Plasma microRNA-21 is a potential diagnostic biomarker of acute myocardial infarction

Y. ZHANG¹, Y.-J. LIU¹, T. LIU², H. ZHANG¹, S.-J. YANG³

¹Department of Cardiology, Tianjin Chest Hospital, Tianjin, China
²Department of Molecular Biology, Institute of Angiocardiopathy, Tianjin Chest Hospital, Tianjin, China
³Graduate School of Tianjin Medical University, Tianjin, China

Abstract. – OBJECTIVE: Previous studies have demonstrated that microRNA-21 (miR-21) is involved in the pathogenesis of myocardium infarction and cardiac fibrosis; the present study aimed to investigate its potential role in the diagnosis of acute myocardium infarction (AMI).

PATIENTS AND METHODS: A cohort of patients with AMI and angina pectoris (AP) were studied, plasma miR-21 level was determined by Realtime-PCR.

RESULTS: We found that the plasma miR-21 level was significantly elevated in patients with AMI compared with those with AP or healthy people. Further studies demonstrated the correlation of miR-21 and several traditional markers such as creatine kinase (CK), creatine kinase-MB (CK-MB) and troponin I (cTnI) in study subjects. Finally, receiver-operator characteristic curve (ROC) analysis showed that miR-21 has similar diagnostic ability compared with CK, CK-MB and cTnI.

CONCLUSIONS: Plasma miR-21 may be a novel biomarker for the diagnosis of AMI. Our study may also provide implications for the development of new biomarkers.

Key words: miR-21, AMI, Diagnosis.

Introduction

Despite the advances in the diagnosis and prevention of coronary cardiovascular complications over the past decades, coronary heart disease is still considered to be a severe health threat to with high morbidity and mortality worldwide⁴. Current applications on the diagnosis and monitoring of the myocardium lesion are largely dependent upon a spectrum of biochemical markers such as lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase MB isoform (CK-MB) and cardiac troponin I (cTnI)⁵. However, biomarkers with more accuracy and sensitivity that are able to give an early prediction are still lacking and need to be developed.

microRNAs (miRs) refer to a class of short, single-strand and non-coding RNAs that are broadly existed in eukaryotes; it is extensively involved in the post-transcriptional regulation of genes by specific base pair match within the 3'UTR region of mRNA⁶. Once established as important regulators in development, cancer, and neurodegenerative diseases, miRs are also reported to exert critical roles in the progress of coronary heart disease⁷-⁸. For example, miR-1 and miR-499 have been shown in previous studies to control arrhythmogenesis and apoptosis following acute myocardial infarction (AMI)⁹,¹⁰. A number of recent researches have identified the multifunctional role of miR-21 in cardiovascular system; it has been demonstrated to play a protective role in AMI but to exacerbate fibrosis during pressure-overload¹¹-¹⁴. Although these reports highlighted the crucial role of microRNAs in experimental studies, its potential action with regard to coronary heart disease has been scarcely studied in clinical samples. In light of the previous works in animal models and studies underlining the significance of plasma microRNAs in the diagnosis of cardiovascular diseases¹⁵-¹⁷, we hypothesized that circulating miR-21 might be established as a potential biomarker for coronary heart diseases.

In the present report, we enrolled 66 patients with coronary heart diseases at various stages. To test whether miR-21 could serve as a potential biomarker for coronary heart diseases, miR-21 expression signatures in patients with AMI or angina pectoris (AP) were identified, and its diagnostic accuracy was compared with the conventional biochemical markers. The information provided by our study may shed some new light on the clinical management of patients with coronary heart diseases in acute phase.
Patients and Methods

Patients

We observed 66 patients at the age of 40-75 from Tianjin Chest Hospital between May 2013 and July 2013, among which 10 people who were admitted to hospital for their complaints of precordial discomfort. It was further confirmed by cardiac catheterization or coronary CT angiography that these 10 people turned out to have no coronary artery lesions; therefore, they were selected as healthy controls. Cardiac catheterization or coronary CT angiography (Sensation 64; Siemens Medical Solutions, Erlangen, Germany) was also applied to the remainder 56 patients; the coronary artery lesions were confirmed by a 50% or greater stenosis in one or more main branches [left main coronary artery, left anterior descending artery (LAD), left circumflex artery (LCX) and right coronary artery (RCA)]. 17 and 39 patients of these patients were then included in AMI and AP subgroups, respectively, based on the consensus criteria defined by The Joint European Society of Cardiology/American College of Cardiology Committee2, i.e. increased cTnI and CKMB as well as pathological Q waves and ST segment elevation or depression on ECG recordings. The diagnosis of AP was based on the typical clinical symptoms as detailedly described in Canadian Cardiovascular Society (CCS) grading of stable angina pectoris and manifestations of transient ischemic ST-T changes on ECG without significant elevation of CK. Individuals with significant comorbidities such as valvular heart disease (VHD), acute or chronic infections disease, hematomatous disease, tumor, systemic autoimmune disease as well as hepatic and renal insufficiency were excluded from this study. 2 mL of fasting blood sample of each individual was collected in EDTA-anticoagulant tubes. The samples were centrifuged at 3000 rpm for 10 min, the supernatant was transferred into an RNase-free eppendorf tube and stored at -80°C for RNA isolation or the measurement of biochemical markers. We obtained informed consents and approval from all the individuals and the Ethics Committee of Tianjin Chest Hospital with regard to the study protocol, respectively.

Measurement of biochemical markers

Plasma CK, CKMB, cTnI, homocysteine (HCY) and fibrinogen (FIB) levels were measured using assay kits, and all the kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China).

miR-21 quantification

The plasma level of miR-21 was quantified by real-time PCR. The total RNA was isolated by a RNAprep pure Blood Kit from Tianjin Biotechnology Co. Ltd. (Beijing, China), followed by a reverse transcription by a QuantScript RT Kit (Tianjin, Beijing, China) to synthesis the first strand of complementary DNA (cDNA). The cDNAs were then subjected to real-time PCR amplification using a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), the TaqMan gene expression assay kit for miR-21 (catalog number 000397) and the internal control cel-miR-39 (catalog number 000200) were obtained from Applied Biosystems (Foster City, CA, USA). The relative expression level of miR-21 was determined by 2-DD
cT method.

Statistical Analysis

Data were expressed as means ± SD, the baseline characteristics were expressed as means or medians. The levels of miR-21 between groups of healthy control, AP and AMI were compared with one-way ANOVA followed by Tukey’s test. The means between two groups were compared with student’s t-test. The categorical variables were compared with χ2 test. Correlations between miR-21 and biochemical parameters were performed with Pearson correlation analysis. Receiver-operator characteristic (ROC) analyses were plotted by Graphpad Prism 5.0 and the areas under the curve (AUC) were calculated. A two tailed p value less than 0.05 was taken as statistical significance.

Results

Clinical characteristics and parameters of study population

Since miR-21 has been shown to be involved in lipid metabolism18,19, to exclude the possible factors that are able to influence its expression, we compared general characteristics and clinical parameters in control group, AP group and AMI group. No statistical difference was observed regarding age, sex, life habits and medical history (p>0.05); additionally, no difference in basic clinical parameters was found (p>0.05). The detailed data were shown in Table I.

The plasma level of miR-21 in healthy control and patients

We next sought to compare the difference of miR-21 levels between groups. As displayed in
Table I. Comparison of the general characteristics of patients with different clinical types.

<table>
<thead>
<tr>
<th>Project</th>
<th>control group (n=10)</th>
<th>AP group (n=39)</th>
<th>AMI group (n=17)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>5 (50)</td>
<td>32 (82.05)</td>
<td>12 (70.59)</td>
<td>0.109</td>
</tr>
<tr>
<td>Smoking history (%)</td>
<td>3 (30)</td>
<td>22 (56.41)</td>
<td>12 (70.59)</td>
<td>0.125</td>
</tr>
<tr>
<td>Drinking history (%)</td>
<td>3 (30)</td>
<td>6 (35.90)</td>
<td>8 (47.06)</td>
<td>0.637</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>4 (40)</td>
<td>25 (64.10)</td>
<td>11 (68.75)</td>
<td>0.361</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>1 (10)</td>
<td>11 (28.21)</td>
<td>4 (23.53)</td>
<td>0.498</td>
</tr>
<tr>
<td>Family medical history (%)</td>
<td>3 (30)</td>
<td>16 (41.03)</td>
<td>6 (35.29)</td>
<td>0.812</td>
</tr>
<tr>
<td>age (year)</td>
<td>56.2±5.5</td>
<td>59.7±8.4</td>
<td>62.8±7.8</td>
<td>0.116</td>
</tr>
<tr>
<td>BMI (kg/m)</td>
<td>25.248±1.803</td>
<td>25.249±2.296</td>
<td>24.904±1.778</td>
<td>0.157</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>2.311±1.414</td>
<td>2.118±1.643</td>
<td>1.852±0.8601</td>
<td>0.697</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>4.956±1.147</td>
<td>4.457±1.125</td>
<td>5.054±1.105</td>
<td>0.153</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.178±0.307</td>
<td>1.109±0.198</td>
<td>1.161±0.261</td>
<td>0.605</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.886±0.891</td>
<td>2.530±0.929</td>
<td>3.196±0.914</td>
<td>0.050</td>
</tr>
<tr>
<td>ApoA1 (g/l)</td>
<td>1.202±0.162</td>
<td>1.132±0.247</td>
<td>1.170±0.278</td>
<td>0.679</td>
</tr>
<tr>
<td>ApoB (g/l)</td>
<td>1.023±0.278</td>
<td>0.931±0.264</td>
<td>1.119±0.285</td>
<td>0.062</td>
</tr>
<tr>
<td>HCY (μmol/L)</td>
<td>12.345±4.479</td>
<td>15.872±7.387</td>
<td>14.218±6.393</td>
<td>0.271</td>
</tr>
<tr>
<td>GLU (mmol/L)</td>
<td>5.892±1.049</td>
<td>6.376±1.931</td>
<td>7.486±2.075</td>
<td>0.337</td>
</tr>
</tbody>
</table>

Figure 1, we found that the relative expression levels of circulating miR-21 in AP and AMI group were both significantly higher than that in control group. Particularly, patients with AMI had a more pronounced elevation compared with AP patients (p<0.05), suggesting its potential value in predicting coronary heart disease.

**Association between plasma miR-21 and conventional biomarkers**

Myocardial enzymes were also analyzed, as shown in Figure 2, plasma levels of CK, CKMB and cTnI were all significantly higher in AMI group compared with the AP or control group. These parameters were found to be significantly correlated with plasma miR-21 level in the study subjects (p<0.001) (Figure 3).

**Plasma miR-21 is a potential diagnostic marker of AMI**

Since we observed the correlations between plasma miR-21 and the conventional biochemical markers of myocardium injury, we then performed ROC analysis to evaluate the diagnostic power of circulating miR-21. Comparisons of the plasma miR-21 levels in control group and AMI group were performed. As displayed in Figure 4, the AUC of miR-21 was 0.892 (95% confidence interval: 0.823-0.961, p<0.05), which was comparable to the parameters of CK and CKMB. This result suggested that circulating miR-21 might be an independent or a complementary biomarker for AMI.

**Discussion**

In the current study, we supported the potential clinical value of circulating miR-21 as novel a biomarker for AMI. We showed that plasma miR-21 level was greatly elevated in patients with AMI; and more importantly, high correlations between certain indicators of myocardium injury and this microRNA were found. ROC analysis further highlighted its diagnostic functions in AMI. Taken together, our study demonstrates that circulating miR-21 might be a useful biomarker for the diagnosis of AMI, and reinforces the emerging concept that integrates the
application of circulating microRNAs in disease diagnosis and prognosis.

Since heart muscle is vulnerable and irreversible after ischemic injury, and AMI is still the main cause of the mortality for patients with cardiovascular complications, the precise management for acute coronary artery syndrome is of vital importance. Blood biochemical markers such as LDH, CK, CKMB and cTnI remain the golden standards for assessing the severity of myocardium injury in clinical practice. However, several enzymes such as CK isoform and CKMB have been proven to be outdated due to the non-specificity, low sensitivity or imprecise cut-off value. Besides, with the fact that most of the existing markers are enzymes and proteins, it is of considerable challenge to develop new protein based assay protocols for rapid detection. The discovery of the microRNAs not only represents a great revolution in biomedical research but also opens a new era in the field of clinical diagnosis. Numerous studies demonstrated that circulating microRNAs are important indicators of various diseases, especially in the heart. For example, miR-10a, miR-31, miR-92a, and miR-155 have been identified as biomarkers of heart transplant rejection, which might represents a novel method for non-invasive assessment of rejection response. The concept that microRNAs are promising as biomarkers probably lies on the advantages of its quantitative PCR (qPCR) based detection method. Unlike the enzyme reactions or ELISA, qPCR offers high reliability and sensitivity with little amount of specimen required. Moreover, the assay protocol can be generalized for other microRNAs, which would represent a more efficient solution for the accurate diagnosis of diseases including cardiovascular complications.

Figure 2. The plasma level of CK, CKMB and cTnI in different groups. *p<0.05 vs. control group, #p<0.05 vs. AP group.

Figure 3. The correlation of circulating miRNA-21 with cardiac parameters in the study subjects.
It is believed that miR-21 expresses at very low level in normal myocardium, and its strong elevations were found to play critical roles in settings of ischemic pathological changes such as hypertrophy and heart failure\textsuperscript{13}. Although this work has been challenged by a study demonstrating no involvement of miR-21 in the setting of cardiac stress\textsuperscript{25}, several papers have still identified miR-21 as a multifunctional molecule that is implicated in a lot of critical processes. For example, miR-21 has been reported to have higher level in the border zone, whereas exhibited decreased expression in infarcted area\textsuperscript{26}. A recent animal study\textsuperscript{11} has also proposed a protective effect of miR-21 on heart ischemia-reperfusion (IR) injury. Such discrepancy indicates that the role of miR-21 might be context dependent. Our study, which reports the elevation of plasma levels of miR-21 in AMI patients, is in agreement with the Northern blot and \textit{in situ} hybridization data obtained from heart biopsy of MI patients in the previous study\textsuperscript{26}. Just as notably, we found that miR-21 was also differentially presented in the blood of patients with different extent of

Figure 4. The ROC curves of different parameters.
coronary artery stenosis; and more notably, by means of ROC analysis, we identified that miR-21 exhibited similar diagnostic accuracy with traditional markers including CK, CKMB and cTnI. Given the aforementioned disadvantages of the traditional biochemical markers, we put forward that miR-21 can be used independently or at least complementarily to evaluate the severity of myocardial injury. Our findings, therefore, supported the perspective proposed by a recent study.27

In spite of the recent report showing circulating miR-21 reflects left ventricular fibrosis and the present demonstration that miR-21 could serve as a potential biomarker for AMI by us, one of the key issues lies in the application of miR-21 as a biomarker for AMI is to address its cardiac specificity. As reported by others, miR-21 is involved in multiple organs by targeting numbers of mRNAs and circulating miR-21 has exhibited certain diagnostic power in several types of cancer.29-32 Moreover, miR-21 has been shown to be significantly involved in common comorbidities such as diabetes and pulmonary hypertension.13-35

Conclusions

The application of this microRNA to AMI diagnosis should be accommodated to different complex clinical scenarios because circulating miR-21 originated from other organs may confound the interpretations. It should be noted that our results were obtained from a relatively small population. In future, a study with larger sample size would better validate this issue. Another limitation is that we did not follow the AMI patients after they have discharged, this study thus did not illustrate whether miR-21 have a prognostic function for AMI. Regardless of the limitations, our study highlighted the importance of circulating miR-21 in the diagnosis of AMI. Due to the unique and irreplaceable advantages of the detection system for microRNAs, our study might lay a foundation for the development of other circulating microRNAs for the diagnosis of AMI or other cardiovascular diseases.

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Conflict of Interests

The Authors declare that they have no conflict of interests.

References

miR-21 is a biomarker for AMI


