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The Predictive Role of MicroRNA19-b in Myocardial Infarction in Rat

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ABSTRACT

Objective(s): Cardiovascular disease is one of the leading causes of death worldwide. Acute myocardial infarction (AMI) is the most common and deadly disease of all types of cardiovascular disease. Many biomarkers such as troponin, CK-MB and various microRNAs have been shown to be involved in a variety of diseases, especially cardiovascular disease. Circulating MicroRNAs may be important and early biomarkers for the prognosis of cardiovascular disease. The aim of this study was to investigate the predictive role of miRNA-19b as a potential biomarker for AMI.

Methods: In this case control method we divided 20 rats in Wistar breed (weight 250-300 g) into two groups of myocardial infarctions and healthy. Standard conditions such as a temperature of 25 ° C, enough water and food were created in the animal room of Mashhad Medical School. 150 mg of isoprenaline was dissolved in 2 ml of injected distilled water and the solution was injected subcutaneously for two days. 24 hours after the last injection, myocardial infarction was confirmed by assessment of (CK-MB and troponin).

Results: Based on the findings, levels of troponin I and also CK-MB were higher in the group of rats induced myocardial infarction compared to healthy rats (P<0.001). In addition, the analyzes showed a significant up-regulation for miR-19b expression in the Treat group (P<0.001).

Conclusion: These findings indicate that circulating miRNAs, especially microRNA 19b, could be excellent candidates as biomarkers for heart disease, especially EMI.

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Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide. Global studies have estimate that nearly 30% of the world's deaths in recent years have been due to cardiovascular disease (1). Atherosclerosis is a progressive inflammatory disease that is detected by the accumulation of LDL in the walls of arteries. Atherosclerotic plaques formed in the coronary artery wall can eventually increase the risk of acute cardiovascular events such as myocardial infarction (MI) and stroke.(2) Acute myocardial infarction (AMI) is the most common and deadly disease of all types of cardiovascular disease. Today, the disease is diagnosed based

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on findings from electrocardiograms as well as measurements of biomarkers such as cardiac troponins (cTns) (3).

In recent years, a new class of RNA molecules called microRNAs (miRNAs) has emerged. MiRNAs are short non-coding RNAs that can reduce the expression of genes encoding proteins (4). These molecules bind to a specific sequence in the 3′ untranslated regions (3′ UTRs) in their target mRNA, leading to decapping, translational reppression, and mRNA deadenylation (5, 6). In addition, miRNAs can induce transcription by binding to other regions of the mRNA, including the promoter (7). Whereas, binding to coding regions as well as 5′ UTRs has the effect of silencing gene expression (8, 9). MiRNAs can be released from dying cells as well as living cells and act as paracrine agents (4). Also, in the heart, miRNAs are widely expressed and regulate multiple physiological and pathological pathways such as apoptosis, fibrosis and angiogenesis (10). In several animal studies and small-scale human studies, it has been reported that many microRNAs are released from cardiac myocytes that die after AMI. In addition, it has been demonstrated the level of blood circulation in muscle enriched with microRNA-1 and microRNA-133E is significantly increased in patients with hypertrophic obstructive cardiomyopathy. Therefore, the findings of the studies confirm this hypothesis that the microRNAs released from the heart may be the primary biomarkers of AMI diagnosis (10).

MicroRNA-19b (miR-19b) is part of the miR-17-92 cluster and is located on chromosome 13 (11). The findings show that overexpression of this miRNA increases proliferation, inhibits apoptosis, and differentiates embryonic carcinoma cells (12). In addition, various studies have reported that decreased miRNA-19b expression increases CCL, increases myocardial lysyl oxidase protein (LOX) as well as left ventricular stiffness (13). Decreased miRNA-19b levels in the myocardium of heart patients suggest that this miRNA may affect heart function (11).

The aim of study was to the predictive role of microRNA19-b in myocardial infarction.

Methods

Study population

In present study, we divided twenty Wistar male rats (weight = 250-300 g) into the with AMI and Healthy groups. Standard conditions such as temperature 25C°, and enough water and food were established in the animal room in Mashhad Faculty of Medicine. The present study was approved by the ethics committee of Mashhad University of Medical Sciences with the code IR.MUMS.MEDICAL.REC.1397.468 and all experimental protocols were performed according to the guidelines approved by the ethical committee of Mashhad University of Medical Sciences. In the first step, 150 mg isoproterenol was dissolved in 2 ml of injectable distilled water. Then, 150 mg/kg of this solution was injected subcutaneously for two days. Measuring weight rats and as well as Isoproterenol was done with an electronic scaler. Myocardial infarction was confirmed 24 hours after the last injection, by measuring serum levels of CK-MB and troponin. In order to balance the injection between rats with AMI and healthy, an equal volume of normal saline was injected into healthy rats for two days.

Blood sampling

First, rats were anesthetized with 50 mg/kg Ketamine and 10 mg/kg Xylene. Then, we collected 2 ml blood by inserting a capillary tube through the sinus membrane of the eye. In the end, samples were centrifuged at 2800 rpm for 5 min to separate the serum, and it was kept at -80C° for future analysis.

Isolation of total RNA

Total RNA was isolated by using the RNX-PLUS kit based on the protocol of the manufacturer at the Mashhad University of Medical Science. We added 1ml of ice-cold RNX-PLUS solution to 2 ml of the homogenized sample in a tube, and the resulting solution vortexed for 10 secs and incubated at room temperature for 5 min. After adding chloroform, incubation at 4C° for 5min and centrifuging (12000 rpm, 4C° for 15min) were performed. We transferred the aqueous phase to another tube. Following incubation on ice for 15 min and centrifuging again (12000 rpm, 4C° for 15 min), the supernatant was removed. Subsequently, 1ml Ethanol 75% was added to the supernatant and shortly vortexed and centrifuged at 4C°, 7500 rpm for 8 min. Next, the supernatant was discarded and the precipitates were dried at room temperature for 5 min. Finally, the pellet was dissolved in 50 µl of DEPC treated water.

Polyadenylation reaction

We mixed 5 µg total RNA, 1 µl rATP(10mM), 2µl 10 poly-A polymerase buffer, 0.1 µl poly-A polymerase, and 20 µl Rnase-free water, and Vortex them. At the end of the reaction, we incubated the result solution at 37C° for 30 min before we made the reaction inactive by incubation at 65C° for 20 min.

Table 1. Primer for the real-time PCR reaction

Primer's Name	TM	Forward Primers	Reverse Primer (Universal)
miR-19b	59	GGTGGCTGAGCAAA	GAGCAGGGTCCGAG
U87	62	ACTTATGTTTTTGCCGTT	

Comparison of the number and percentage ΔCt of the studied samples

Synthesis of cDNA

For every polyadenylated RNA sample, we performed as follows:

12 µl polyadenylated RNA was mixed with BON-RT adaptor primer (10 µM) and made the tube volume to 13 µl by RNase-DNase free water, then we used the PCR system Veriti thermocycler (Applied Biosystems, USA) at 75C° for 5 min. After that, we put the tubes on ice immediately before using the thermocycler again. We prepared 20 µl total volume constituting 1 µl RT enzyme, 2 µl dNTP, 4 µl 5*RT buffer, and RNase-free water for the remnant. Finally, we used thermocycler in three cycles that were respectively at 25C° for 10 min, at 42C° for 60 min, and at 70C° for 10 min.

Real-time PCR

The assessment of RNA expression was performed by the BON-miR QPCR kit according to the manufacturer's protocol. Synthesized cDNA was applied in quantitative real-time PCR (QRT-PCR). To complete this reaction, we mixed 1 µl cDNA, 0.5 µl miRNA-specific forward primer, 0.5 µl universal reverse primer, 6.5 µl miRNA QPCR master mix, and 13 µl H2O (table 1).

Statistical analysis

Data analysis was undertaken using the Statistical Package for Social Sciences software (SPSS Inc., IL, USA). For normal distributed variables, the T-student test was used. The Mann–Whitney U test was used for continuous variables if they were not normal distributed. We reported results of RT-PCR with fold change and analyzed them in GraphPad Prism version 8. To prove the potential of miR-21 as a biomarker, ROC-curve was used. An AUC>70 is considered as a good index for a biomarker with a high ability to correctly classify samples. Significance level in the present study was considered <0.05.

Results

In this study, 20 samples of Wistar rats were examined. Of these, 10 samples were in the control group and 10 samples were in the treated group (figure 1). Among these, one sample was died due to lack of suitable capacity for dose of the studied

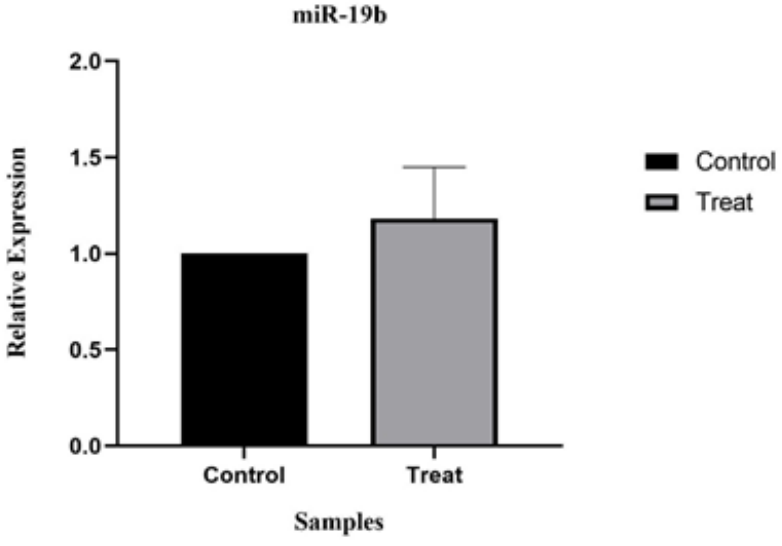

Figure 1. Comparison of expression between the control group and induced myocardial infarction group

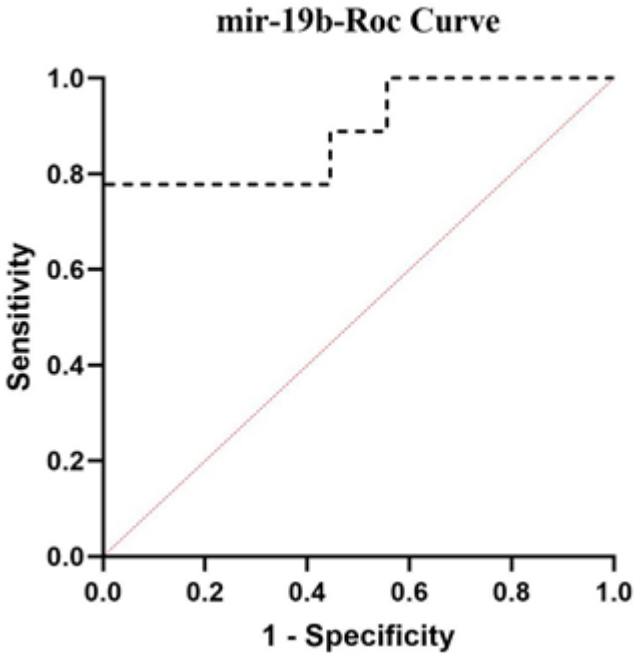

Figure 2. ROC curve analysis is used to determine the potential role of miR-19b. in rats, with Area Under the Curve (AUC) of 0.8889 observed significant differences in AMI rats rather than normal rats (sensitivity and specificity were 100%)

materials. Analyzes demonstrated that, CK-MB was significantly higher in AMI rats compared with healthy group (298±7 (IU/l) vs. 73±4 (IU/l), P<0.001). Also, higher levels of troponin I was observed in AMI rats relative to healthy rats (1.6 (ng/ml) vs. 0.4 (ng/ml), P<0.001).

The serum assessment in rats showed there is a significant up-regulation for the relative expression of miR-19b in rats with AMI than in normal rats (P<0.05) (figure 2).

Discussion

In summary, according to the results of the present study, we found that the levels of troponin I and also CK-MB were higher in the group of rats induced myocardial infarction compared to healthy rats. In addition, the analyzes showed a significant up-regulation for miR-19b expression in the Treat group.

Myocardial infarction is the leading cause of coronary artery disease (14) and can lead to cardiac hypertrophy, myocardial fibrosis, and in acute cases lead to sudden death (15, 16). In recent years, various approaches such as bypass surgery as well as percutaneous coronary intervention (PCI) have been used to treat myocardial infarction (17, 18), while these factors are only able to reduce the severity of the disease (19, 20). Therefore, reducing the risk of MI-induced myocardial cell death requires the adoption of new treatment strategies. Studies show that many miRNAs have a potential role in cardiac myocardial pathology after MI (21).

In recent years, studies on various miRNAs have elucidated their pathophysiological role in coronary heart disease (22, 23). Circulating miRNAs can be secreted from dying cells, such as heart cells after AMI, so they can form a new class of AMI biomarkers (24). In contrast, some other studies with extensive studies in patients with AMI and chest pain have reported that circulating miRNAs cannot be used as biomarkers compared to traditional markers, including cTns (25). The study of Wang et al. (2016) after examining 18 patients with AMI as well as 20 healthy individuals as a control group, indicated that the expression of miRNA-19b-3p in the early stages of AMI was significantly up-regulated and its expression after admission (T0) reaches its maximum value. It should be noted that this miRNA will gradually decrease after reaching the peak of expression, and the exact cause has not yet been found. The results showed that the circulating miRNA-19b-3p has a strong positive correlation with cTnI. Although this miRNA did not show a high ability to detect AMI, it was surprisingly found that combining miR-19b-3p with miR-134-5p and miR-186-5p increased the power of differentiation compared to single terms (26). MiRNA-134 regulates neuronal cell death (due to reperfusion) by targeting CREB and HSPA12B protein (27, 28). Published studies have indicated that both miRNA-134 and miRNA-186 are up-regulated in patients with AMI or unstable angina pectoris (UAP) and can be used as biomarkers of

these diseases (29-31). In a case-control study of 198 patients, it was concluded that circulating miR-22 levels could be a predictor of mortality from ischemic and non-ischemic heart failure (32). Another study that looked at individuals with unstable angina pectoris reported that miRNA-19b was up-regulated in these patients (33). This miRNA may also play an antithrombotic role by acting on tissue factor influencing blood coagulation (34). By acting on the ABCA1 transporter, miRNA-19b leads to the development of aortic atherosclerosis and by suppressing the PGC-1α receptor, leads to endothelial cell dysfunction and the progression of atherosclerosis (24, 25).

Although the use of circulating miRNAs as biomarkers of AMI prognosis can be very useful, but it is necessary more and more detailed studies to be done and diagnostic methods need to be substantially improved so that they can be used in the clinical setting with complete confidence. One of the limitations of the present study was the small sample size, so, to ensure the accuracy of the results obtained, it is recommended that more samples be evaluated in future studies.

Conclusion

In this study, we have shown that circulating miRNAs, especially micro-RNA-19B, may be used as biomarkers, but it cannot be conclusively stated that microRNAs, especially micro-RNA-19. B. can act as a prognostic and early indicator. Although miRNAs have diagnostic value for a wide range of diseases, especially cardiovascular disease, more and more extensive studies are needed to reach a definitive conclusion.

Conflict of interest

The authors have no conflict of interest to disclose.

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