus work as biological markers for early diagnosis. MiR-122 has been shown to have up-regulation in acute coronary syndrome especially acute myocardial infarction (AMI), but leaving its potential diagnostic value unattended. This study thus investigated the value of miR-122 in AMI diagnosis. A total of 78 AMI patients were recruited along with 72 non-cardiovascular disease patients. Real-time fluorescent quantitative PCR (qRT-PCR) was employed to detect serum miR-122 levels at 0, 4, 8, 12 and 24 hours after admitting. Enzyme linked immunosorbent assay (ELISA) and immunosuppressant method were used to measure serum cardiac troponin I (cTnI) and creatine kinase isoenzyme (CK-MB) levels. Receiver operating characteristic (ROC) curve was plotted to predict the diagnostic value of miR-122 on AMI patients. Serum miR-122 level was significantly higher in AMI patients compared to control group ($P < 0.05$), with higher level in NSTEMI patients than STEMI ones. Most significant elevation of miR-122 occurred at 8 hours after admitting. ROC analysis revealed the area under curve as 0.861, 0.895, 0.92, 0.877 and 0.859 at 0, 4, 8, 12 and 24 hours after admitting. At all time point there were statistical significance ($P < 0.01$). MiR-122 has higher diagnostic values for AMI, especially at 8 hours after admitting. It may thus work as one biological marker for early diagnosis of AMI.

Keywords: MicroRNA-122, acute myocardial infarction, diagnostic value

Introduction

With the rapid aging of total population, cardiovascular disease has now become one major public health issue threatening life quality. Acute myocardial infarction (AMI) is featured with myocardial tissue necrosis due to persistent severe hypo-perfusion [1]. It has high incidence and high mortality, thus causing heavy burdens for people's health [2, 3]. Based on the presence of ST-segment elevation in two consecutive leads on electrocardiogram (ECG), AMI can be further divided into ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI) [4]. In those patients with atypical ECG features and clinical symptoms, creatine kinase isoenzyme (CK-MB) and cardiac troponin I (cTnI) have been utilized as biological markers for diagnosis. CK-MB was once

believed to be the golden standard of AMI, as its contents usually doubles within 5~6 hours after the manifestation of primary symptom, and reaches a peak at 12~24 hours. Although with shorter observing window, CK-MB displayed satisfactory application values in diagnosing recurrent infarction [5]. Its implication in AMI, however, remained debated. Cardiac troponin (cTnI/cTnT) has higher tissue histocompatibility and sensitivity, and is accepted as the gold standard for AMI diagnosis [6]. However, due to the occurrence of cTnI usually between 6 and 12 hours after coronary occlusion, even high-sensitive cTnI can only be observed 3 hours after infarction [7, 8]. Therefore the establishment of one early diagnostic method using those markers is of critical importance.

Micro RNA (miR) is one type of small molecules for regulating body growth and progression of

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Table 1. Clinical information between AMI and control group

Clinical index	AMI (78)	Control (72)	P value
Sex (Male/female)	43/35	42/30	0.69
Age (years)	56.34±12.31	54.54±11.32	0.35
Risk factors			
Systolic pressure (mmHg)	135.32±8.45	132.45±10.21	0.06
Diabolic pressure (mmHg)	79.01±4.32	78.21±5.43	0.31
Diabetes (Yes/No)	19/59	17/55	0.91
Smoking (Yes/No)	32/46	30/42	0.93
Drinking (Yes/No)	34/44	32/40	0.91
Heart rate (min ⁻¹)	85.32±8.96	83.21±6.75	0.11
Lab tests			
TC (mmol/L)	3.85±0.89	3.56±1.02	0.07
TG (mmol/L)	1.43±0.92	1.39±0.73	0.76
HDL (mmol/L)	1.12±0.23	1.07±0.18	0.14
LDL-C (mmol/L)	2.45±1.09	2.31±1.20	0.46
Serum marker			
CK-MB (IU/L)	230.04±13.21	10.24±5.04	<0.01
cTnI (ng/ml)	14.32±1.98	0.02±0.01	<0.01

Note: TC, total cholesterol; TG, total triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; CK-MB, creatine kinase isoenzyme; cTnI, cardiac troponin I.

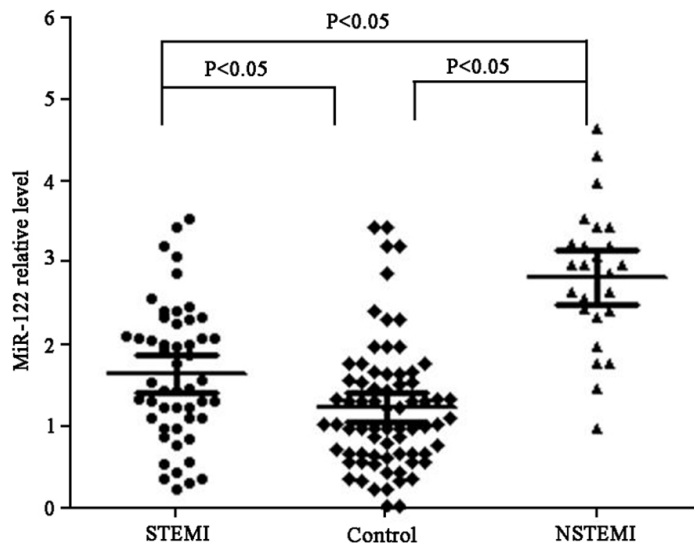


Figure 1. MiR-122 levels.

multiple diseases. It has the length at about 19 to 24 nucleotides. MiR has been shown to participate in the progression of tumors, cardiovascular diseases, and auto-immune disease. Meanwhile, the highly conserved sequence of miR also makes it one potential biomarker. Most previous studies have shown the significant elevation of miR-21, miR-34a, miR-328,

and miR-126 in AMI patients, suggesting its potency to be drug targets in AMI diagnosis [9-12].

MiR-122, as one liver-specific miRNA, plays critical role in lipid metabolism [13, 14]. In heart tissues, it has relatively lower levels or even is absence under normal conditions. However, miR-122 expression level in myocardial tissues was significantly elevated in AMI mice [15]. The elevated miR-122 during myocardial infarction-induced heart failure facilitates the occurrence of atrial fibrillation. Moreover, serum miR-122 and miR-3149 levels were elevated in acute coronary syndrome (ACS) patients especially AMI patients, suggesting their potency as AMI diagnostic indexes [16]. The diagnostic value of miR-122 in AMI, however, has not been studied. We thus aimed to illustrate the correlation between serum miR-122 levels and predictive values for early diagnosis of AMI.

Material and methods

General information of research objects

A total of 78 AMI patients from June 2014 to June 2015 were recruited in this study as disease group and another cohort of 72 patients in other departments of The First Affiliated Hospital of Shantou University Medical College was selected as the control group. The diagnosis of AMI followed the ESC/ACCF/AHA/WHF standards in 1983. Inclusive criteria: (1) With typical features such as chest pains caused by infarction; (2) Altered ECG; (3) Dynamic changes of serum markers for myocardial infarction,

such as CK-MB or cTnI. A patient cannot be diagnosed as AMI unless two or more symptoms occurred. Exclusive criteria: (1) History of major illness such as tumor, cardiovascular disease or mental issues; (2) Having liver-kidney illness. This study has been pre-approved by the ethical committee of The First Affiliated Hospital of Shantou University Medical College

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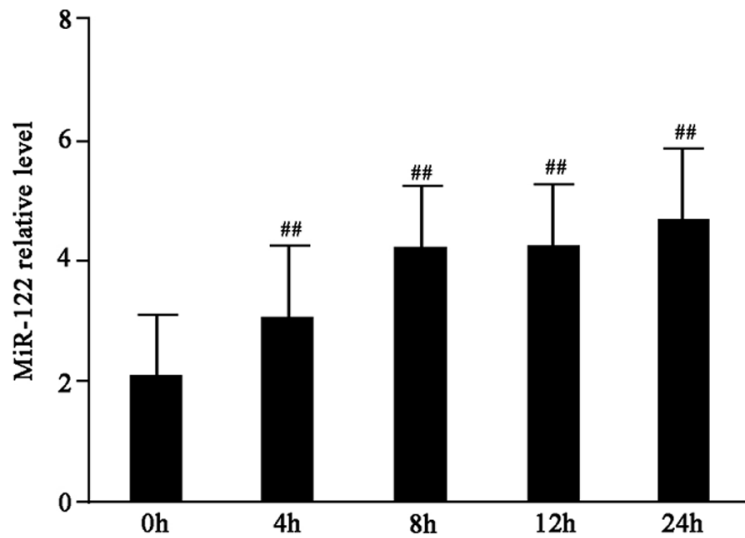


Figure 2. MiR-122 levels at different time points. ##, $P < 0.05$ compared to values at 0 h.

and has obtained written consents from all participants.

Major reagents and equipments

Real-time fluorescent quantitative RT-PCR kit was produced by TaKaRa (Japan). Reverse transcription kits and Trizol were purchased from Invitrogen (US). Serum cTnI assay kit was provided by Beckman Coulter (US). CK-MB test kit was a product of Lideman (Beijing, China). ABI7500 fluorescent quantitative PCR cyclor was produced by Applied Biosystem (US). High-speed cold centrifugation was a product of Eppendorf (US).

Serum RNA extraction

5 mL venous blood samples were collected at different time points (0 h, 4 h, 8 h, 12 h and 24 h after admitting), and were centrifuged at 3000 g for 10 min to extract serum for further use. Total RNA extraction kit (Qiagen, US) was used to extract total RNA, which was dissolved in DEPC-treated water. RNA purity and concentration were determined by UV spectrometer. RNA integrity was observed in 1% agarose gel electrophoresis.

CK-MB and cTnI assay

Enzyme linked immunosorbent assay (ELISA) was used to quantify serum cTnI level while CK-MB level was measured by immunosup-

pressant method using test kits following manual instruction.

Real-time fluorescent quantitative PCR

Reverse transcription kit was used to synthesize DNA based on miRNA sequence following manual instruction. DNA products were then used for real-time PCR in a 20- μ L reaction mixture including 9 μ L 2XSYBR Green Mixture, 2 μ L of each primer (at 5 μ M), 2 μ L DNA and 5 μ L ddH₂O. Primer sequences were: miR-122-Forward, 5'-TTGAA TTCTA ACACC TTCGT GGCTA CACGC G-3'; miR-122-Reverse, 5'-TTAGA TCTCA TTTAT

CGAGG GAAGG ATTG -3'; U6-Forward, 5'-CTCGC TTCGG CAGCA CA-3'; U6-Reverse, 5'-AACGC TTCAC GAATT TCGGT-3'. The reaction conditions were: 95°C denature for 30 sec, followed by 40 cycles each containing 95°C denature for 30 sec, 55°C annealing for 30 sec and 72°C elongation for 60 sec. Using U6 snRNA as the internal reference, triplicated experiments were performed. Relative level of miR-122 was determined by $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Measurement data were presented as mean \pm standard deviation (SD) and were analyzed by SPSS 16.0. Student t-test and analysis of variance (ANOVA) were used to compare means across data sets fitted normal distribution with equal variance. All statistical analysis was performed in two-tailed manner. The significant level was pre-determined as 0.05. Receiver operating characteristic (ROC) was used to predict the diagnostic implication of miR-122 on AMI.

Results

Clinical information

A statistical analysis of clinical information on all participants (**Table 1**) revealed no significant difference regarding age, sex, blood pressure (systolic and diastolic), diabetes, smoking & drinking, heart rate and lab tests (total choles-

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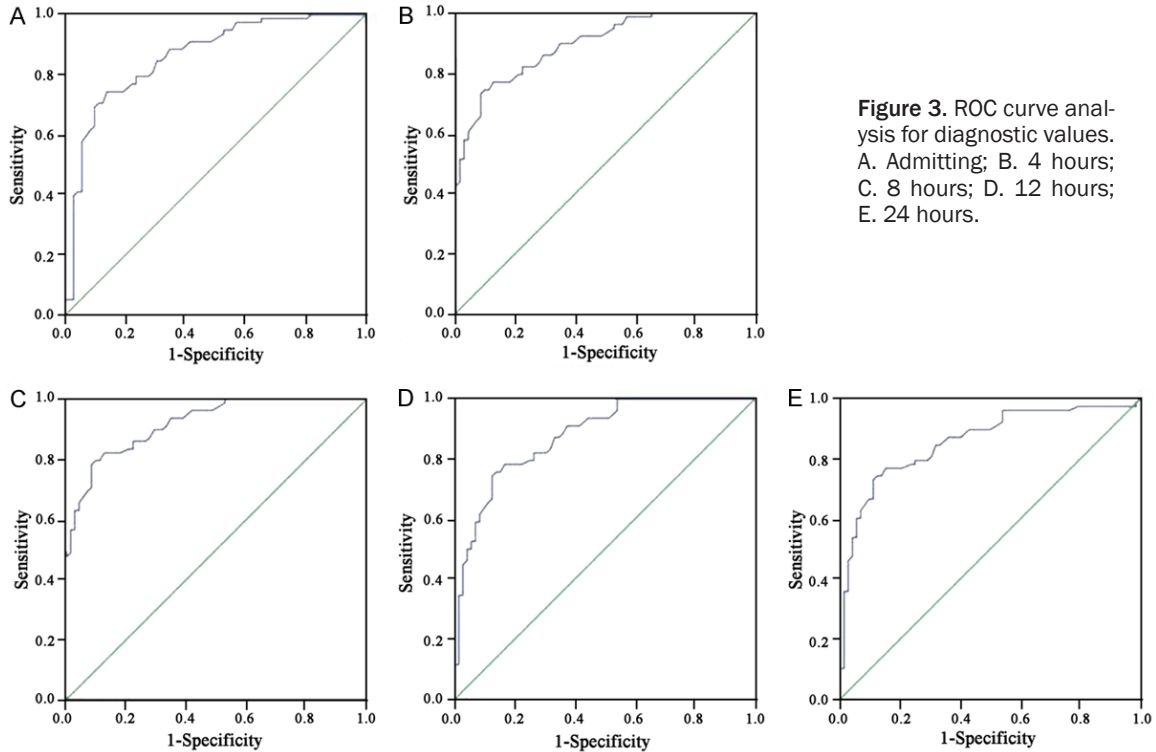


Figure 3. ROC curve analysis for diagnostic values. A. Admitting; B. 4 hours; C. 8 hours; D. 12 hours; E. 24 hours.

terol, triglyceride, high density lipoprotein and low density lipoprotein) between two groups ($P>0.05$), suggesting homogeneity between groups. CK-MB and cTnI levels, however, showed statistically significant difference between AMI and control group.

miR-122 expression level

We firstly analyzed serum miR-122 levels at the time of admission using qRT-PCR. Results showed significantly elevated miR-122 level in AMI patients compared to control group. Within AMI patients, NSTEMI ones had higher miR-122 than STEMI individuals ($P<0.05$, **Figure 1**). We further analyzed miR-122 levels at different time points after admitting and found the peak level at 8 hours (**Figure 2**).

ROC curve analysis

Using ESC/ACCF/AHA/WHF diagnostic standard (2000 version), we analyzed the diagnostic value of miR-122 levels at different time points in AMI patients. As shown in **Figure 3**, areas under the curve were 0.861 (95% CI, 0.802~0.921, $P<0.01$), 0.895 (95% CI, 0.847~0.943, $P<0.01$), 0.92 (95% CI, 0.88~0.961, $P<0.01$), 0.877 (95% CI, 0.824~0.931, $P<0.01$)

and 0.859 (95% CI, 0.798~0.919, $P<0.01$) for miR-122 values at 0 h, 4 h, 8 h, 12 h and 24 h after admitting. Therefore, miR-122 had relatively higher diagnostic value for AMI, especially at 8 h after admitting.

Discussion

The early diagnosis of AMI is crucial for improving treatment efficiency and patient's life quality. Recent studies have revealed the diagnostic value of circulated miRNAs on AMI diagnosis [17, 18]. DNA microarray screening has revealed more than two-fold increase of serum miR-122, miR-140-2p, miR-144, miR-720, miR-2861 and miR-3149 compared to healthy people. Using animal models, it has been found that miR-122 and miR-3149 levels were elevated earlier than cTnI, indicating their efficiency in early diagnosis [16]. MiR-3149, however, has only been shown to have down-regulation [19]. Therefore we selected miR-122 to test its diagnostic value in AMI.

We used two serum markers, CK-MB and cTnI as AMI diagnostic criteria combining with ECG and clinical symptoms. We found significantly elevated serum miR-122 levels in AMI patients compared to control ones. NSTEMI patients

had even higher miR-122 levels than STEMI individuals, suggesting the better candidate for diagnosing NSTEMI. Previous study has shown 17.8-fold increase of miR-122 in AMI patients. The ROC under-curve area was 0.838. Meanwhile, in pig model of AMI, miR-122 expression level was also elevated in both atrial fibrillation and non-fibrillation types [16]. This study also proposed the possible correlation between miR-122 & miR-3149 with atrial fibrillation post AMI. Based on such information and our results, miR-122 may work as one novel biomarker for AMI, especially for NSTEMI patients. Past studies about miR-122 mainly focused on liver cancer and liver cirrhosis, with less attention on cardiovascular disease. This study revealed the peak level of miR-122 at 8 hours after admitting. Normal serum indexes such as CK-MB and cTnI, however, only achieved the peak level 12~24 hours after onset. Therefore, miR-122 has better efficiency for early diagnosis compared to serum indicators. ROC curve analysis also revealed high specificity and sensitivity at 8 hours.

Most *in vivo* and *in vitro* studies have revealed critical roles of miR-122, miR-370 and miR-33a/b in lipid metabolism. The silencing of miR-122 can increase TC and TG in serum [20, 21]. Other studies have revealed the correlation between miR-122 and miR-370 up-regulation and the occurrence of coronary heart disease [22]. Therefore miR-122 elevation may be one reason underlying myocardial infarction. Another study has revealed the modulation of lipid metabolism by miR122 by inducing the expression of related proteins [23]. As lipid metabolism disorder may become one risk factor for AMI, miR-122 may participate in the pathogenesis of AMI via affecting lipid metabolism in liver.

Due to the relatively small sample size in this study, large-sample experiment is necessary in future to validate proposed model. Moreover, the expression of serum miR-122 beyond 48 hours after admitting needs to be elucidated. Due to the liver-specificity of miR-122, further studies are required to determine if miR-122 can work in AMI patients complicated with liver dysfunctions.

Disclosure of conflict of interest

None.

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