European Review for Medical and Pharmacological Sciences 2017; 21: 4649-4653

# Study on the expression and mechanism of plasma microRNA-21 in patients with ischemic cardiomyopathy

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**Abstract. – OBJECTIVE: To investigate the expression and mechanism of plasma microRNA-21 in patients with ischemic cardiomyopathy (ICM).**

**PATIENTS AND METHODS: 56 cases of ICM patients were selected in our hospital from February 2010 to March 2016 as the observation group, and 60 cases of healthy patients were selected as control group. Real-time quantitative polymerase chain reaction (qRT-PCR) was used to detect the expression of microRNA-21 in two groups. Then, differences of the total cholesterol (TC), triglycerides (TG), left ventricular ejection fraction (LVEF), high and low density lipoprotein cholesterol (HDL-C, LDL-C), left ventricular end-diastolic volume (LVEDV), N terminal B type brain natriuretic peptide (NT-proBNP) and other clinical indicators of the two groups, were compared. The correlation between the plasma microRNA-21 level and the clinical indices was analyzed, and the value of microRNA-21 in the diagnosis and treatment of ICM was evaluated.**

**RESULTS: The levels of LDL-C, HDL-C and LVEF in the observation group were lower than those in the control group ($p<0.05$). Plasma microRNA-21, TG, NT-proBNP and LVEDV were higher than those in the control group; the difference was statistically significant ($p<0.05$). Logistic regression analysis showed that the plasma microRNA-21 level was positively correlated with NT-proBNP and LVEDV ($p<0.05$).**

**CONCLUSIONS: The expression of microRNA-21 in plasma of patients with ICM was significantly increased. And the expression of micro RNA-21 in plasma was positively correlated with NT-proBNP and LVEDV. Through the ventricular remodeling in ICM patients, it can be used as a new target for the diagnosis and treatment of ICM and a new biomarker for risk assessment.**

Key Words:
Ventricular remodeling, N terminal B type natriuretic peptide, Ischemic cardiomyopathy, MicroRNA-21.

## Introduction

Due to lack of myocardial blood supply, ischemic cardiomyopathy (ICM) is considered to cause myocardial tissue dystrophy, atrophy or a large area of infarction and fibrous tissue hyperplasia of myocardial dysfunction disease[1-3]. ICM is also the most common cause of chronic heart failure. With the aging of the population in recent years, ICM has become the major cause of chronic heart failure in China[4-6]. MicroRNA-21 is widely distributed in a variety of organizations. Previous reports showed that the expression level of microRNA-21 changed in various cardiovascular diseases, and was increased level in myocardial remodeling induced by myocardial ischemia, hypertrophy and fibrosis, and participated in the regulation of a variety of cardiovascular diseases[7]. The purpose of this work was to investigate the expression of microRNA-21 in ICM patients and its role in the pathophysiology of ICM.

## Patients and Methods

### Patients

56 cases of ICM patients were selected in our hospital (Hangzhou Red Cross Hospital, Hangzhou, Zhejiang, China) from February 2010 to March 2016 as the observation group. 60 cases of healthy patients were selected as control group. ICM diagnoses were referring to the Guidelines for Cardiovascular Social Consensus Conference (2009)[8].

Inclusion criteria: 1. NYHA cardiac function classification was grade III and IV; 2. Coronary angiography displayed that the main coronary artery and the branches ≥50% stenosis of more than 1 branch; 3. The expansion of the heart and LVEF ≤ 40%.

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Exclusion criteria: 1. Coronary heart disease and its complications; 2. Heart enlargement and heart failure caused by other heart disease; 3. ICM history was unknown, and occurrence of malignant tumors, autoimmune disease, infection, severe liver and kidney dysfunction, cerebrovascular disease and blood system diseases.

Two groups of patients signed informed consent and the Ethics Committee approval was granted by our hospital. Control group had: 29 male cases, 31 female cases, age range of 45-76, average (69.5±8.7) years old, 13 patients with smoking history, 1 patient with diabetes mellitus, average diastolic pressure (73.5±8.7) mmHg, average systolic pressure (119.5±6.7) mmHg. Observation group had: 25 male patients, 31 female patients, age range of 44-73, average (67.8±8.6) years old, 14 patients with smoking history (25%), 5 patients with diabetes mellitus, average diastolic pressure (73.6±8.5) mmHg, average systolic pressure (119.6±6.5) mmHg. There was no significant difference between the two groups in the clinical data such as diabetes, gender, blood pressure, age, smoking history ($p>0.05$), as shown in Table I.

## Methods

### Major Reagents and Appliances

RAN extraction reagent was TRIR reagent BD purchased from MRC Gene (Cincinnati, OH, USA); Automatic biochemical analyzer was purchased by Roche C8000 (South San Francisco, CA, USA); qRT-PCR was from TaKaRa (Dalian, Liaoning, China); 500 Fast Real-time PCR (ABI Company, Vernon, CA, USA); UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

### Specimen Collection and Detection

After the admission of the patient, 4 mL fasting venous blood was taken in the morning. The TC, TG, HDL-C, LDL-C and other lipid profiles were determined by the Roche colorimetric assay. The level of NT-proBNP was determined by Roche enhanced chemiluminescence (ECL). The LVEDV and LVEF were determined by cardiac color Doppler ultrasound (Thermo Fisher Scientific, Waltham, MA, USA).

### Determination of microRNA-21 Expression by qRT-PCR

Blood samples were centrifuged and 200 µl plasma were obtained, TRIR reagent BD was used to extract total RNA which was then tested by the photometer (Thermo Fisher Scientific, Waltham, MA, USA) for the purity. The absorbance ratio was selected from 1.8 to 2.1, and the samples were prepared for subsequent experiments. RNA reverse transcription was performed in strict accordance with the RT reverse transcription kit (Ruibo Company, Guangzhou, Guangdong, China) specification for cDNA operation. The total reaction system of qRT-PCR was 20 µl. The loop parameters were set to 95ºC 30 s, 95ºC 3 s, 60ºC 30 s, for a total of 40 loops. U6 was used as the reference gene, 7500 Fast System SDS was used for calculating the Ct value, and the calculation method was $2^{-\Delta\Delta Ct}$.

### Statistical Analysis

Statistical analysis was using SPSS18.0 software (SPSS Inc., Chicago, IL, USA), measurement data was expressed by`x±s. The t-test was used between groups. Count data was expressed in n %, using the c2-test. Logistic regression analysis was used to analyze the correlation between the clinical parameters and the expression level of

**Table I.** Comparison of general data.

| Group | f/m | Age (yr) | Smoking history | Diabetes mellitus | Diastolic pressure (mmHg) | Systolic pressure (mmHg) |
|---|---|---|---|---|---|---|
| Control | 29/31 | 69.5±8.7 | 13 | 1 | 73.5±8.7 | 119.5±6.7 |
| Observation | 25/31 | 67.8±8.6 | 14 | 5 | 73.6±8.5 | 119.6±6.5 |
| $t/x^2$ | 0.159 | 1.057 | 0.180 | 3.114 | -0.063 | -0.081 |
| *p* | 0.690 | 0.293 | 0.671 | 0.078 | 0.950 | 0.935 |
| Mean | 1.8 | 34.3 | 2.3 | 5.8 | | |
| Range | 0-19.5 | 0-287.1 | 0-16.1 | 0-44.8 | | |

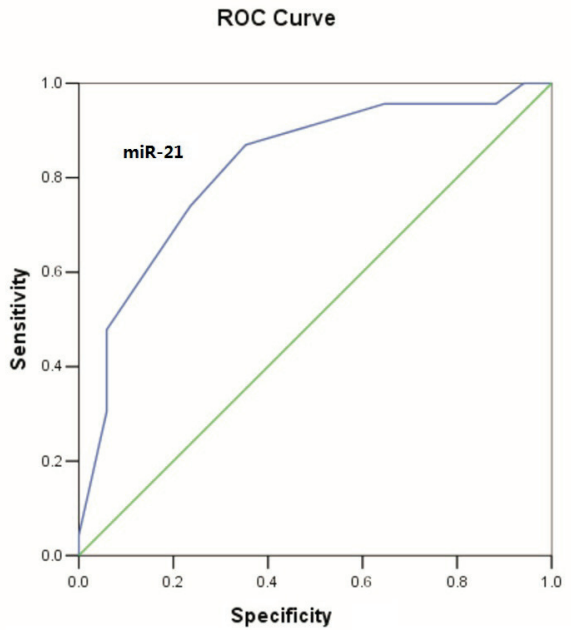


**Figure 1.**

microRNA-21. With $p<0.05$, the difference was statistically significant.

## Results

### Comparison of Serum Lipids, Cardiac Function and microRNA-21 Expression in two Groups

The LDL-C, HDL-C and LVEF in the observation group were lower than those in the control group ($p<0.05$), Plasma microRNA-21, TG, NT-proBNP, and LVEDV were higher than those in control group. The differences were statistically significant ($p<0.05$), as shown in Table II.

### Logistic Regression Analysis

Logistic linear regression analysis showed that the plasma microRNA-21 level was positively correlated with NT-proBNP and LVEDV in the observation group ($p<0.05$), as shown in Table III.

### ROC Curve Analysis

The sensitivity and specificity were analyzed by plotting ROC curve. Our results showed that the sensitivity was 0.870 while the specificity was 0.765. The Youden index was 0.696. Moreover, we calculated the AUCROC, which suggested that miR-21 was of good diagnostic value (AUCROC=0.877) (Figure 1).

## Discussion

ICM is a severe myocardial dysfunction caused by coronary artery occlusion and long-term myocardial ischemia. Myocardial ischemia can result in the decrease of myocardial oxygen supply, necrosis, and apoptosis of myocardial cells, serious lack of energy metabolism substrate, myocardial stunning or hibernation. Moreover, it causes as well as secondary myocardial cell reduction, large area of infarction after the increase of fibrous tissue and pathological ventricular remodeling[9,10]. Although in recent years there were good effects in the clinical treatment of ICM, the prognosis of the severe potential cardiovascular complications (such as ischemic heart failure and stroke) was still insufficient[11,12]. Only a few researches brought up new methods of treatment. Therefore, the treatment of ICM has always been the hotspot. Cardiovascular disease included a variety of mechanisms, as in the latest discovery of microRNAs suggested that they are involved in the occurrence of cardiovascular diseases[13]. MicroRNA, which is a type of single-stranded small molecule RNA formed by the non-coding region, is widely found in plants and animals[14]. A large number of papers have shown that MicroRNAs can participate in many physiological and pathological processes by regulating the target gene[15,16]. MicroRNA-21 is one of the most widely studied microRNAs, and is highly expressed in all tissues, playing an important role in pathogenesis of cardiovascular disease. Previous studies[17] have shown that the miRNAs are closely related to the pathogenesis of

**Table II.** Comparison of serum lipids, cardiac function and microRNA-21 expression in two groups ($\bar{x}\pm s$).

| Group | LDL-C (mmol/l) | HDL-C (mmol/l) | TG (mmol/l) | TC (mmol/l) | microRNA-21 | NT-proBNP (ng/l) | LVEDV (ml) | LVEF (%) |
|---|---|---|---|---|---|---|---|---|
| Control | 2.66±0.34 | 1.47±0.63 | 0.89±0.59 | 4.19±0.21 | 4.21±1.19 | 187.63±49.13 | 112±22 | 60±15 |
| Observation | 2.18±0.36 | 1.08±0.68 | 1.43±0.58 | 4.22±0.25 | 8.09±1.01 | 833.77±50.74 | 124±22 | 30±12 |
| *t* | 7.385 | 3.207 | 4.966 | 0.702 | 18.867 | 69.671 | 2.936 | 11.841 |
| *p*-value | 0.000 | 0.002 | 0.000 | 0.484 | 0.000 | 0.000 | 0.000 | 0.000 |

**Table III.** Multivariate logistic regression analysis.

| Program | OR | *p*-value | Wald | 95% CI |
|---|---|---|---|---|
| NT-proBNP | 1.897 | 0.031 | 0.069 | 0.413-2.052 |
| LVEDV | 2.433 | 0.016 | 10.423 | 1.407-4.452 |
| LVEF | 1.676 | 0.051 | 1.355 | 0.311-1.792 |
| LDL-C | 0.387 | 0.611 | 0.083 | 0.383-1.975 |
| HDL-C | 0.467 | 0.603 | 8.098 | 2.419-12.154 |
| TG | 1.092 | 0.078 | 3.128 | 1.234-6.129 |

ICM, such as miR-126, miR-130a, etc. Van Rooij et al[18] observed the expression of microRNAs in different parts of the heart of acute myocardial ischemia mice and patients. The results indicated that the expression level of microRNA-21 was significantly increased in the ischemic border area, and non ischemic area of the experimental mice and patients. Subsequently, Dong et al[19] confirmed the findings. Furthermore, it was found that the expression level of microRNA-21 was decreased in the ischemic region, and the expression of microRNA-21 by virus transfection can reduce the ischemic area and the degree of heart failure after 2 weeks. Sayed et al[20] suggested that in transgenic mice and the wild-type mice of over expression of cardiac-specific microRNA-21 and after the treatment of myocardial ischemia. The myocardial ischemia area of the transgenic mice and the symptoms of heart failure after 4 weeks of ischemia were significantly lower than those of the wild-type mice. Qin et al[21] showed that microRNA-21 can decrease the myocardial apoptosis induced by myocardial ischemia reperfusion and slow down the process of ventricular remodeling. MicroRNA-21 played an important role in cardiac remodeling induced by myocardial hypertrophy, ischemia, and fibrosis. This work suggested that the levels of LDL-C, HDL-C and LVEF in the observation group were significantly lower than those in the control group, and the levels of TG, NT-proBNP, microRNA-21 and LVEDV were significantly higher than those in the control group. Thus, ICM patients all had conditions of dyslipidemia and cardiac remodeling. Nevertheless, the correlation analysis showed that microRNA-21 was positively correlated with NT-proBNP and LVEDV in the observation group, which indicated that microRNA-21 may be involved in ICM ventricular remodeling and used to control the process of ventricular remodeling, which is an important anatomic marker of ICM ventricular remodeling. All analysis results were consistent with the experimental results of the current researches[22,23].

## Conclusions

The expression of microRNA-21 in plasma of patients with ICM was significantly increased, and the expression of LVEDV was positively correlated with NT-proBNP and LVEDV. MicroRNA-21 plays a role in ventricular remodeling in patients with ICM and serves as a new target for the diagnosis and treatment of ICM and a new biomarker for risk assessment.

### Conflict of interest

The authors declare no conflicts of interest.

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