Effective microRNAs in Ischemia/Reperfusion Before and After Bypass Graft Surgery in Coronary Artery Patients

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Research

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Abstract

Background

Cardiovascular diseases (CVD) are among the causes of morbidity and mortality in the world. Significant advances have been made in the diagnosis, treatment and prognosis of CVD. New biomarkers and therapeutic targets are needed to reduce the incidence of this disease. Recently, there is growing evidence that circulating microRNAs can be used as diagnostic biomarkers in this disease.

Methods

We compared five microRNA (*hsa-miR-21-5p*, *hsa-mi181a-5p*, *hsa-miR-199a-5p*, *hsa-miR-199b-5p* and *hsa-miR-320a*) expression levels associated with ischemia/reperfusion before and after bypass graft surgery in serum samples of patients (N=46) with coronary artery disease and healthy control subjects (N=48). Expression measurements were made for each miRNA preoperatively and postoperatively at 1. and 24. hours, and then compared with the control subjects. Troponin I, creatine phosphate kinase and creatine kinase myocardial band cardiac markers were measured before and 1 and 24 hours postoperatively and compared to miRNA expressions and controls. Quantitative real-time PCR was used for expression analysis. The data were analyzed by Mann-Whitney test, chi-squared test, Logistic Regression analysis, and Kruskal-Wallis test with the statistical package SPSS.

Results

The five miRNAs were down-regulated compared to controls. The expression level for miR-199a at 24 h postoperatively was significantly lower than at 1 h (p=0.001). Receiver operating characteristic analysis showed that the area under the curve of miR-199a-5p was 0.810 (sensitivity 87% and specificity 68.5%) in preoperative patients.

Conclusions:

miR-199a and miR-199b in serum are a novel non-invasive biomarker candidate for coronary artery disease.

Background

Currently, cardiovascular disease (CVD) is the most important cause of mortality in the middle and old age group and constitutes 31% of global deaths. It is estimated that CVD causes approximately 17.7 million deaths each year in the world [1]. The number of people dying from CVD is estimated to be more than 24 million by 2030 [2]. According to data TEKHARF (Prevalence of Heart Disease and Risk Factors in Turkish Adults) of 2017, 43% of deaths in Turkey is caused by coronary heart disease. There are approximately 3.5 million coronary heart patients in our country. It is estimated that this number increases by 4% annually and 210 thousand people die annually from coronary heart disease [3].

Ischemic damage due to coronary heart disease leads to permanent damage to the cardiac tissue, and a decrease in contractility set the stage for heart failure. In heart failure, hypertrophy of myocytes at cellular level, inflammation, mitochondrial dysfunction, cardiac fibrosis, arrhythmia, vascular defects, endothelial cell viability and decreased cardiomyocytes are observed. Factors that influence these results at the molecular level include a variety of gene regulatory networks, including transcription factors, co-activators, repressors, promoter DNA regions, promoter elements, and chromatin exchange enzymes [4].

MicroRNAs (miRNAs) are a small class of non-encoded RNAs of 20–25 nucleotides in length that act as a molecular switch for gene expression. They are involved in many important cellular processes such as proliferation, differentiation, cell metabolism, apoptosis and angiogenesis. They regulate gene expression by inhibiting protein translation from mRNA or by degrading mRNA at the post-transcriptional level [4, 5].

To date, the studies examining the association between miRNAs and vascular disease have shown that there are few studies evaluating miRNAs in patients with coronary artery bypass grafts. Therefore, we have selected five miRNAs (miR-21, miR-199a, miR-199b, miR181a and miR320a) that may be associated with ischemia/reperfusion (I/R) in coronary artery disease patients that are not adequately investigated. We aimed to compare the levels of these miRNAs both in the healthy control group and before the surgical treatment in which the coronary vessels could not provide sufficient oxygen in the patients who would undergo bypass graft surgery due to coronary artery disease and after the surgical treatment in which reperfusion was provided. Another aim was to determine whether there is a correlation between miRNA levels and cardiac markers (cardiac troponin I and creatinine kinase MB). Thus, we think that miRNAs may change due to reperfusion of the heart and we can obtain concrete data about these changes. We hope that these data will be evaluated as biomarkers in patients with coronary artery disease in the coming years and will provide us with predictions about the prognosis of coronary artery disease.

Materials And Methods

Selection of volunteers

The study was conducted on 46 patients and 48 healthy volunteers who applied to Cardiovascular Surgery Department of Sanko University Research and Application Hospital in Turkey. The patient group consisted of volunteers aged between 18-65 years with a history of coronary artery disease, myocardial infarction, coronary artery stenosis greater than 70%, left ventricular ejection fraction between 30% and 55% and without diabetes mellitus, malignant diseases and chronic renal failure. The control group consisted of healthy volunteers between 18-65 years of age, without coronary artery disease and discomfort that could affect related miRNAs. Peripheral blood samples were obtained from 46 patients who accepted to undergo coronary artery bypass surgery preoperatively. Peripheral blood samples were collected preoperatively and at the 1st and 24th hours after the operation. Blood samples of healthy individuals were collected from the same clinic and who had no disease that could affect coronary artery disease and related miRNAs. Informed consent was obtained from each patient and the study protocol

conforms to the ethical guidelines of the 2013 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. This study was approved by Gaziantep University Medical School Medical Ethics Committee.

Plasma Sampling

5 ml blood samples from each individual were taken into tubes containing 7.5% EDTA. The tubes were centrifuged at 2000 rpm for 15 min. Then, the approximately 1000 microliter plasma was centrifuged again at 2000 rpm for 5 minutes and placed into a 200 µL clean micro centrifuge tube without touching the supernatant from the top. Micro centrifuge tubes were labeled with the patient's barcode label. A total of 192 plasma samples were stored at -80 °C until RNA isolation. Also, biochemical parameters (LDH, BUN, Glucose, Troponin I, CK, and CK-MB) of the blood samples of the patient and control groups were measured in the Biochemistry Laboratory (Sanko University Research and Application Hospital in Turkey). Plasma samples were coded as pre-op, 1-hour post-op (OP1), 24-hours post-op (OP24) and control group (K).

RNA Extraction

Total RNAs containing microRNAs were obtained from plasma using the miRNeasy RNA Isolation Kit (Qiagen, Hilden, Germany). cel-miR-39 was used as spike-in control. RNA was obtained according to the manufacturer's instructions and then stored at -80°C for further studies.

Complementary DNA (cDNA) reaction preparation

Isolated RNAs were transformed into cDNA using the Qiagen miScript II kit. 50 microliters of reaction mix were prepared as 5x miScript Hispec buffer, 10x miScript Nucleics mix, DNase-RNase free water and 2 ng RNA. Samples were amplified by holding at 37 °C for 60 min. and at 95 °C for 5 min. The obtained cDNA samples were diluted with nuclease-free water in 1/5 ratio and stored at -20 °C until expression.

Reverse Transcription Reactions

Total RNA samples (3.5 ml) were converted to cDNA using the Qiagen miScript II Kit (Cat.No:218161). At this stage, 5x miScript Hispec buffer, 10x miScript Nucleics mix, DNase-RNase free water and miScript Rev. A transcriptase mix was added to the total RNA and the total reaction volume was completed to 7 ml. Samples were placed in the thermal cycler (Bioer GenePro Thermal Cycler) and the reverse transcription reaction was performed at 37 °C for 60 min. 95 °C for 5 min. 4 Set to °C ¥. Then, cDNA samples were diluted 1/5 with nuclease-free water. The Qiagen miScript Microfluidics PreAMP kit and the Qiagen miScript Primary Assay [cel-miR-39 (MS00080247), miRTC (218161), miR-21-5p (MS00009079), miR-199a-5p (MS00006741), miR-199b-5p (MS00006741), miR-181a-5p (MS00008827), miR-320a (MS00014707)] were used for the pre-amplification reaction. MiRTC is a miRNA reverse transcription control and was used to evaluate reverse transcription performance. Another control is cel-miR-39 and

exogenously spiked cel-miR-39 was used for alternative data normalization. The pre-amplification mix were added to diluted cDNA samples. Samples were placed in thermal cycler (Bioer GenePro Thermal Cycler). Thermal program for pre-amplification reaction 15 min for 95 °C, 30 min for 94 °C 12 min for 3 min for 60 °C, ¥ for 4 °C (rest) and then 15 min for 37 °C, 5 min for 95 °C , 4 °C is set to ¥. At the end of the cycles, the samples were diluted 1/5 with nuclease-free water.

Quantitative Reverse Transcriptase PCR (qRT-PCR)

qRT-PCR were performed using a high-throughput Biomark Real-Time PCR system (Fluidigm, South San Francisco, CA). 4 ml pre-Mix (20X DNA Binding Dye Sample Loading Reagent) (Fluidigm, PN 100-3738), qPCR Master mix and Nuclease free water) were pipetted into 96-well wells. Pre-amplified samples were diluted 1/5. 2 ml of these samples were pipetted into 96 well plate wells and a total of 6 ml of sample mix was prepared. The prepared Assay mix (2X Assay Loading Reagent, 1x DNA Suspension Buffer and 100 M forward and reverse primer) was pipetted as 3.5 ml into the sample wells of the 96.96 Dynamic Array chip. 96.96. Dynamic Array Chip IFC loaded into controls. Then array chip was removed from the IFC controller and placed in the Fludigm Biomark. Thermal cycler protocol was set for 120 sec for 50 °C, 1800 sec for 70 °C and 600 sec for 25 °C, 600 sec for start-up temperature 600 sec for 95 °C, and then 94 °C for 15 sec (Denaturation), 30 sec for 55 °C (Binding), for 30 sec 70 °C (Elongation) and 30 cycles.

Statistical Analysis

Shaphiro wilk test was used to test the normal distribution of the numerical data in the patient and control groups. Student t test was used to compare the variables with normal distribution in two groups and Mann Whitney U test was used to compare the variables with normal distribution in two groups. In order to compare the measurements of miRNA expression levels before and after the operation in the patient group, repeated measures analysis of variance and LSD multiple comparison tests in normally distributed variables were used. Friedman 2-way analysis of variance and all pairwise multiple comparison tests were used for non-normally distributed variables. The relationship between the expression levels of microRNAs and cardiac markers was tested with spearman correlation coefficient. Analyzing real-time PCR data by the comparative DCT method [6]. Cut off values were determined as a result of ROC analysis for the variables of miRNA expression levels before the operation. SPSS 22.0 software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used in the analysis. $P<0.05$ was considered significant.

Results

Demographic and clinical findings of the patient and control group

When the patient and control groups were compared in terms of demographic characteristics, significant differences were found between age, LDH, blood urea nitrogen (BUN) ($p < 0.001$) and glucose ($p < 0.002$)

values. Cardiac troponin, creatinine phosphokinase (CPK) and creatinine kinase-MB (CK-MB) values of preoperative patient group and control group were compared and significant differences were found between CK-MB and cardiac troponin values ($p < 0.001$). Demographic and clinical findings of the patient and control groups are shown in Tables 1 and 2, the operation data of these groups are given in Table 3.

Table 1
Demographic and clinical data of patient and control groups.

Demographic and Clinical Features	**Patient (M = 20, F = 26) MEAN±STD**	**Control (M = 16, F = 32) MEAN±STD**	***P***
Age	62.630 ± 11.249	42.340 ± 11.249	**0.001 ***
LDH	228.159 ± 65.975	192.438 ± 23.828	**0.001 ***
BUN	18.620 ± 8.684	13.790 ± 9.908	**0.001 ***
Glucose	100.304 ± 8.684	94.708 ± 9.908	**0.002 ***

LDH, Lactate Dehydrogenease; BUN, Blood Urea Nitrogen, M, Male; F, Female, P-values < 0.05 are indicated in bold

Table 2
Cardiac marker values of the patient and control groups before the operation.

	Group	**N**	**Mean**	**Std. Deviation**	***P***
Troponin I	Control	48	0.021	0.144	**0.001 ***
	Patient	46	0.862	2.800	
CPK	Control	48	79.125	40.256	0.812
	Patient	46	87.976	57.070	
CK-MB	Control	48	18.102	4.738	**0.001 ***
	Patient	46	27.902	17.597	

CPK, creatine phosphokinase; CK-MB, creatine Kinase myocardial band; N, number of individuals, * P-values < 0.05 are indicated in bold.

Table 3
Operation data of patient group

Intensive care (hour)	**49.956 ± 8.817**
Time Spent in the hospital after surgery (day)	5.544 ± 0.836
Weight (kg)	80.348 ± 7.218
Cardiopulmonary bypass time (minute)	54.174 ± 9.481
Kross Clamp Time (minute)	37.696 ± 7.387
Bypass number	3.304 ± 0.866

There was a significant decrease in the expression values of miR-21, miR-199a-5p, miR-199b-5p, miR-181a, miR-320a in the preoperative patient and control groups ($p < 0.001$). When miRNA changes were evaluated in the patient group before and after the operation, the significant differences were found only between the 1st and 24th hours for miR-199a-5p ($p = 0.044$). Mean ΔCT and standard deviation values is shown in Table 4. ROC value belong to patients and controls are shown in Table 5.

Table 4
Mean ΔCT and standard deviation values of patient and control groups.

miRNA name	Groups	N	Mean ΔCt	Std. Deviation	P
miR-21	Control	48	2.393	1.353	**0.001 ***
	Pre-Op	46	3.732	2.389	
	Post-op 1	46	3.850	2.662	
	Post- op 24	46	3.860	2.272	
miR-199a-5p	Control	48	7.639	2.499	**0.001 ***
	Pre-Op	46	10.994	3.725	
	Post-op 1	46	10.480	3.778	
	Post- op 24	46	11.727	3.424	
miR-199b-5p	Control	48	8.246	1.570	**0.001 ***
	Pre-Op	46	10.560	3.496	
	Post-op 1	46	10.285	3.310	
	Post- op 24	46	10.810	3.170	
miR-181a	Control	48	9.061	2.245	**0.001 ***
	Pre-Op	46	11.580	3.533	
	Post-op 1	46	10.390	3.580	
	Post- op 24	46	10.680	3.410	
miR-320a	Control	48	2.016	1.587	**0.001 ***
	Pre-Op	46	3.555	3.113	
	Post-op 1	46	3.901	3.606	
	Post- op 24	46	3.618	3.190	

Pre-op; before from operation, post-op; after from operation, N, number of individuals, * P-values < 0.05 are indicated in bold.

Table 5
The Receiver Operating Characteristics Curve Analyses of the Five microRNA.

Compared Groups	miRNAs	AUC	95% CI	P-Value	Specificity (%)	Sensitivity (%)	Criterion
Coronary artery patients vs Control	miR-21	0.777	[0.680–0.857]	< 0.0001	0.875	0.652	> 14.691
	miR-199a-5p	0.810	[0.716–0.883]	< 0.0001	0.687	0.869	> 19.283
	miR-199b-5p	0.808	[0.714–0.882]	< 0.0001	0.792	0.826	> 20.301
	miR-181a	0.784	[0.687–0.862]	< 0.0001	0.854	0.630	> 21.580
	miR-320a	0.784	[0.688–0.863]	< 0.0001	0.813	0.791	> 13.735

AUC, area under the curve; CI, confidence interval.

Correlation Between Cardiac Biomarkers And Mirnas

We evaluated the relationship between changes in plasma markers of cardiac markers and miRNA expression. There was a positive weak correlation between miR-21 and cardiac troponin levels in the patient group (r = 0.344, p = 0.019). However, no correlation was found between miR-21 and CK-MB values. There was a negatively weak correlation between miR-181a and cardiac troponin values measured at 1 hour and 24 hours (r=-0.331, p = 0.025, -r = 0.361, p = 0.014). There was a weak positive correlation between miR-181a values measured 1 hour postoperatively and CK-MB values measured before and 1 hour postoperatively (r = 0.306, p = 0.039, -r = 0.321, p = 0.030). In the patient group, a moderate negative correlation was found between miR-199a-5p values measured 24 hours postoperatively and cardiac troponin values measured 1 hour postoperatively (r=-0.469, p = 0.001). There was no significant relationship between miR-199a and CK-MB values. There was no significant relationship between miR-199b and cardiac troponin and CK-MB levels before and after the operation. There was a weak positive correlation between miR-320a and cardiac troponin levels in the patient group (r = 0.318, p = 0.031). However, no significant relationship was found between miR-320a and CK-MB values.

Discussion

Coronary artery disease is the most common cardiovascular disease. Studies on the positive and negative effects of miRNAs on the pathogenesis of the disease show that circulating miRNAs can be used as biomarkers in the diagnosis of coronary artery disease. Therefore, in our study, the role of

miRNAs in the diagnosis of coronary artery disease and its relationship with the parameters in the clinical evaluation process were investigated.

miRNAs have gene regulatory effects [7]. In studies conducted in patients with myocardial infarction, serum levels of miR-1 and miR-133a are high [8, 9]. Heart-related studies of microRNAs have mostly focused on their effects on cardiac remodeling processes. It has been shown that miRNAs have significant potential in evaluating the early and late stages (early detection of a heart attack) of cardiac damage, regeneration, rhythm regulation, cardiac growth, and myocardial infarction [10–12]. miR-1, miR-133 and miR-208 have also been shown to be effective in many heart related conditions. These have been identified as "cardio-specific miRNAs" [5, 13, 14]. Therefore, we investigated human plasma samples that have not previously been studied in the presence of clinical data. We evaluated miRNA levels under ischemic conditions and obtained data that these miRNAs can be evaluated as markers in ischemic conditions in patients with coronary artery disease.

miR-21 is one of the miRNAs considered to be oncogenic due to its proliferative and anti-apoptotic effect in cancer cells [15, 16]. MiR-21 inhibition in cultured cardio myocytes was found to have a negative effect on cardio myocyte hypertrophy [17]. When MiR-21 is inhibited by antagomir approach, cardiac hypertrophy and fibrosis are reduced [18], as well as protective effects against heart damage caused by I/R [19]. This effect, which was initially useful in preventing cardiac damage, becomes harmful when the effects that lead to damage persist or recur. MiR-21 levels are high in unbalanced angina patients [20] and atherosclerosis model mice [21]. Patients with heart failure have high levels of miR-21, brain natriuretic peptide and miR-21 in serum samples collected from both the peripheral vein and coronary sinus [22]. In patients suffering from acute myocardial infarction, there is a strong correlation between miR-21 and cardiac markers such as cardiac troponin, CK and CK-MB [23].

In our study, a positive weak correlation was found between preoperative miR-21-5p and cardiac troponin values in the patient group. While there was no correlation between cardiac troponin and miR-21-5p values measured at postoperative hour 1, there was a negative and weak correlation between cardiac troponin measured at 1 hour and miR-21-5p measured at 24 hours. However, no correlation was found between CK-MB and miR-21-5p values at the 1st and 24th hours. Our patient group was not acute and was selected from individuals with a history of myocardial infarction. Therefore, we think that the miR-21 levels were lower in patients with coronary artery disease ($p < 0.001$). In addition, there was no significant difference between preoperative and postoperative miR-21-5p levels in patients with coronary artery disease ($p < 0.05$). These results were found to be different according to the literature data that miR-21 levels were higher in patients with coronary artery disease and heart failure compared [19–23]. This difference may be thought to occur because miR-21-5p levels correlate with cardiac markers. The diagnostic value of miR-21 in the patient group in our study could be considered as moderate. From this point of view, our results are consistent with the literature [24] and the nomination of miR-21 for diagnosis can be confirmed by repeated studies.

miR-199a-5p is localized in the introns of the dynamin genes and is expressed in cancer, brain, liver and cardiovascular system, particularly in cardio myocytes and endothelial cells [25, 26]. It is also associated with cancer and cardiovascular diseases [27, 28]. miR-199a-5p decreased in cardio myocytes exposed to hypoxia [27, 29[. High levels of miR-199a in circulating micro vesicles are associated with a reduced risk of cardiovascular events in patients with stable coronary artery disease [30]. In our study, miR-199a-5p levels were lower in patients with coronary artery disease than in the control group ($p < 0.001$). When miR-199a-5p changes were evaluated in the patient group before and after the operation, significant differences were found between the 1st and 24th hours ($p < 0.044$). The increase in the level of miR-199a 1 hour after the operation may be due to the saturation of the Hif-1alfa in the medium to achieve saturation and then to decrease its levels. MiR-199a-5p values measured in the patient group at 24th postoperative hour and cardiac troponin levels measured at 1 hour postoperatively were negatively moderately correlated. There was no significant relationship between miR-199a-5p and CK-MB values ($p > 0.005$). When we evaluate the obtained data, we think that changes in miR-199a-5p levels may be an important biomarker for coronary artery disease.

One of the miRNAs involved in cardiac hypertrophy is miR-199b-5p and plays an important role in the development of various organs such as the heart [31, 32]. miR-199b has been shown to activate the calcineurin/NFAT signaling pathway by targeting double-specific tyrosine phosphorylation-regulating kinase 1A [31] and improving heart function when miR-199b is inhibited [33]. It has been reported that miR-199b-5p regulates the nuclear factor kappa-B (NF-κB) signal pathway in the inflammatory response of the nervous system and suppresses neurodegenerative disease [34]. In another study, monocytes treated with lipopolysaccharide have been shown to have a significant decrease in miR-199b-5p levels and an increase in GSK3β expression and anti-inflammatory effect by activation of the NF-κB pathway [14]. In our study, miR-199b-5p levels were lower in patients with coronary artery disease compared to the control group ($p < 0.001$). Low miR-199b-5p levels result in the inability to inhibit NFκ-B protein and inflammation. Therefore, it can be said that it contributes to the etiopathogenesis of coronary artery disease. However, there was no significant difference between miR-199b-5p levels before and after surgery in patients with coronary artery disease ($p > 0.05$). There was no significant relationship between miR-199b-5p and cardiac troponin and CK-MB values for before and after operation in the patient group. According to ROC data, miR-199b-5p levels can be an important marker for coronary artery disease. We have seen in the literature that miR-199b-5p levels have not been studied in patients with coronary artery disease. To the best of our knowledge, this is the first study to investigate the correlation of miR-199b-5p levels with cardiac markers before and after bypass grafting in patients with coronary artery disease. Further studies are needed to evaluate miR-199b-5p levels in patients with coronary artery disease and to support our results.

miR-320a has been mostly studied in cancer and heart disease and also varies in heart disease, but has been studied in a limited number of patient groups [35, 36]. It suppresses cardio myocyte apoptosis of downregulation of miR-320a and protects against myocardial ischemia and reperfusion injury by targeting insulin-like growth factor 1 (IGF-1) [37]. In another study, it has been shown that downregulation of miR-320a suppresses cardio myocyte apoptosis and protects against myocardial ischemia and

reperfusion damage by targeting insulin-like growth factor 1 (IGF-1) [38]. miR-320a is down-regulated in patients with myasthenia gravis [39]. In these patients, the expression of cyclooxygenase-2 (COX-2) is high, and over-expression of pro-inflammatory cytokines has been observed [40]. In our study, miR-320a levels were lower in patients with coronary artery disease compared to the control group ($p < 0.004$). There was no significant difference between the preoperative and postoperative miR-320a levels in patients ($p > 0.05$). There was a weak positive correlation between preoperative miR-320a levels and cardiac troponin levels in patients and there was no significant relationship between miR-320a and CK-MB. The first ROC analysis for miR-320a in patients with coronary artery disease was conducted in this study, and we believe it can be used to diagnose the disease, according to our data.

MiR-181a is highly expressed in the mammalian brain [41]. It plays an important role in various biological events, such as immune modulation and inflammation [42]. Inhibition of miR-181a has been found to increase neuronal loss due to ischemia and bcl-2 levels, which are anti-apoptotic protein [43] and upregulated in atherosclerotic vessels compared to normal vessels [44]. In the neurogenic hypertension model, miR-181a levels were low and renin mRNA levels were high [45]. In a study of unstable angina and acute myocardial infarction in circulation, MiR-181a levels were higher in the myocardial infarction group [14]. In our study, miR-181a levels were lower than the control group ($p < 0.001$). There was no change in miR-181a levels according to preoperative values after reperfusion with bypass graft ($p > 0.05$). There was a negative weak correlation between miR-181a-5p and cardiac troponin values after surgery in patients with coronary artery disease and weakly positive. There was a correlation between postoperative miR-181a-5p and CK-MB. The role of MiR-181a in cardiovascular diseases has not been described. As a result, miR-181a-5p has diagnostic potential in coronary artery disease. Therefore, we think that it can be considered as a biomarker for this disease if it is supported by new studies.

Conclusions

In our study, the expression levels of miR-21-5b, miR-199a-5p, miR-199b, miR-181a and miR-320a were lower in patients with coronary artery disease than in the control group. Moreover, miR-199a-5p was higher in patients with coronary artery disease than in the first hour and 24th hour after the operation. Accordingly, it can be said that decreased levels of these miRNAs may pave the way for the pathogenesis of coronary artery disease. miR-199a can be used to predict future adverse events in coronary artery by-pass graft surgery patients, optimize patient care, and improve the patient clinic. Clinicians need to know the morbidity and mortality of patients, and miR-199a-5p has the potential to respond to this need as an epigenetic marker.

Limitations

This is a case-control study with a relatively small sample size. MiRNAs' extracellular assets, molecular processes, oscillations, transports, molecules and mechanisms mediating these processes have not been fully elucidated. Techniques and skills are required to detect circulating miRNAs and use them in routine

laboratories. In addition, miRNAs are found in the entire circulation and more research is needed for tissue expression.

Abbreviations

CVD
Cardiovascular disease
TEKHARF
Prevalence of heart disease and risk factors in Turkish adults
miRNAs
microRNAs
I/R
Ischemia/reperfusion
LDH
Lactate dehydrogenase
BUN
Blood urea nitrogen
CK
Creatinine kinase
CK-MB
Creatinine kinase myocardial band
CPK
Creatinine phosphokinase
ROC
Receiver operating characteristic
AUC
Area under the ROC curve
NFAT
Nuclear factor of activated T-cells
NF-κB
Nuclear factor kappa B
GSK3β
Glycogen synthase kinase 3β
IGF-1
Insuline growth factor 1
COX-2
Cyclooxygenase-2

Declerations

Ethics approval and consent to participate

Gaziantep University Faculty of Medicine Ethics Committee approved the working procedure. All sampling was carried out in accordance with the 2013 Helsinki declaration guidelines and ethical rules.

Consent for publication

The manuscript is approved by all authors for publication.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

EK, ŞGY, MY, NB, and MN designed the research. EK, and ŞGY performed the experiments. MY and NB provided the resources. EK and ŞGY wrote the original draft. EK, ŞGY, MY, NB, and MN review and editing the paper. All authors read and approved the final manuscript.

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