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Expression of serum microRNA-155 and its clinical importance in patients with heart failure after myocardial infarction

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Abstract

Objective: This study was conducted to investigate the level of microRNA-155 (miRNA-155) in patients with heart failure (HF) after myocardial infarction (MI) and its clinical importance.

Methods: Serum miRNA-155 levels were measured using quantitative reverse transcription (qRT)-PCR. The left ventricular ejection fraction (LVEF), left ventricular posterior wall thickness, and left ventricular end-diastolic diameter were measured by echocardiography. Serum amino-terminal pro-B-type natriuretic peptide (NT-proBNP) and other parameters were also analyzed.

Results: miRNA-155 levels in patients with HF were significantly higher than in control and MI groups. The area under the receiver operating characteristic curve of serum miRNA-155 in the diagnosis of HF after MI was 0.941, the cutoff value was 1.77, sensitivity was 92.73%, and specificity was 92.14%. NT-proBNP levels were significantly higher and LVEF was lower in patients with high versus low miRNA-155 expression.

Conclusions: Patients with HF after MI had elevated miRNA-155 levels and poor cardiac function, suggesting that determining miRNA-155 expression could be used to assess the severity of the disease.

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Myocardial infarction, heart failure, microRNA, ventricular dysfunction, left ventricular ejection fraction, amino-terminal pro-B-type natriuretic peptide

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Introduction

The pathogenesis of heart failure (HF) is complex and clinical symptoms and signs of the disease are often non-specific, resulting in high morbidity, hospitalization rates, and mortality. Additionally, routine examinations are often insufficient for patient evaluation.[1] Therefore, biomarkers are highly desirable as alternative or supplementary tools to diagnose HF.

MicroRNAs (miRNAs) have been shown to mediate a variety of pathophysiological processes in cardiovascular diseases such as coronary atherosclerotic heart disease and HF.[2,3] However, Cruz et al.[4] showed that clinical evaluation remains the most accurate diagnostic method for ischemic HF because known biomarkers have high costs, are difficult to use for early diagnosis, and have low specificity. Nevertheless, a number of miRNAs have been analyzed for use as potential biomarkers for post-myocardial infarction (MI) HF.

miR-155 was previously shown to be expressed in B cells, T cells, and endothelial cells, and to participate in cell proliferation, differentiation, and apoptosis.[5] miR-155 levels were elevated in patients with coronary heart disease,[6] and its inhibition protected against lipopolysaccharide-induced cardiac dysfunction and apoptosis in mouse models.[7] miR-155 has also been reported to be a biomarker for a diverse range of conditions such as cancers,[8,9] male fertility,[10] and hyperandrogenic polycystic ovary syndrome.[11]However, its use as a biomarker for HF is not known, and its levels and clinical importance in patients with HF after MI are unclear.

In this study, we investigated the level of miR-155 in patients with HF and examined its clinical importance in the diagnosis of cardiac function. Our findings provide new tools for the assessment of disease severity and will aid the diagnosis and treatment of patients.

Materials and methods

Study population

Consecutive patients with and without HF after old myocardial infarction (OMI) treated at the Second Xiangya Hospital, Central South University, Changsha from January 2014 to December 2017 as well as healthy volunteers were recruited for the study. Participants were divided into three groups: the HF group, consisting of patients who had HF after OMI; the MI group, consisting of patients who did not have HF after OMI; and the control group of healthy volunteers. Patients in the HF group were diagnosed based on Framingham HF diagnostic criteria.[12] Patients had a history of underlying cardiovascular disease, and experienced fatigue, dyspnea, and lower extremity edema during rest or exercise. They also had symptoms such as shortness of breath, tachycardia, hepatic hydrothorax, increased jugular venous pressure, lung rales, peripheral edema, and hepatomegaly. Some had functional and structural abnormalities such as a heart murmur, third heart sound, enlarged

heart cavity, abnormal echocardiography, and elevated levels of natriuretic peptide, characteristic of systolic or diastolic HF.

The MI group consisted of patients who had acute MI for at least 8 weeks (OMI) but did not develop HF. Patients were excluded if they were pregnant or lactating, younger than 18 years, had severe liver or kidney dysfunction, congenital heart disease, hypertrophic cardiomyopathy, and other cardiac diseases such as aortic dissection, cardiogenic shock, severe arrhythmia, a history of cardiogenic syncope, malignant tumors, and severe infectious diseases (Figure 1).

The study complied with the Declaration of Helsinki and was approved by the institutional ethics committee (approval no. CSU-C1823-3). Written informed consent was obtained from all patients.

Methods

Demographic data and laboratory findings were collected from the hospital's electronic database systems, and included patient age, sex, and records of examination and treatments (Table 1).

To measure serum levels of miRNA-155, peripheral venous blood was collected from participants and mixed with the anticoagulant ethylenediaminetetraacetic acid. This was then centrifuged at $500 \times g$ for 10 minutes to separate the serum. Total RNA

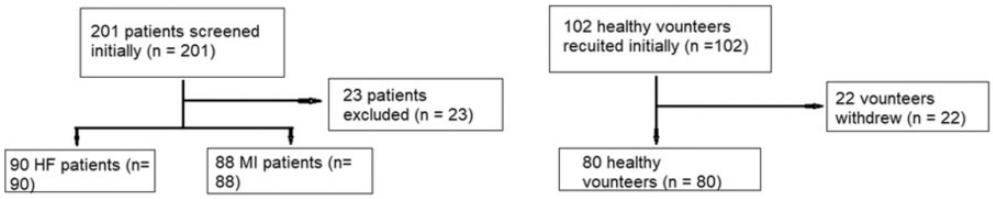

Figure 1. Flow chart of participating patients and volunteers.

Table 1. Baseline characteristics and laboratory findings of subjects and controls.

Characteristics	Heart failure group	Myocardial infarction group	Controls	*P* value
No. of patients	90	88	80	0.423
Male	48	46	41	
Female	42	42	39	
Age (years)	64.31 ± 7.99	62.31 ± 7.39	62.91 ± 6.79	0.423
Body mass index (kg/m^2)	22.91 ± 3.14	22.11 ± 3.18	22.23 ± 3.16	0.782
Fasting blood sugar (mmol/L)	6.39 ± 1.15	6.15 ± 1.95	6.26 ± 1.20	0.223
High-density lipoprotein (HDL) cholesterol (mmol/L)	2.49 ± 0.15	2.55 ± 0.25	2.16 ± 0.22	0.123
Total Cholesterol/HDL ratio	4.41 ± 1.75	4.21 ± 1.85	4.11 ± 1.70	0.123
Creatinine (μmol/L)	98.41 ± 22.75	91.41 ± 20.75	96.41 ± 21.75	0.223
Triglyceride (mmol/L)	1.11 ± 0.55	1.71 ± 0.15	1.66 ± 0.25	0.423
Smoker (%)	18.3	19.3	18.7	0.223
Systolic blood pressure (mmHg)	126.44 ± 17.80	127.44 ± 19.80	121.44 ± 17.80	0.132
Diabetes (%)	13.9	14.7	14.1	0.432
Hypertension (%)	10.9	11.7	10.1	0.563
Serum miRNA-155	1.05 ± 0.13	1.46 ± 0.17	1.89 ± 0.14	0.000

was extracted using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA), and reverse-transcribed into cDNA for quantitative (qRT)-PCR using a SYBR® Green RT-qPCR kit according to the manufacturer's instructions (Sigma-Aldrich, St Louis, MO, USA). miRNA-155 primers were: forward 5′-TGCCTCG AACTGCACTCGTAG-3′ and reverse 5′-GCGAGCAGAATAATAGGAC-3′. U6 was used as an internal reference. PCR conditions were: pre-denaturation at 95°C for 5 seconds, followed by 40 cycles of denaturation at 60°C for 20 seconds, annealing at 62°C for 1 second, and a final extension at 72°C for 1 second. Data were managed using the RQ Manager v1.2.1 software (Applied Biosystems, Foster City, CA, USA). Relative expression was calculated using the comparative Ct method and obtaining the fold-change value ($2-\Delta\Delta Ct$) according to a previously described protocol.[13]

The left ventricular ejection fraction (LVEF), left ventricular posterior wall thickness (LVPW), and left ventricular end diastolic diameter (LVDd) were measured using the iE 33 ultrasound system (Philips Health care, Best, the Netherlands) and were determined as linear anteroposterior dimensions that could be obtained in M-mode or 2D in the parasternal long-axis view. The LVEF was measured by echocardiography, radionuclide ventriculography, cardiac magnetic resonance, or LV angiography. Blood samples were drawn from all participants for measurements of serum creatinine, amino-terminal pro-B-type natriuretic peptide (NT-proBNP), and other parameters.

Statistical analysis

The normality of distribution of continuous variables was tested by the one-sample Kolmogorov–Smirnov test. Continuous variables, presented as means ± standard deviations, were compared using the t-test or variance analysis. The LSD-t test was used for paired comparisons. Categorical variables were compared by the χ^2 test. The Pearson correlation was used for numerical data, and the Spearman correlation was used for nominal data. Optimal dichotomous values were identified using receiver operating characteristic (ROC) curve analysis, and the area under the ROC curve was calculated to examine the discriminatory value of miRNA-155 levels. A value of $P < 0.05$ was considered to be statistically significant.

Results

Serum miRNA-155 level

There were 258 participants in the study, including 90 HF patients (48 men and 42 women, with an average age of 64.31 ± 7.99 years), 88 MI patients (46 men and 42 women, with an average age of 62.31 ± 7.39 years), and 80 healthy controls (41 men and 39 women, with an average age of 62.91 ± 6.79 years) (Figure 1). As described above, HF and MI patients were those who did or did not have HF following at least 8 weeks of MI. Table 1 shows that demographic characteristics such as sex, age, body mass index, fasting blood sugar levels, percentage of smokers, and patients with diabetes or hypertension were similar among the study groups. Levels of high density lipoprotein (HDL) cholesterol, the cholesterol/HDL ratio, creatinine, triglyceride, and systolic blood pressure also did not differ significantly among groups. Average serum miRNA-155 levels were 1.05 ± 0.13, 1.46 ± 0.17, and 1.89 ± 0.14 in control, MI, and HF groups, respectively. These differences were significant ($F = 682.162$, $P = 0.000$), with levels being significantly higher in the HF than the MI group ($P < 0.05$) and in the MI than the control group ($P < 0.05$). Multivariate regression

analysis showed that other factors listed in Table 1 were not significantly correlated with the expression of miRNA155.

Diagnostic value of serum miRNA-155

We next calculated the diagnostic value of miRNA-155 levels for HF. ROC curve analysis showed that the area under the curve (AUC) was 0.941 (95% confidence interval [CI]: 0.852, 0.973), and the cutoff value was 1.77 (95% CI: 0.852, 1.963). At the optimal cutoff point, the sensitivity and specificity of diagnosis were 92.73% (95% CI: 0.575, 0.964) and 92.14% (95% CI: 0.792, 0.976). A total of 57 and 31 patients were above and below the cutoff, respectively (Figure 2).

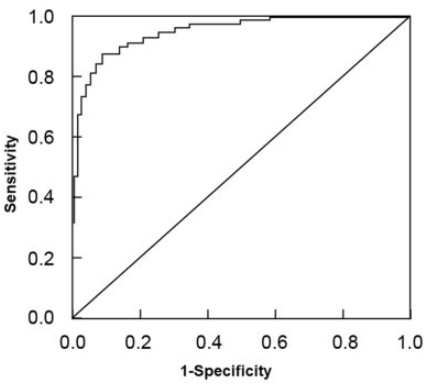

Figure 2. Receiver operating characteristic curves by miR-155 for predicting heart failure after old myocardial infarction.

Relationship between serum miRNA-155 and HF-related parameters

Analysis of the relationship between serum and HF-related parameters revealed no significant difference in age, sex, fasting blood sugar, HDL cholesterol, low-density lipoprotein cholesterol, creatinine, or triglyceride between patients with high and low miRNA-155 expression. However, the level of NT-proBNP was significantly higher in patients with high versus low miRNA-155 levels (Table 2, $P < 0.000$). Correlation analysis also showed that miR-155 levels were positively related to NT-proBNP (Table 3).

Relationship between serum miRNA-155 and ultrasonography findings

In patients with high versus low miRNA-155 expression, LVEF was significantly lower ($P < 0.00$) while LVDd was significantly higher ($P < 0.00$). LVPW was similar between the two groups (Table 4). Correlation analysis showed that miR-155 levels were negatively related to LVEF and positively related to LVDd (Table 3).

Relationship between serum miRNA-155 and cardiac function grading

The χ^2 test showed that there was higher percentage of grade III and IV patients with high than low miRNA-155 level ($\chi^2 = 23.854$, $P = 0.000$, Table 4). Correlation analysis

Table 2. Comparison of baseline characteristics and laboratory findings between patients with high and low miRNA-155 levels.

miR-155 level	*n*	Age (years)	Male/ female	FPG (mmol/L)	HDL-C/ (mmol/L)	LDL-C/ (mmol/L)	Cr/ (mmol/L)	TG/ (mmol/L)	BNP/ (pg/L)
≥1.77	57	63.24 ± 7.31	35/22	6.28 ± 1.56	1.11 ± 0.27	2.42 ± 0.77	92.13 ± 12.46	1.06 ± 0.31	5314.32 ± 636.24
<1.77	31	63.38 ± 7.26	19/12	5.97 ± 1.83	1.02 ± 0.26	2.53 ± 0.81	88.54 ± 13.41	1.14 ± 0.26	4215.47 ± 642.75
t/x^2 value		0.083	0.036	0.802	1.452	0.603	1.205	1.174	7.399
P value		0.934	0.849	0.425	0.150	0.548	0.232	0.244	0.000

FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Cr, creatinine; TG, triglyceride; BNP, B-type natriuretic peptide.

Table 3. Relationship between miR-155 level and cardiac parameters.

Variable	r^2	*P* value
Left ventricular ejection fraction	–0.762	0.031
Left ventricular posterior wall thickness	0.223	0.652
Left ventricular end-diastolic diameter	0.613	0.021
High density lipoprotein cholesterol	0.112	0.780
Cholesterol/HDL ratio	0.109	0.456
Creatinine	0.334	0.125
Triglyceride	0.217	0.221
Amino-terminal pro-B-type natriuretic peptide	0.782	0.028
Cardiac function grading	0.633	0.049

HDL, high-density lipoprotein

Table 4. Comparison of ultrasonography findings between patients with high and low miRNA-155 levels.

miR-155 level	*n*	Left ventricular ejection fraction (%)	Left ventricular posterior wall thickness (mm)	Left ventricular end-diastolic diameter (mm)
≥1.77	57	28.78 ± 1.87	11.53 ± 1.67	71.24 ± 8.57
<1.77	31	32.12 ± 1.94	10.94 ± 1.82	54.35 ± 8.46
t/x^2value		7.577	1.470	8.513
P value		0.000	0.146	0.000

Table 5. Comparison of cardiac function grading between patients with high and low miRNA-155 levels.

miR-155 level	*n*	No. (%) of cardiac patients	
		I and II grade	III and IV grade
≥1.77	57	21 (36.8)	36 (63.1.59)
<1.77	31	26 (83.9)	5 (16.1)

showed that miR-155 level was positively related to cardiac function grading (Table 5).

Discussion

Coronary heart diseases are the main cause of death in middle-aged and older people. Among them, acute MI can be very serious, but with the development of angioplasty and new drugs, most patients can be treated effectively to avoid HF. However, in some patients, ventricular remodeling is poor, leading to an increased chance of HF after MI.[14] Therefore, early diagnosis and treatment are important. Currently, clinical tools and methods to assess the severity of HF include ultrasound, electrocardiogram, VEF, walking tests, and endurance exercise. Because of atypical clinical symptoms and signs, however, these conventional methods may be ineffective or insufficient to assess the severity of the condition, so non-invasive diagnostic biomarkers are highly desirable.

miR-155 is an anti-oncogene that is abnormally expressed in many malignant tumors.[15,16] It also plays an important role in hematopoiesis and immune regulation,[17,18] promotes pulmonary fibrosis,[19] and was shown to be involved in the pathogenesis of coronary heart disease by

regulating macrophage apoptosis.[20] In the present study, we showed that miR-155 levels were significantly elevated in HF after MI. miRNA levels were previously found to be up- or down-regulated depending on the disease condition,[21] with miR-296-5p expression significantly down-regulated in patients with hypertension and up-regulated in those with white coat hypertension compared with the normotensive group. Additionally, some circular RNAs were reported to have a protective role in pathological hypertrophy and HF by targeting miRNA. For example, miR-223 was shown to be target of a circular RNA, and its down-regulation resulted in attenuated hypertrophic responses.[22] However, the molecular mechanisms of miR-155 in cardiac function are unclear.

NT-proBNP is secreted by cardiac myocytes in response to excessive distension and stretching of the cardiac wall, and has a half-life of about ~90 minutes (range, 60–120 minutes).[23] It is regarded as the best biomarker for evaluating the severity of HF, showing high sensitivity and reliability, with high levels indicating faster progression of HF[24]. Our finding from ROC curve analysis that serum miRNA-155 has an AUC of 0.941 in the diagnosis of patients with HF after OMI suggests that miRNA-155 is comparable in its ability to predict HF post-MI to NT-proBNP. Although miRNA-155 was shown to reduce cardiac injury by inhibiting the nuclear factor κB pathway during acute viral myocarditis,[25] and appears to be involved in fetal and adult cardiac remodeling,[26] its mechanism in HR remains unknown. Furthermore, our findings should be validated in a more diverse patient population.

LVEF is an important indicator in the diagnosis of HF, which sensitively responds to the functionality of patient ventricles. An LVEF >50% represents poor cardiac function,[27] but this is inconsistent with our current results that LVEF correlated negatively with miR-155 levels. LVDd is also used to evaluate cardiac function, and elevated levels of LVDd suggest a decreased cardiac function.[28] Higher cardiac grading reflects a poor overall cardiac function status.[29] The results of our study indicate that miR-155 levels are associated with these cardiac function parameters, suggesting that it has diagnostic value for HF after OMI. However, we did not find different LVPW values in patients with varying miRNA-155 levels, although this may reflect the limited sample size.

Other limitations of our study include the fact that all patients were Chinese. Future work would therefore involve a larger patient sample with diversified disease conditions. Second, we did not fully compare the predictive function of miRNA-155 with classical methods in a clinical setting.

Conclusion

We have demonstrated that serum miRNA-155 has diagnostic value for HF after MI, with elevated levels of miRNA-155 suggesting a higher chance of HF. Therefore, miRNA-155 has the potential to be used as a marker for the diagnosis of disease and assessment of its severity. However, because our results were obtained from a single center, they should be validated in a larger, multi-center randomized study.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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