Int J BioMed Public Health. 2018; 2(2):41-45
10.22631/ijbmph.2019.164756.1087

International Journal
of
BioMedicine and Public Health
http://www.ijbmph.com

Review Article

MiRNA-21: a potential biomarker for the diagnosis of acute myocardial infraction

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ARTICLE INFO

Article History:
Received 29 December 2018
Revised 2 May 2019
Accepted 20 May 2019

Keywords:
Myocardial infarction
Gene expression
miRNAs
miR-21
Diagnose

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ABSTRACT

Introduction: Myocardial infarction is the most important cause of death in cardiovascular patients. Recently, biomarkers play a key role in the diagnosis of cardiovascular disease, including myocardial infarction. Several recent studies have suggested that microRNAs (miRNAs), including miR-21, play a pivotal role in acute myocardial infarction (AMI). Therefore, we aimed to investigate the expression levels of miR-21 in patients with AMI.

Methods: A total of 73 patients with AMI and 73 healthy controls who were between 25 and 85 years old were enrolled in this study. Serum levels of total cholesterol, triglycerides, high density lipoprotein-cholesterol, and low-density lipoprotein- cholesterol, cardiac troponin I (cTnI), activities of creatine kinase, creatine kinase-MB, lactate dehydrogenase, aspartate aminotransferase, Prothrombin Time and Partial Thromboplastin Time were measured using commercial kits. The expression levels of miR-21 were measured by quantitative real time –polymerase chain reaction.

Results: Serum levels of cardiac enzymes, including CPK, and CPK-MB, cTnI were significantly higher in the AMI group ($P < 0.05$), but no significant difference was observed in, LDH, PT, and PTT levels between AMI and control group ($P > 0.05$). Plasma miR-21 levels in the AMI group was statistically higher than the control group ($P < 0.05$).

Conclusion: Data suggested that miR-21 can be used as a specific independent or complementary biomarker for AMI diagnosis. The current diagnostic method is proposed for the early diagnosis of heart disease, especially AMI.

Introduction

Acute myocardial infarction (AMI), is the leading cause of mortality and morbidity worldwide, and is considered as the secondary pathological response to coronary artery occlusion (1-3). The major consequence of AMI is cardiomyocyte death due to abrupt occlusion of blood flow that leads to contractile dysfunction (3). Scar generation at the site of the infract and interstitial fibrosis of adjacent myocardium hinder myocardial repair machinery and involves in the loss of pump function and susceptibility to arrhythmias (4, 5). The most important problem in cardiovascular diseases including AMI, is finding an appropriate diagnostic biomarker with high sensitivity and specificity, to identify subjects who are at risk for the development of these disorders (6). Despite the enormous effort in this area, no proper marker has yet been identified. Therefore, there is a critical clinical need for complementary biomarkers to evaluate the risk for cardiovascular diseases (7). MicroRNAs (miRNAs) are small non-coding RNA molecules with 19 to 25 nucleotides. The main function of miRNAs is participation in the regulation of gene expression at post-transcriptional level, through binding to the 3′-untranslated regions of target mRNAs (8, 9). Interaction of miRNA and mRNA results in the induction of mRNA degradation, as well as reduction in translation efficiency of specific transcripts (10). Approximately 1000 miRNAs are predicted to exist in the human genome, which have the ability to target hundreds of miRNAs (11). Recently, it was reported that miRNAs are present in biological fluids, including blood (12, 13), and the level of specific miRNAs could be used as a diagnostic and prognostic marker in particular diseases, including heart failure, coronary artery disease, and diabetes (14-17). An increasing body of studies have demonstrated that miRNAs are the key regulators of cardiac growth, vascular development, and angiogenesis (18, 19). MiR-21 is one of the most important miRNA molecules with major contribution to the regulation of various aspects of cardio-myocytes, including necrosis, fibrosis, and especially apoptosis (20, 21). MiR-21

regulates the expression of a large number of genes that directly or indirectly participate in the intrinsic or extrinsic apoptosis pathways (22, 23). Significant changes in the expression levels of miR-21 due to cardiovascular damage and stress can be detected in various tissues and blood (24, 25). Although several studies have shown an essential role for miR-21 in laboratory studies, the potential of miR-21 in the diagnosis of AMI has rarely been studied in clinical specimens (25). A mouse pressure-overload-induced disease model via reserve of sprouty homolog 1 (Spry1) in fibroblasts revealed that fibroblast survival can be promoted with miR-21 and that miR-21 can aggravate the size of interstitial fibrosis and cardiac hypertrophy (26). MiR-21 was also found to have a defensive action against cardiomyocyte apoptosis after myocardial ischemia– reperfusion (I/R) injury via the PTEN/Akt signaling pathway and inhibition of FasL (27). Therefore, in this study, we aimed to evaluate the plasma levels of miR-21 in patients with AMI and healthy controls to assess whether miR-21 could be used as a potential biomarker for AMI diagnosis.

Methods

Participants

In this case- control study, 73 patients with AMI (59 males, 14 females) and 73 healthy controls (57 males, 16 females) who referred to Shahid Madani Medical Research and Training Hospital in 2016 were enrolled based on random sampling method. The inclusion criteria were, the age range of 25 to 85 years old, willingness to cooperate in research by signing the consent form, and not having a history of other chronic diseases. The exclusion criteria were pregnancy, breastfeeding, irregular menstruation, chronic smoking (average smoking of more than 5 cigarettes per day for more than 48 months) or alcohol consumption, taking medications including tamoxifen, raloxifene, tibolone, methotrexate, sulfasalazine, metformin, cyclosporine, theophylline, diuretics, anticonvulsant and contraceptive over the past two years, use of protein supplements, phytoestrogens, folate and vitamin B12 supplements within 1 month before the diagnosis of the disease, type 1 diabetes mellitus with glomerular hyperfiltration, kidney disease, hyperthyroidism, chronic inflammation (rheumatoid arthritis involvement of joint, multiple sclerosis, skin inflammation, etc.), benign breast cancer, fibroenthomas, breast abscesses, ductal inflammation or fat tissue necrosis. All participants were instructed regarding the study and were then requested to complete the study questionnaire.

Ethical consideration

The study protocol was approved by the ethics committee of the Tabriz University of Medical Sciences (ethical code: IR.TBZMED.REC.1395. 1219), and informed consent obtained from each patient before enrollment.

Blood sampling

The ST AMI was diagnosed based on electrocardiogram findings and cardiac markers for heart muscle cell damage, including creatine kinase (CPK), creatine kinase -MB (CPK-MB). Peripheral venous blood samples were collected into tubes containing ethylenediaminetetraacetic acid (EDTA). Blood collection was performed immediately after AMI diagnosis prior to any drug intervention, especially heparin blood samples were used to assess all mentioned biochemical and coagulation tests, as well as measurement of miRNA levels. Blood samples were centrifuged at 3000 rpm for 5 minutes at room temperature (25°C) to extract plasma. The prepared samples were immediately frozen in aliquots at −80°C until analysis.

Baseline clinical and laboratory investigations

Serum levels of total cholesterol (TC), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C), and low density lipoprotein- cholesterol (LDL-C), were measured using Pars Azmun kit (Pars Azmun, Iran).

Cardiac marker enzymes assay

Activities of creatine kinase (CPK), creatine kinase -MB (CPK-MB), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) in the serum were measured using commercial kits (ParsAzmun, Iran). Prothrombin Time (PT) and Partial Thromboplastin Time (PTT) were investigated using the commercial kits (STAG, France). The troponin level was measured using ELISA kit (sandwich method).

Quantification of microRNA expression by qRT-PCR

Total RNA including the miRNA fraction was isolated using miRNeasy Serum/Plasma Kit, according to the manufacturer's protocol (Qiagen, USA). The cDNA was synthesized using miScript PCR System kit (Qiagen, USA), and miRNA levels were quantified with miScript SYBR Green PCR Kit and the $2^{(-\Delta\Delta ct)}$ relative quantification method. The primer sequences were: R 5′-CAGTGCAGGGTCC GAGGT-3′ and F 5′-GCCCGCTAGCTTATCAGACTGATG-3′. 1× SYBR premix Ex Taq mix (Qiagen, USA). Then, 2 µl RT products and 10 nM of each forward and reverse primer were mixed. Reactions were incubated at 95°C for 30 sec, followed by 45 cycles of 95°C for 15 sec and 60°C for 21 sec. To confirm the specificity of the amplification products, dissociation from 65 to 95°C was conducted. The miR-191 was used as reference gene for data normalization.

Statistical analysis

Normality of distribution was assessed using the Kolmogorov-Smirnov test. Mean and standard deviation (SD) were used to present normally distributed variables while the independent t-test was used to compare normally distributed variables between groups. Median (interquartile range) and the Mann-Whitney *U* test were used for presentation and comparison of non-normally distributed variables respectively. Correlations between the miRNA levels and cardiac biomarkers were evaluated using the Pearson correlation coefficient. Data analysis was performed using the Statistical Package for Social Sciences (SPSS) software package version 20.0. p value<0.05 was considered as statistically significant.

Results

The baseline characteristics of study population were shown in Table 1. All characteristics were similar in AMI and control groups and no statistically significant difference existed between groups (Table 1). In addition, the serum levels of cardiac enzymes, including CPK (692.25 ± 186 U/L vs. 230 ± 54 U/L; P < 0.005), and CPK-MB (109.82 ± 22 U/L vs. 38.66 ± 8 U/L;

Table 1. Baseline characteristics and measured parameter levels of study population.

Parameters	Levels in patients with AMI	Levels in healthy controls	P value [a]
Age (year)	59±1	58±1	≥0.05
Gender (male/ female)	58/11	57/16	≥0.05
BMI (Kg/m2) *	28.1±3.08	24.5±1.3	<0.05
Triglyceride	156±82	154±76	≥0.05
LDL	121±44	120±41	≥0.05
HDL	38±10	40±10	≥0.05
Cholesterol	186±54	185±53	≥0.05
PTT (second)	48.89±14.83	29.7±3.8	≥0.05
PT (second, INR)	13.4±7.6	13±1.5	≥0.05
CPK (U/L) *	692.25±186	230±54	<0.05
CPK-MB (U/L) *	109.8±22	38.66±8	<0.05
cTnI(ng/ml)	0.04±0.01	1±0.02	<0.05

[a] P value was significant at level of <0.05

P < 0.05) were significantly higher in the AMI group compared to control group. There was no statistically significant difference in LDH, PT, and PTT between AMI group and control group (P > 0.05). As shown in Figure 1, miR-21 expression was significantly higher in the AMI group compared to control group (P < 0.05), which suggested the potential for miR-21 to predict cardiovascular disease. In addition, miR-21 levels were positively correlated with CPK and CPK-MB levels in AMI group. There was no correlation between cTnI, LDL levels and serum miR-21 levels (Table 2). The standard curve for real-time PCR was shown in Figure 3. Furthermore, comparison of plasma miR-21 levels between patients based on body mass index (BMI) demonstrated that the expression of miR-21 was significantly higher in in BMI≥30 compared to BMI≤30 (Table 3).

Discussion

It is well-known that dysregulation of multiple genes is a common event in the AMI. Since miRNAs are the key regulators of over 30% of genes in a cell, it is not surprising to hypothesize that these small non-coding RNAs are involved in AMI. The current study demonstrated that miR-21 was aberrantly expressed in serum of patients with AMI. More interestingly, we found a positive correlation between serum levels miR-21 and cardiac enzymes, including CPK and CPK-MB. High expression levels of miR-21 has been reported in all types of cardiovascular cells, including vascular smooth muscle cells (VSMC) (28), endothelial cells (29), cardio-myocytes (30), and cardiac fibroblasts (31). In addition, miR-21 is one of the most important miRNAs with dysregulated expression pattern in many cardiovascular diseases (32, 33). A large body of studies has recently focused on elucidating the expression signature of miR-21 in AMI. For instance, Rooij et al. (34) investigated the expression levels of various miRNAs including miR-21 in the late phase of AMI (3 and 14 days after AMI). They observed that the expression levels of miR-21 was increased in the border zone of the infracted area in mice. Dong et al. (35) examined the miRNA expression signature in rats myocardium 6 h after AMI. They found 38 miRNAs with differentially expression pattern in infracted areas and 33 miRNAs with aberrant expression levels in the border areas. Among these miRNAs, the expression levels of miR-21 was significantly decreased in infracted areas, but was increased in border areas. Cardiac protective methods including ischemic preconditioning was shown to reverse AMI-induced changes in the expression of miR-21. Overexpression of miR-21 via adenovirus expressing miR-21 (Ad-miR-21) decreased myocardial infract size. In another study by Roy et al. (31) it was reported that ischemic reperfusion (IR) rapidly induced miR-21 in the heart, and elevated miR-21 levels were noted on day 7 post-IR as well. In the heart, miR-21induction in response to IR was limited to fibroblasts, where phosphatase and tensin homologue (PTEN) were identified as target genes for miR-21. They showed that IR-induced miR-21 limits PTEN function and therefore causes activation of the Akt pathway and increased metalloprotease-2expression in fibroblasts of the infract region of the IR heart. In a prospective study by Zampetaki et al. (36), a total of 19 candidate miRNAs were quantified in 820 participants and their relation to risk of AMI was investigated. They found four miRNAs with consistent and significant relation to incidence of AMI, among which miR-126 and miR-21 showed a positive association whereas miR-223 and miR-197 were inversely associated with disease risk (36) . These studies were among several studies that showed a protective role for miR-21 in AMI. It is believed that the expression levels of miR-21 in normal cardiac cells are very low, and it's up-regulation reflects the pathological changes in the heart, including hypertrophy and heart failure, which result in the cellular damage and increase the expression levels of protective miRNAs, including miR-21. In accordance with the mentioned studies, our findings showed that miR-21 expression was significantly increased in the AMI group compared to the control group. Based on the data from this study and similar studies, miR-21 can be used as a specific independent or complementary biomarker for AMI. The findings of this study further strengthen the currently proposed diagnostic methods for the early diagnosis of heart disease, especially AMI.

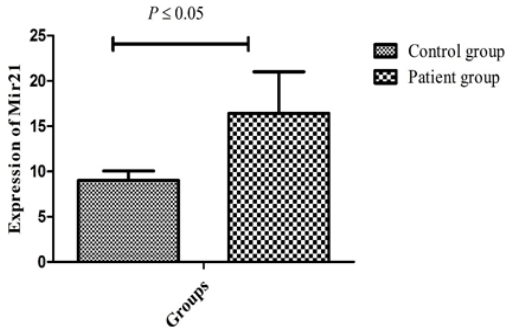

Figure1. Comparison of plasma miR-21 levels between the AMI and control groups

Table 2. Correlation between miR-21 levels and CPK and CPK-MB, cTnI , LDL levels in AMI group.

	R	P value
CPK	0. 427	0.016
CPK-MB	0.483	0.042
cTnI	0.276	0.212
LDL	0.201	0.310

Table 3. Comparison of plasma miR-21 levels between patients based on body mass index (BMI).

	BMI<30 (N=62)	BMI>30 (N=11)	Healthy Control (N=19)	P value*
mir-21	20.3±0.9**	19.6±0.3**	7.7±0.3**	<0.05

Conclusion

Since miR-21 is expressed in other tissues, including endothelial cells and white blood cells, any dysfunctions in these cells may interfere with the diagnosis of AMI based on miR-21. Therefore, more specialized studies are required to justify the clinical potential for miR-21 in diagnosis of AMI. Furthermore, this study was based on a small number of clinical specimens. It is suggested that further studies be implemented on larger samples in order to confirm the findings of this study. However, considering the available facilities and the results of this study, miR-21 can be considered as a highly sensitive biomarker for diagnosis of AMI.

Acknowledgements

The authors are exceptionally thankful to all patients who participated in the study. The authors declare that there is no conflict of interest.

Ethical disclosure

In this study, tests that threatened the health of individuals were not used.

Author contributions

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding/Support

None declared.

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