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The clinical value of circulating miR-99a in plasma of patients with acute myocardial infarction

S.-Y. YANG[1], Y.-Q. WANG[1], H.-M. GAO[1], B. WANG[1], Q. HE[2]

[1]Department of Intensive Care Unit, Tianjin First Center Hospital, Tianjin, China
[2]Department of Cardiology, Tianjin First Center Hospital, Tianjin, China

Abstract. – OBJECTIVE: Acute myocardial infarction (AMI) contributes to be a significant public health problem, and it is the leading cause of morbidity and mortality in the world. However, the molecular mechanisms underlying AMI is poorly known. The purpose of this study was to investigate the change of plasma microRNA-99a (miR-99a) level in AMI patients and its potential clinical value.

PATIENTS AND METHODS: Plasma samples from 54 patients with AMI and 30 healthy volunteers were collected. Quantitative real-time PCR (qPCR) and ELISA assays were used to detect the expression of circulating plasma miR-99a and of cardiac troponin I (cTnI)/creatine kinase-MB (CK-MB), respectively. AMI patients were categorized into subgroups according to the number of stenosed coronary vessels, and the association between the plasma miR-99a level and the severity of AMI was analyzed. The expression level of miR-99a was also evaluated in patients receiving percutaneous coronary intervention (PCI).

RESULTS: The expression of miR-99a was significantly downregulated in patients with AMI compared with the normal controls ($p < 0.01$). In the AMI patients, miR-99a level had a negative correlation with cTnI level ($r = -0.8202$, $p < 0.01$) and CK-MB ($r = -0.6924$, $p < 0.01$). Also, the expression of miR-99a was markedly lower in patients with more stenosed coronary vessels ($p < 0.01$). The relative expression level of miR-99a in AMI patients was significantly increased after receiving PCI ($p < 0.01$).

CONCLUSIONS: The expression level of plasma miR-99a was remarkably reduced following AMI and closely associated with the severity of AMI. Therefore, it was a promising novel diagnostic biomarker for AMI.

Key Words:
Acute myocardial infarction, Biomarker, MicroRNA-99a.

Introduction

Acute myocardial infarction (AMI) is the main complication of cardiovascular diseases with high morbidity and mortality[1]. The highest risk of fatality occurs within the initial hours of onset of AMI. Therefore, early and correct diagnosis of AMI is an effective strategy for inhibiting the progression AMI as well as improving the clinical outcome of the patients[2]. Cardiac troponin I (cTnI) and creatine kinase-MB (CK-MB) are common biomarkers currently used for diagnosis of AMI in the clinical setting[3]. However, they are not sensitive and specific enough as their expression levels can also increase in patients without AMI. Thus, it is urgent and important to explore novel biomarkers for early diagnosis of AMI[4].

MicroRNAs (miRNAs) are small non-coding single-stranded RNA molecules (21-23 nucleotides) which negatively regulate gene expression by binding to 3'UTR of mRNA and promoting its degradation[5,6]. miRNAs have been demonstrated to play crucial roles in various essential biological processes including, but not limited to, proliferation, survival, migration and differentiation[7,8]. In addition, dysregulated expression of miRNAs contributes to a variety of diseases such as cancer and cardiovascular diseases[9-11]. Many studies have shown that miRNAs are promising biomarkers for diagnosis and prognosis of AMI. Yao et al[12] reported that the expression level of circulating miR-122-5p was increased in AMI patients compared with the controls. In addition, it has a positive correlation with cTnI concentration and considerable diagnostic accuracy for AMI. Similarly, Lv et al[13] showed that the expression levels of circulating miR-208b and miR-34a are associated with left ventricular remodeling after AMI, indicating that these two miRNAs could be used to predict the risk of mortality or heart failure.

Corresponding Author: Shuying Yang, MD; e-mail: shuyingyangtjh@sina.com

MiR-99a seems to play an important role in cardiomyogenesis. Kehle et al[14] showed that the expression level of plasma miR-99a was significantly upregulated in patients with congenital heart defects, indicating miR-99a is involved in cardiac malformation and may serve as a biomarker during fetal development. In addition, overexpression of miR-99a attenuated ventricular remodelling, cardiac hypertrophy and improved cardiac performance after myocardial infarction, suggesting miR-99a might involve in the pathogenesis of AMI[15,16]. Therefore, the purpose of this study was to investigate the expression level of plasma miR-99a in AMI patients and then evaluate its potential clinical significance.

Patients and Methods

Study Population and Sample Collection

54 patients with AMI and 30 healthy volunteers were recruited into the study. The investigation was approved by the Ethics Committee of the Tianjin First Center Hospital, and written informed consent was obtained from all participants. The diagnosis of AMI was confirmed by emergency coronary angiography or laboratory analyses. The healthy controls have no clinical manifestation or medical history of cardiovascular diseases.

Up to 5 mL of venous blood was drawn with EDTA from each participant. The plasma fraction was prepared immediately by centrifugation at 1500 × g for 20 min at 4 °C. Then samples were divided into aliquots in cryovials and stored at -80°C until total RNA extraction.

Quantitative Real-time PCR(qPCR)

Total RNA was isolated from 200 L plasma samples using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer instructions. Primescript RT Regent Kit (Takara, Dalian, China) was used to convert the RNA into first strand cDNA. Real-time PCR was performed using SYBR Premix DimerEraser (Takara) and on a 7500 Real-time PCR System (Applied Biosystems, Foster City, CA, USA). The amplification conditions were as follows: 95°C for 12 min, followed by 40 cycles of 94°C for 15 s, 55°C for 30 s, and 70°C for 30 s. All reactions were performed in triplicate and U6 snRNA was used as an internal control. The relative expressions of plasma miR-99a were normalized to the levels of U6 snRNA and analyzed via using the $2^{-\Delta\Delta}$Ct method. The qPCR primers were as following:

miR-99a primers	
Sense	5 -GGCAAACCCGTAGATCCGA-3
Anti-sense	5 -TCCGTTGGTTGTCCCATAGACT-3
U6 snRNA	
Sense	5 -CTCGCTTCGGCAGCACATATACT-3
Anti-sense	5 -ACGCTTCACGAATTTGCGTGTC-3

Plasma cTnI and CK-MB Determination

The concentration of plasma cTnI and CK-MB were measured by ELISA assay according to the manufacturer's protocol (Beckman Coulter, Fullerton, CA, USA).

Statistical Analysis

All the data were expressed as mean ± SEM. The data of miR-99a, cTnI and CK-MB were first analyzed by the Kolmogorov-Smirnov test to evaluate whether they obeyed the normal distribution. If the data subjected to the normal distribution, then student's *t*-test and the ANOVA are conducted. Otherwise, Mann-Whitney U test and two-tailed Kruskal-Wallis tests are used. As all the data fit the normal distribution, student's t-test and ANOVA followed by Tukey's HSD test were performed for data analysis in this study. All statistical analyses were performed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Differences were considered as significant at $p < 0.05$.

Results

Plasma miR-99a was Reduced in AMI Patients

The qRT-PCR results showed that the expression level of plasma miR-99a was significantly decreased in patients with AMI compared with the normal controls ($p < 0.01$) (Figure 1).

Correlation of Plasma miRNA-99a with cTnI and CK-MB

The results revealed that a significant negative correlation was found between plasma miR-99a and cTnI (r= -0.8202, $p < 0.01$). Similar findings were detected between the expression level of miR-99a and CK-MB (r = -0.6924, $p < 0.01$) (Figure 2).

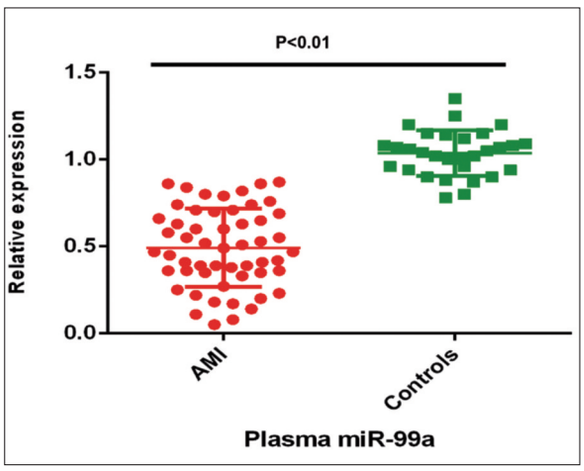

Figure 1. The expression level of plasma miR-99a in AMI patients and controls.

The Association Between the Number of Stenosed Coronary Vessels and Plasma miR-99a Expression Level in Patients with AMI

Thirty-seven of fifty-four AMI patients had received coronary angiography. These 37 patients were divided into three subgroups based on the number of stenosed coronary vessels (n = 1, 2, or 3). The results showed that the expression level of plasma miR-99a was significantly lower in AMI patients with a greater number of stenosed coronary vessels than those with less stenosed coronary vessels (all $p < 0.01$) (Figure 3).

Plasma miR-99a was Increased After Percutaneous Coronary Intervention (PCI)

Thirty of fifty-four patients underwent emergency PCI. As shown in Figure 4, the relative expression level of plasma miR-99a in AMI patients was significantly increased after receiving PCI ($p < 0.01$).

Discussion

AMI occurs when blood stops flowing properly to a part of the heart, and the heart muscle is injured because it is not receiving enough oxygen. AMI causes approximately two million deaths annually worldwide. Early detection of AMI plays a crucial role in determining the course of treatment to preserve and prevent further damage to the myocardial tissues[17]. The existing biomarkers for AMI diagnosis, including CK-MB, myohemoglobin (MYO), cTnI, and N-terminal pro-brain natriuretic peptide (NT-proBNP), have limited specificity and sensitivity. Therefore, there is an urgent need for identifying novel biomarkers that can guide AMI patient management at an early stage. In recent years aberrant expression of miRNAs has been associated with the initiation and development of AMI. In addition, these small molecules show great promise for AMI diagnosis[18,19].

Our results showed that the expression level of plasma miR-99a was significantly decreased in patients with AMI in comparison with the healthy volunteers, and it had a negative correlation with plasma cTnI and CK-MB concentrations. In addition, plasma miR-99a level was associated with the severity of AMI. Moreover, the expression level of plasma miR-99a in AMI patients was remarkably upregulated after receiving PCI. Our data suggested that plasma miR-99a was reduced in AMI patients and it might be a

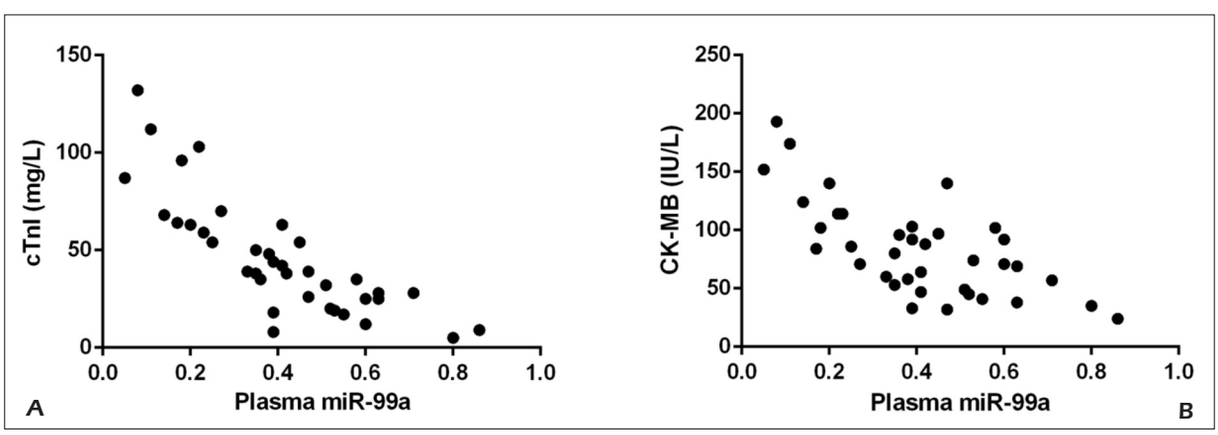

Figure 2. ***A,*** The correlation between plasma miR-99a level and cTnI ***(A)***/CK-MB ***(B)*** concentration.

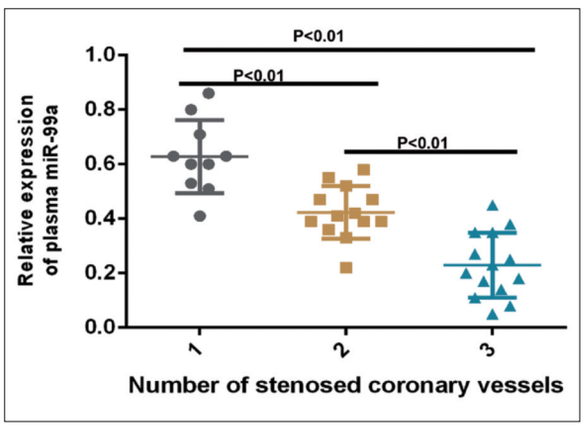

Figure 3. The association between plasma miR-99a level and number of stenosed coronary vessels.

promising biomarker for AMI diagnosis. To the best of our knowledge, this is the first work to reveal the clinical value of plasma miR-99a in AMI. Consistent with our study, Dong et al[20] compared the expression of miRNAs among the infarcted, non-infarcted and border area of rat hearts at 6 h after AMI, the expression level of tissue miR-99a was significantly downregulated in infarcted and border area compared with the non-infarcted area. Wang et al[21] profiled genome-wide miRNAs that differentially expressed between in a cohort of AMI patients and non-AMI patients. The expression level of miR-99a was remarkably reduced in the heart tissues derived from patients with AMI.

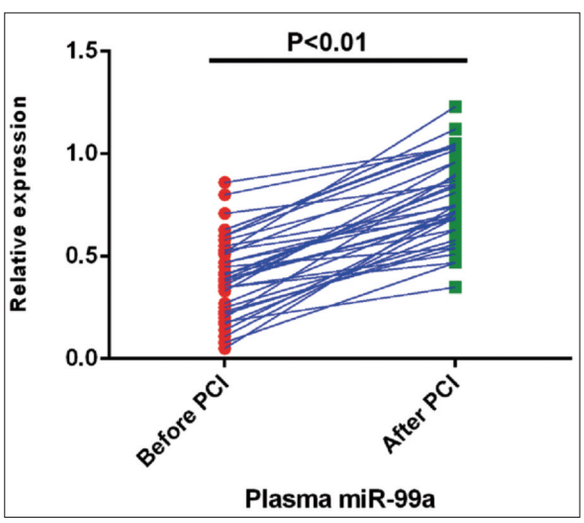

Figure 4. The expression level of plasma miR-99a after receiving PCI.

The AMI patients with more stenosed coronary vessels had lower plasma miR-99a level. The possible underlying reason is that normal expression of miR-99a is important for maintaining normal heart function, thus reduced miR-99a expression was a poor indicator of AMI. Li et al[15] reported that ectopic expression of miR-99a in pressure-overloaded heart could preserve the myocardial structure, reduce myocardial fibrosis and apoptosis, attenuate cardiac hypertrophy and improve cardiac function. The research group also demonstrated that miR-99a overexpression contributed to better both cardiac function and survival ratio by inhibiting cell apoptosis and increasing autophagy *via* an mTOR/P70/S6K signaling pathway[16]. Furthermore, Coppola et al[22] showed that miR-99a/let-7c cluster, located on human chromosome 21, was involved in the control of cardiomyogenesis by changing epigenetic factors, indicating miR-99a plays an important role in regulating cardiac development. Therefore, it is reasonable to speculate that the synthesis and secretion of miR-99a that derived from cardiac muscle cells might be significantly decreased following AMI.

However, there are some limitations of our study. Firstly, the clinical sample size was relatively small. Further research is necessary to provide more evidence to elucidate the clinical significance of plasma miR-99a in early detection of AMI. Secondly, investigations should be conducted to reveal the molecular mechanism of miR-99a in regulating pathogenesis of AMI.

Conclusions

The plasma miR-99a level is reduced in patients with AMI and serves as an indicator of the severity of this deadly disease, indicating plasma miR-99a might be a promising diagnostic biomarker for AMI.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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