Correlation of serum miR-148b expression with myocardial injury and myocardial fibrosis in patients with myocardial infarction

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ABSTRACT
Objective: To study the correlation of serum miR-148b expression with myocardial injury and myocardial fibrosis in patients with myocardial infarction.
Methods: A total of 130 patients who were diagnosed with acute myocardial infarction and 100 healthy subjects who received physical examination in Hanzhong Central Hospital between March 2013 and October 2016 were selected and enrolled in AMI group and control group respectively. Serum was collected, fluorescent quantitative PCR kit was used to detect miR-148b expression, and enzyme-linked immunosorbent assay kit was used to detect the contents of myocardial injury markers and myocardial fibrosis markers.
Results: Serum miR-148b expression as well as CK-MB, cTnT, H-FABP, PICP, PIIINP, CTX-I, TGF-β1 and GDF-15 levels in AMI group was significantly higher than those in control group; serum CK-MB, cTnT, H-FABP, PICP, PIIINP, CTX-I, TGF-β1 and GDF-15 levels in AMI patients with high miR-148b expression were significantly higher than those in AMI patients with low miR-148b expression.
Conclusion: Highly expressed miR-148b in serum of patients with myocardial infarction can promote myocardial injury and myocardial fibrosis.

1. Introduction
Myocardial infarction is a common disease of cardiovascular system, coronary atherosclerosis is the pathological basis of the disease, and atheromatous plaque rupture, thrombosis and lumen stenosis can cause myocardial ischemic hypoxic injury. Although thrombolytic therapy and interventional therapy have developed rapidly in recent years and patients are able to receive reperfusion therapy in a timely manner after myocardial infarction, there will be different degrees of cardiac hypofunction after treatment[1,2]. The current research on cardiac hypofunction after myocardial infarction shows that the myocardial cell damage caused by ischemia hypoxia and ischemia reperfusion will directly affect the myocardial diastolic and systolic function, and the myocardial fibrosis after the restore of blood supply will also significantly affect the myocardial diastolic and systolic function. Myocardial injury and myocardial fibrosis in myocardial infarction are regulated by complex factors, and it has been proven that miR-148b can influence myocardial fibrosis in myocardial infarction animal model[3,4]. In the following study, the correlation of serum miR-148b expression with myocardial injury and myocardial fibrosis in patients with myocardial infarction was specifically analyzed.

2. Subjects and methods
2.1 General information of research subjects
A total of 130 patients who were diagnosed with acute myocardial infarction and 100 healthy subjects who received physical examination in Hanzhong Central Hospital between March 2013 and
October 2016 were selected as subjects. All patients with myocardial infarction were diagnosed by clinical symptoms, myocardial enzyme spectrum and electrocardiogram, with disease attack for the first time and included in the AMI group of the research; all healthy subjects were proven healthy after physical examination, without history of coronary heart disease, myocarditis, cardiac valvular disease and so on, and included in the control group of the research. There were 71 men and 59 women in the AMI group, and they were 43-63 years old; there were 58 men and 42 women in the control group, and they were 42-60 years old. There was no significant difference between the two groups of subjects (P>0.05).

2.2 miR-148b expression detection

5-6 mL of cubital venous blood was collected from AMI group of patients on admission, 5-6 mL of cubital venous blood was collected from the control group during physical examination, the blood was centrifuged to separate serum, serum miRNA extraction kit was used to separate total miRNA, the dedicated miRNA reverse transcription kit was used for cDNA synthesis, miR-148b primers were designed at last for PCR amplification, and U6 gene was used as reference to calculate miR-148b expression.

2.3 Serum myocardial injury and fibrosis marker detection

Serum samples were taken from the AMI group and control group, and the contents of CK-MB, cTnT, H-FABP, PICP, PIIINP, CTX-I, TGF-β1 and GDF-15 were detected by enzyme-linked immunosorbent assay kit.

2.4 Statistical methods

SPSS 20.0 statistical software was used for analysis, miR-148b expression and serum marker analysis between two groups was by t test and P<0.05 indicated statistical significance in differences.

3. Results

3.1 Serum miR-148b expression

Serum miR-148b expression in AMI group was (2.37±0.41), and serum miR-148b expression in control group was (1.03±0.16). After t test, serum miR-148b expression in AMI group was significantly higher than that in control group, and differences in serum miR-148b expression were statistically significant between two groups of subjects (P<0.05).

3.2 Serum myocardial injury molecule levels and their correlation with miR-148b expression

Analysis of serum myocardial injury molecules CK-MB (U/L), cTnT (ng/mL) and H-FABP (pg/mL) levels between two groups of subjects was as follows: serum CK-MB, cTnT and H-FABP levels in AMI group were significantly higher than those in control group, and differences in serum CK-MB, cTnT and H-FABP levels were statistically significant between two groups of subjects (P<0.05). Analysis of serum CK-MB, cTnT and H-FABP levels in AMI group with different miR-148b expression was as follows: serum CK-MB, cTnT and H-FABP levels in AMI patients with high miR-148b expression were significantly higher than those in AMI patients with low miR-148b expression, and differences in serum CK-MB, cTnT and H-FABP levels were statistically significant between AMI group with different miR-148b expression (P<0.05).

3.3 Serum myocardial fibrosis molecule levels and their correlation with miR-148b expression

Analysis of serum myocardial fibrosis molecules PICP (ng/mL), PIIINP (ng/mL), CTX-I (pg/mL), TGF-β1 (ng/mL) and GDF-15 (ng/mL) levels between two groups of subjects was as follows: serum PICP, PIIINP, CTX-I, TGF-β1 and GDF-15 levels in AMI group were significantly higher than those in control group, and differences in serum PICP, PIIINP, CTX-I, TGF-β1 and GDF-15 levels in AMI group were significantly higher than those in control group, and
Significantly higher than those in AMI patients with low miR-148b expression were H-FABP levels in AMI patients with high miR-148b expression were analyzed in the study, and the results showed that serum miR-148b expression in AMI group was significantly higher than that in control group. This indicates that the high expression of miR-148b is closely related to the occurrence and development of myocardial infarction.

In the progression of acute myocardial infarction, ischemia hypoxia damage to myocardial cells will cause the release of a variety of catalyzing enzymes and structural proteins from cells to blood circulation, which will become the markers to reflect the degree of myocardial injury. CK-MB, cTnT and H-FABP are the most common myocardial injury markers in clinical practice. CK-MB is the isoenzyme of creatine kinase, which is highly expressed in myocardial cells and participates in the regulation of energy metabolism and ATP production; cTnT is a class of troponin, which is involved in the regulation of the striated muscle contraction by intracellular calcium ions; H-FABP is a fatty acid-binding protein highly expressed in myocardial cells and it is involved in the fatty acid transport and bio-utilization in cells.

In the study, analysis of the difference in serum contents of the myocardial injury markers between patients with myocardial infarction and healthy subjects showed that serum CK-MB, cTnT and H-FABP levels in AMI group were significantly higher than those in control group. This indicates that the high expression of miR-148b in AMI group was significantly higher than that in control group. However, the miR-148b change in patients with myocardial infarction is not yet clear. In order to define the effect of high miR-148b expression on myocardial cell injury, the correlation between serum miR-148b expression and myocardial injury marker levels in AMI patients further analyzed in the study, and the results showed that serum CK-MB, cTnT and H-FABP levels in AMI patients with high miR-148b expression were significantly higher than those in AMI patients with low miR-148b expression. This indicates that the high expression of miR-148b in AMI group is closely related to the occurrence and development of myocardial infarction.

The myocardial fibrosis is closely related to the fibroblast...
proliferation and collagen synthesis after reperfusion therapy for myocardial infarction patients. Both GDF-15 and TGF-β1 are members of the transformation growth factor family, which have a significant catalytic effect on the activation and proliferation of fibroblasts in myocardial tissues, and thus accelerate the formation of fibrous scar[13-15]. At the same time, the abnormal fibroblast proliferation will affect myocardial extracellular matrix synthesis and degradation, type I collagen is the most abundant collagen in the myocardial extracellular matrix, and the generation of PICP, PIINP, CTX-I and other collagen metabolites significantly increase in the process of type I collagen synthesis and deposition[16,17]. In the study, analysis of serum levels of myocardial fibrosis markers in myocardial infarction patients and healthy subjects showed that serum PICP, PIINP, CTX-I, TGF-β1 and GDF-15 levels in AMI group were significantly higher than those in control group. This suggests that after myocardial infarction, the fibroblast proliferation and collagen increase are closely related to myocardial fibrosis. In order to define the effect of high miR-148b expression on myocardial fibrosis, the correlation between serum miR-148b expression and myocardial fibrosis marker levels in AMI patients was further analyzed in the study, and the results showed that serum PICP, PIINP, CTX-I, TGF-β1 and GDF-15 levels in AMI patients with high miR-148b expression were significantly higher than those in AMI patients with low miR-148b expression. This means that high expression of miR-148b in serum of patients with myocardial infarction is closely related to the rise of serum myocardial fibrosis marker levels, and it also confirms that miR-148b has a promoting effect on myocardial fibrosis process.

Based on above discussion, it is concluded that the serum miR-148b expression increases significantly in patients with myocardial infarction; the high expression of miR-148b is closely related to myocardial injury and myocardial fibrosis.

References