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Determination of microRNAs associated with adverse left ventricular remodeling after myocardial infarction

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Abstract

Increasing evidence indicates that microRNA (miRNA) regulated mechanisms in myocardial healing and ventricular remodeling following acute myocardial infarction (AMI). We aim to comprehensively investigate changes of exosomal miRNA profile during the post-MI period and determine potential miRNAs associated to adverse left ventricular remodeling (ALVR). We prospectively evaluated ST-elevated MI patients with cardiac magnetic resonance imaging at the 2 weeks and 6 months after AMI ($n=10$). ALVR was defined as an increase in LV end-diastolic and end-systolic volume > 13%. The blood samples were taken for miRNA measurements at the baseline, 2 and 6 weeks after AMI. In the miRNA profile assessment, 8 miRNAs were identified that were associated ALVR (miR-199a-5p, miR-23b-3p, miR-26b-5p, miR-301a-3p, miR-374a-5p, miR-423-5p, miR-483-5p and miR-652-3p). Three of them (miR-301a-3p, miR-374a-5p and miR-423-5p) differed significantly between patients with and without ALVR during follow-up period and the rest of them during the acute phase of AMI. The detection of these miRNAs, which have different role in various pathways, necessitate future mechanistic studies unravel the complex remodeling process after AMI.

Keywords Adverse left ventricular remodeling · Myocardial infarction · MicroRNA · Exosome

Introduction

The pathophysiological basis of adverse left ventricular remodeling (ALVR) after acute myocardial infarction (AMI) includes the ionic, genomic, cellular, and extracellular levels changes that occur during the process of wound healing [1]. If cardiac stress conditions are persistent, the process can become irreversible and cellular and extracellular changes such as apoptosis, necrosis, and fibrosis can occur [2]. Depending on these changes, the fate of infarct healing can either be adaptive (reverse) or maladaptive (adverse) remodeling, respectively preserving or worsening of cardiac function and geometry [3]. However, the molecular mechanisms underlying left ventricular remodeling after AMI are not yet fully understood.

MicroRNAs (miRNAs) are non-coding RNA molecules of 19–25 nucleotides that join in gene expression regulation at the post-transcriptional level [4]. Reprogramming of gene expression underlies the pathophysiology of ALVR [5]. Therefore, miRNAs play a key role in physiological-pathological adaptations of heart [6]. miRNAs are expressed in multiple cell types including cardiomyocytes, fibroblasts, endothelial cells and inflammatory cells [7]. It is thought that these miRNAs may have a role in the regulation of basic processes such as excessive myocardial fibrosis, pathological cardiomyocyte hypertrophy and myocardial cell apoptosis. Numerous studies have shown that dysregulation in the expression of microRNA (miRNA) molecules leads to changes in various pathological processes in the heart, which are associated with post-AMI transition from cardiac hypertrophy to heart failure. Previous panel miRNA studies have shown that miR-1, miR-21, miR-24, miR-27a, miR-29 family

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members (miR-29a and miR-29b), miR-101, miR-133a, and miR-208a/b miRNAs have been implicated in left ventricular dysfunction after AMI [8–11]. It is thought that these miRNAs may have a role in the regulation of basic processes such as excessive myocardial fibrosis, pathological cardiomyocyte hypertrophy and myocardial cell apoptosis [12].

MiRNAs can be packaged in microparticles such as exosomes. Exosomal miRNAs are more stable in biological fluids compared to plasma miRNAs [13]. It is reasonable to consider that exosomal miRNAs are not released passively from cells selectively loaded into exosomes to serve specific functions, as exosomal miRNAs have a specific expression pattern under altered pathophysiological conditions [14]. However, there are limited studies about the role of exosomal miRNAs in ALVR after AMI. Therefore, an increasing interest emerges in miRNAs as potential diagnostic biomarkers and a new therapeutic target involved in the pathophysiology of AR after AMI. In the present pilot study, we explored the temporal changes of exosomal miRNAs using an extensive assessment protocol in patients after AMI. The aim was to find novel miRNAs of exosomal origin that may have a role in pathophysiological mechanisms and/or could potentially be useful as a biomarker for ALVR.

Materials and methods

Study population

This study was conducted as a multi-center prospective study between June 2015 and 2016. Patients (≥ 18 years of age) who were admitted to the hospital with STEMI for the first time ever, and who underwent primary percutaneous coronary intervention (pPCI) within 12 h after onset of chest pain were evaluated. The definition of STEMI was according to the 3rd universal definition of MI [15] and was managed according to the European Society of Cardiology guidelines [16]. Inclusion and exclusion criteria are shown in Table 1. A total of ten patients were included in the study.

Study protocol

Clinical, demographic, laboratory and radiological findings were timely recorded in patient files during follow-up. After inclusion, a follow-up cardiac magnetic resonance (CMR) imaging was performed at baseline (2 weeks) and 6 months after index event. The assessment of miRNAs expression and was conducted at baseline (1 day), 2 and 6 weeks after index event. All blood samples were collected and immediately processed at similar timepoints (08:00 and 12:00 A.M.) to prevent, if any, circadian rhythm-associated variation in miRNA expression. Serum and plasma samples were stored at − 80 °C until assayed. After collecting the serum specimens of the whole sample, parameters were measured by the same device, in the same session and by the same laboratory staff in Tissue Typing Laboratory and Genetic Diagnosis Center of Ankara Diskapi Training and Research Hospital.

Ethical approval and informed consent

The study was executed according to the principles of the Declaration of Helsinki 2013 and approved by the local ethics committee at each participating center (Decision Date, No: 06.2013, 2013/106). Written informed consent was obtained from all patients.

Biochemical parameters

Venous blood samples were taken from the antecubital vein at the 24th h of the percutaneous intervention. Afterward, the blood sample was centrifuged at 1500 rpm for 10 min to measure the determined parameters. Total cholesterol was measured on the Hitachi Modular P800 (Roche Diagnostics Corp. Indiana, Indianapolis, USA) autoanalyser by a homogeneous enzymatic colorimetric method. Low-density lipoprotein cholesterol was calculated using the Friedewald method [17].

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
STEMI diagnosis according to the 3rd universal definition of MI	Cardiogenic shock (defined as systolic blood pressure ≤ 90 mmhg) or patients in need of hemodynamic support
Admission to the emergency department between 08:00 and 12:00 A.M.	Previous silent ischemia/infarction
	Any kind of systemic inflammatory disease or autoimmune disease
	History of chronic corticosteroid or anti-inflammatory drug use
	Pregnancy or delivery within the previous 90 days
	Coronary artery bypass grafting after initial angiography
	Myocardial re-infarction during follow up

Analysis and relative quantification of miRNA

Selection of miRNAs is based on a database of disease-associated SNPs and microRNA target sites on 3′UTRs of human genes (http://mirdsnp.ccr.buffalo.edu/search.php# and http://www.mir2disease.org) and literature before 2016 (Supplementary Table 1). Exosomes were precipitated from the plasma miRCURY™ Exosome Isolation Kit—serum and plasma (Exiqon, Copenhagen, Denmark). Total RNA was extracted from the exosome pellet using the miRCURY™ RNA isolation kit-biofluids. The isolation protocol was carried out in accordance with the manufacturer's directives. The exosomes were transferred to a new microcentrifuge tube and 60 µL of Lysis solution BF containing 16.67 µg/mL of MS2 bacteriophage RNA and RNA spike-in template mixture was added to the sample. The tube was mixed and incubated for 3 min at room temperature, followed by addition of 20 µL Protein Precipitation solution BF. The tube was mixed, incubated for 1 min at room temperature and centrifuged at 11,000×*g* for 3 min. The clear supernatant was transferred to a new collection tube, and 270 µL isopropanol was added. The solutions were mixed and transfer to a binding column. The column was incubated for 2 min at room temperature, and emptied using a vacuum-manifold. After 100 µL wash solution 1 Biwa's added to the spin columns. The liquid was removed using a vacuum-manifold, and 700 µL wash solution 2 BF was added. The liquid was removed using a vacuum-manifold. To dry the columns entirely, the vacuum was extended to 2 min. The miRNAs were eluted in 50 µL RNase-free H2O and the purified miRNAs were stored at − 80 °C.

cDNA synthesis performed using the miRCURY LNA™ Universal Real-time (RT) microRNA cDNA synthesis kit (Exiqon). 7 µL RNA was reverse transcribed in 35 µL reactions using the miRCURY LNA™ Universal RT microRNA PCR, Polyadenylation and cDNA synthesis kit (Exiqon). cDNA was diluted 50× and assayed in 10 µL PCR reactions according to the protocol for miRCURY LNA™ Universal RT microRNA PCR; each microRNA was assayed once by qPCR on the microRNA Ready-to-Use PCR, Serum/Plasma Focus panel using ExiLENT SYBR® Green master mix. Negative controls excluding template from the reverse transcription reaction was performed and profiled like the samples. The amplification was performed in a LightCycler® 480 Real-Time PCR System (Roche) in 384 well plates. The amplification efficiency was calculated using algorithms similar to the LinReg software. Furthermore, assays must be detected with $Ct < 37$ to be included in the data analysis. All results were expressed as *Ct* and normalized to the calculated mean *Ct* of each sample (ΔCt). The relative expression was calculated using the comparative *Ct* method ($2^{-\Delta\Delta Ct}$). miRNAs with values below the limit of detection were not included in the analysis.

Cardiac magnetic resonance imaging

Cardiac magnetic resonance imaging was performed using a 3-T scanner (Magnetom Skyra, Siemens Medical Systems, Erlangen, Germany) 2 weeks and 6 month, and the imaging protocol entailed acquiring one four-chamber view, cine short-axis sections (10-mm intervals for slice thickness of 6 mm), and one two-chamber view. Left ventricular (LV) systolic function indices were evaluated with the retrospective electrocardiogram-gated turbo-fast low angle shot (turbo-FLASH) sequence. Acquisition settings were: echo time 1.42 ms, repetition time 39 ms, flip angle 57°, voxel size $1.67 \times 1.67 \times 6$ mm. LV function and volumes were measured using Syngo.via imaging software (Siemens Healthineers, Erlangen, Germany). LVEDV and LVESV were calculated with short-axis based planimetry from basal to apical level. Stroke volume was calculated as LVEDV minus LVESV, and LV ejection fraction (LVEF) was calculated as follows: $LVEF = [(LVEDV - LVESV)/LVEDV] \times 100$. The definition of ALVR was $a \geq 13\%$ increase in LVEDV and LVESV at the 6 months after AMI [18].

Statistical analysis

Statistical evaluation was performed using the Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, IL) program. The normal distribution of the data was evaluated with the Shapiro–Wilk test. Normally distributed numerical variables were shown as mean ± standard deviation, while numerical variables not showing normal distribution were shown as median (minimum, maximum). Categorical variables were expressed as numbers and percentages. Chi-Square, and Yates' correction and Fisher's Exact test were used for comparison of categorical data. Student *T*-test or Mann–Whitney *U* test was used to compare numerical variables between two groups according to the distribution of normality. miRNA and CMR levels in post-MI periods were evaluated using mixed model for repeated measurements. $p < 0.05$ (*) value was considered significant in statistical analysis.

Results

The study consisted of 10 male patients with a mean age of the cohort was 51.6 ± 6.0 (43–60) years. Four (40%) patients had a history of smoking, 2 (20%) patients had hypertension and 1 patient (10%) had diabetes mellitus. When compared with baseline LV dimensions, ALVR evaluated by CMR was detected in 5 (50%) patients in the 6th month. There was no significant difference in baseline demographic and laboratory findings in patients with ALVR compared to RLVR (Table 2).

Table 2 Baseline characteristics

Variables	Total population	Remodeling		*p*
		RLVR	ALVR	
	n = 10	*n* = 5	*n* = 5	
Demographic findings				
Age, years	51.6 ± 6.0	51.0 ± 7.0	52.2 ± 5.6	0.773
BMI, kg/m^2	25.6 ± 1.9	25.6 ± 1.8	25.6 ± 2.2	0.991
Smoke, *n* (%)	4(40.0)	2(40.0)	2(40.0)	0.999
Hypertension, *n* (%)	2(20.0)	1(20.0)	1(20.0)	0.999
DM, *n* (%)	1(10.0)	–	1(20.0)	0.999
Clinics findings				
SBP, mm Hg	124.6 ± 17.5	126.6 ± 15.2	122.6 ± 21.2	0.741
DBP, mm Hg	74.2 ± 10.7	73.2 ± 8.0	75.2 ± 13.8	0.786
HR, beat per minute	78.7 ± 8.7	82.6 ± 9.5	74.8 ± 6.5	0.167
Door to balloon time, min	46.0 ± 9.6	45.1 ± 10.3	46.8 ± 10.0	0.809
Symptom to balloon time, min	316.5 ± 70.6	318.0 ± 73.0	315.0 ± 76.6	0.809
Anterior infarct, *n* (%)	7(70.0)	3(60.0)	4(80.0)	0.999
Laboratory findings				
Peak troponin, nq/L	30(15–99)	24(16–33)	40(15–99)	0.151
CK-MB, nq/L	87.5(29–267)	115(29–267)	79(45–89)	0.150
Total cholesterol, mg/dL	201(134–312)	238(155–262)	184(134–312)	0.421
LDL, mg/dL	110(83–170)	122(102–170)	87(83–169)	0.095
HDL, mg/dL	40.9 ± 6.0	38.2 ± 4.8	43.6 ± 6.4	0.170
Triglyceride, mg/dL	192.5(90–312)	198(90–224)	187(116–312)	0.690
WBC, × 10^9/L	12.6 ± 3.0	12.9 ± 3.8	12.3 ± 2.4	0.834
Hemoglobin, g/dL	14.1 ± 0.9	14.1 ± 0.9	14.0 ± 0.9	0.870
Neutrophil, × 10^9/L	70.4 ± 6.6	70.3 ± 7.6	70.6 ± 6.4	0.939
Lymphocytes, × 10^9/L	2.7 ± 0.2	2.8 ± 0.2	2.6 ± 0.1	0.169
Monocytes, × 10^9/L	9.9 ± 1.4	10.5 ± 1.3	9.2 ± 1.3	0.135
Platelets, × 10^9/L	313.5 ± 55.9	296.4 ± 64.3	330.6 ± 46.6	0.363

Categorical variables were shown as number percentages. Numerical variables with normal distribution were shown as mean ± standard deviation, while numerical variables not showing normal distribution were shown as median (min–max)

ALVR adverse left ventricular remodeling; *BMI* Body mass index; *DM* diabetes mellitus; *SBP* systolic blood pressure; *DBP* diastolic blood pressure; *HR* heart rate; *EF* ejection fraction; *LDL* low density lipoprotein; *HDL* high density lipoprotein; *WBC* white blood cell; *RLVR* reverse left ventricular remodeling

At the 2 weeks after AMI evaluation by CMR, the mean EDV, median ESV, and mean LVEF (%) levels were not significantly different between patients with ALVR and RLVR. However, at the 6 months after AMI in ALVR group, mean EDV, and median ESV, were increased, mean LVEF (%) was decreased (Table 3).

A panel of 178 miRNAs (Human Serum & Plasma miScript miRNA PCR Array) was profiled in ten patients (Supplement Table 2). In all candidate exosomes, 8 miRNAs (miR-199a-5p, miR-23b-3p, miR-26b-5p, miR-301a-3p, miR-374a-5p, miR-423-5p, miR-483-5p and miR-652-3p) were associated with ALVR during the follow-up period (Table 4) (Fig. 1). According to this; at the 1st day after AMI, miR-199a-5p (fold change: 0.30; $p = 0.050$), miR-23b-3p (fold change: 0.56; $p = 0.050$), and miR-483-5p (fold change: 0.34; $p = 0.034$) were significantly downregulated and miR-26b-5p (fold change: 2.35; $p = 0.028$) and miR-652-3p (fold change: 1.79; $p = 0.050$) were significantly upregulated in ALVR compared to RLVR groups (Fig. 2). At the 2 weeks after AMI, miR-374a-5p (fold change: 0.28; $p = 0.050$) was significantly downregulated in ALVR compared to RLVR groups. At the 6 weeks after AMI, miR-301a-3p (fold change: 0.24; $p = 0.050$), and miR-374a-5p (fold change: 0.48; $p = 0.050$) were significantly downregulated and miR-423-5p (fold change: 1.76; $p = 0.047$) was significantly upregulated ALVR compared to RLVR group (Fig. 3). The fold changes of miRNAs in ALVR versus RLVR group are shown in Table 4.

Table 3 Baseline and follow-up values for CMRI evaluation

Variables	Remodeling	2 weeks	6 months	p^1	p^2	Δp
Ejection fraction, %	RLVR $n=5$	48.4 ± 13.6	50.4 ± 13.1	0.028*	0.956	0.002*
	ALVR $n=5$	48.8 ± 7.5	41.6 ± 11.3	0.029*		
EDV, mL	RLVR $n=5$	183.4 ± 31.3	178.0 ± 27.0	0.125	0.879	0.012*
	ALVR $n=5$	187.0 ± 40.8	214.6 ± 50.8	0.047*		
ESV, mL	RLVR $n=5$	82.8(53.4–162)	78.8(52.8–143.9)	0.203	0.754	0.019*
	ALVR $n=5$	121.4(61.6–122)	144.6(64–195.3)	0.058		
Stroke volume, mL	RLVR $n=5$	87.4 ± 29.1	109.8 ± 22.1	0.102	0.742	0.227
	ALVR $n=5$	82.3 ± 16.3	79.9 ± 31.5	0.898		

Numerical variables with normal distribution were shown as mean ± standart deviation, while numerical variables not showing normal distribution were shown as median (min–max)

ALVR adverse left ventricular remodeling; *EDV* end-diastolic volume; *EF* ejection fraction; *ESV* end-systolic volume; *LV* left ventricle; *MRI* magnetic resonance imaging; *RLVR* reverse left ventricular remodeling

*$p < 0.05$ shows statistical significance

p^1: 1st day vs 6th week in remodeling groups

p^2: comparison of baseline results (ALVR vs RLVR)

Δp: comparison of the changes in the follow-ups (ALVR vs RLVR)

Discussion

The current study was the first to assess exosomal miRNA expression in more than one period in patients with AMI. We determined that 8 miRNAs from the panel of 178 exosomal miRNA were significantly up- or downregulated in patients with ALVR (miR-199a-5p, miR-23b-3p, miR-26b-5p, miR-301a-3p, miR-374a-5p, miR-423-5p, miR-483-5p, miR-652-3p). The vast majority of these miRNAs differed significantly on the 1st day after AMI. Furthermore, in serial measurements, only miR-374a-5p was downregulated on the 2 and 6 weeks post-MI. MiR-301a-3p was downregulated on the 6 weeks post-MI, and miR-423-5p was upregulated on the 6 weeks post-MI. Serial measurements gave information about the period in which miRNAs play a role in cardiac remodeling.

The stability of circulating miRNAs and their regulation in pathological conditions has sparked interest in their use as biomarkers. The heart reacts to hemodynamic overload and injury by stimulating myocyte hypertrophy, reprogramming of gene expression and remodeling of extracellular matrix [19]. In myocardial infarction pathology, miRNAs are involved in the regulation of different types of cell death, including necrosis and apoptosis [20, 21]. Increasing evidences have shown an important role for microRNA (miRNA)-regulated mechanisms in myocardial healing or ALVR after AMI [22–25]. Overexpression of some miRNAs (miR-133a-3p, miR-1a-3p, miR-27b-3p and miR-208a-3p) preserves cardiac functions, while inhibition has been associated with hypertrophy and dysfunction. On the other hand, some miRNAs (MiR-21-5p, miR-378-5p and miR-223-3p) were found to be overexpressed in heart failure patients [26]. However, there may be as yet unidentified miRNAs that may play a role in both MI and ALVR.

MiR-199a-5p has been shown to be abundant in the myocardium and its downregulation plays an important role in the prevention of cardiac hypertrophy and heart failure [27]. It is reported that miR-199a-5p rapidly decreases in cardiomyocytes exposed to hypoxia [28]. In studies performed in different heart failure models, it has been shown that miR-199a-5p upregulation prevents cell death [29, 30]. In contrast, other studies have been shown that high expression of miR-199a-5p after MI contributes to heart failure development [31–33]. We found that baseline expression of miR-199a-5p was downregulated in ALVR group, while there was no difference in the miR-199a-5p expression between ALVR and RLVR groups in the later periods. Low expression of miR-199a-5p may play a role in ALVR with potential mechanisms such as apoptosis, pathological cardiac myocyte hypertrophy and cardiac homeostasis due to inflammatory response [28, 34–36].

MiR-23b-3p, which plays a role in monocyte/macrophage inflammatory reaction, is upregulated under cardiac stress conditions [37, 38]. Zhang et al. [39] showed that miR-23b-3p was upregulated at the time of acute MI and decreased after percutaneous coronary intervention. This may be due to the post-MI period miR-23b-3p is associated with migration of phagocytes, proliferation of mononuclear leukocytes and cell movement of smooth muscle cells [40]. In our study, baseline expression of miR-23b-3p was downregulated in the ALVR group. These findings suggest that downregulation of miR-23b-3p may be associated with an unfavorable inflammatory response. On the other hand, the excessive expression of miR-26b-5p, which plays a role in the development of cardiac hypertrophy in physiological

Table 4 miRNAs expression values in remodeling groups

miRNAs	Remodeling	First day	2 weeks		6 weeks		p^2
		Mean	Mean	Fold change[2]	Mean	Fold change[3]	
miR-199a-5p	RLVR	−5.09	−3.58	0.35	−4.29	1.64	0.037*
	ALVR	−3.33	−3.97	1.56	−4.88	1.88	0.135
	Fold change[1]	0.30	1.31		1.50		
	p^1	0.050*	0.999		0.806		
miR-23b-3p	RLVR	−1.14	−0.61	0.69	−0.81	1.15	0.165
	ALVR	−0.29	−0.81	1.43	−0.72	0.94	0.472
	Fold change[1]	0.56	1.15		0.94		
	p^1	0.050*	0.917		0.465		
miR-26b-5p	RLVR	−2.96	−3.23	1.20	−2.90	0.79	0.272
	ALVR	−4.19	−3.70	0.72	−3.00	0.60	0.688
	Fold change[1]	2.35	1.40		1.07		
	p^1	0.028*	0.917		0.822		
miR-301a-3p	RLVR	−5.51	−4.88	0.65	−5.80	1.89	0.370
	ALVR	−4.43	−5.37	1.92	−3.77	0.33	0.288
	Fold change[1]	0.47	1.41		0.24		
	p^1	0.317	0.180		0.050*		
miR-374a-5p	RLVR	−5.05	−5.99	1.92	−5.68	0.81	0.338
	ALVR	−5.83	−4.14	0.31	−4.62	1.39	0.415
	Fold change[1]	1.71	0.28		0.48		
	p^1	0.248	0.050*		0.050*		
miR-423-5p	RLVR	−0.77	−1.00	1.17	−0.66	0.79	0.690
	ALVR	−1.28	−1.28	1.00	−1.48	1.15	0.472
	Fold change[1]	1.43	1.21		1.76		
	p^1	0.140	0.917		0.047*		
miR-483-5p	RLVR	−4.41	−5.39	1.97	−5.00	0.76	0.610
	ALVR	−2.86	−4.67	3.51	−3.77	0.54	0.366
	Fold change[1]	0.34	0.61		0.43		
	p^1	0.034*	0.564		0.643		
miR-652-3p	RLVR	−2.90	−3.32	1.34	−3.78	1.38	0.611
	ALVR	−3.74	−3.21	0.69	−2.67	0.69	0.745
	Fold change[1]	1.79	0.93		0.46		
	p^1	0.050*	0.653		0.142		

ALVR adverse left ventricular remodeling; *miRNAs* microRNA; *RLVR* reverse left ventricular remodeling

*$p < 0.05$ shows statistical significance

p^1: comparison of baseline and follow-up values of miRNAs (between remodeling groups)

p^2: comparison of the changes in the follow-ups of miRNAs (within remodeling groups)

Fold change[1]: comparison of baseline values of miRNAs (between remodeling groups)

Fold change[2]: MiRNAs change in 2nd weeks compared to 1st day (within remodeling groups)

Fold change[3]: MiRNAs change in 6th weeks compared to 2nd weeks (within remodeling groups)

conditions possible as an outcome of blocking the initiation of autophagy, has been found to be associated with increased cardiovascular events in patients with AMI [41, 42]. This hypothesis is consistent with the upregulation of miR-26b-5p expression in our study. Previous studies have shown that miR-26b-5p is upregulated in patients with heart failure [43, 44].

MiR-483-5p, which plays a role in the regulation of apoptosis and are upregulated during the acute MI process, were found at high levels in our study, especially in patients with RLVR. This role may be due to the triggering of IGF2 by the presence of miR-483-5p upregulation and thus the involvement of pro-inflammatory mechanisms in the regulation of NF-kB- and interleukin-6 mediated pathways [45]. To the best of our knowledge, we show for the first time in this study that downregulation of MiR-483-5p expression is associated with ALVR. Therefore, the role of miR-483-5p in LV remodeling requires further research, examining

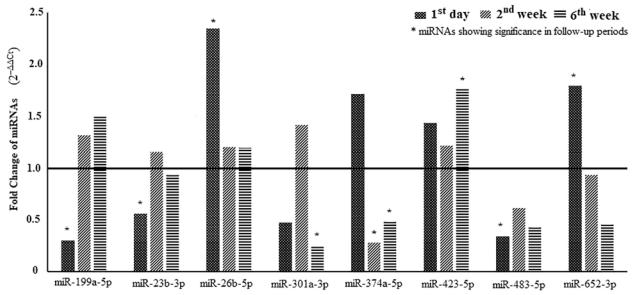

Fig. 1 Fold changes of miRNA regulation in adverse left ventricular remodeling compared to reserve left ventricular remodeling

host gene transcription and protein expression rather than a biomarker.

In the early phase of AMI, the upregulation of MiR-652-3p expression was associated with ALVR. Huang et al. [46] found that the inhibition of miR-652-3p promotes cyclin D2 expression, increasing endothelial repair and decreasing atherosclerosis. Wang et al. [47] showed that an anaerobic condition triggers the accumulation of fragmented mitochondria and leads to apoptotic cell death by a mechanism of MFACR-dependent inhibition of miR-652-3p and uninterrupted expression of the MTP18 protein.

We found that majority of ALVR-associated miRNAs developed significant responses in the early phase AMI. However, in the follow-up period, differences were found in the expression of miR-423-5p, miR-301a-3p, and miR-374a-5p. It is known that the level of circulating miR-423-5p is a useful biomarker in demonstrating LV remodeling after AMI [48]. Bauters et al. [49] found that an increase in miR-423-5p levels over periods compared to baseline post-MI. Consistently, in the current study, it was observed that the miR-423-5p expression at the 6 weeks post-MI were higher in the patients with ALVR. MiR-301a, second late onset miRNA, regulates calsarcin-1 and cofilin-2, which have roles in the development of cardiomyopathy [50]. In addition, miR-374a-5p may have protective effects against myocardial ischemia–reperfusion injury and can be expected to be upregulated in the early phase of acute MI due to percutaneous coronary intervention [51]. It seems consistent that the miR-301a-3p level, which has been shown to be indirectly related to heart failure, showed a significant difference at 6 weeks post-MI, but it was expected that miR-374a-5p would show an increased response in the early period of AMI. Therefore, more evidence is needed to understand the effect of miRNAs in ALVR after AMI.

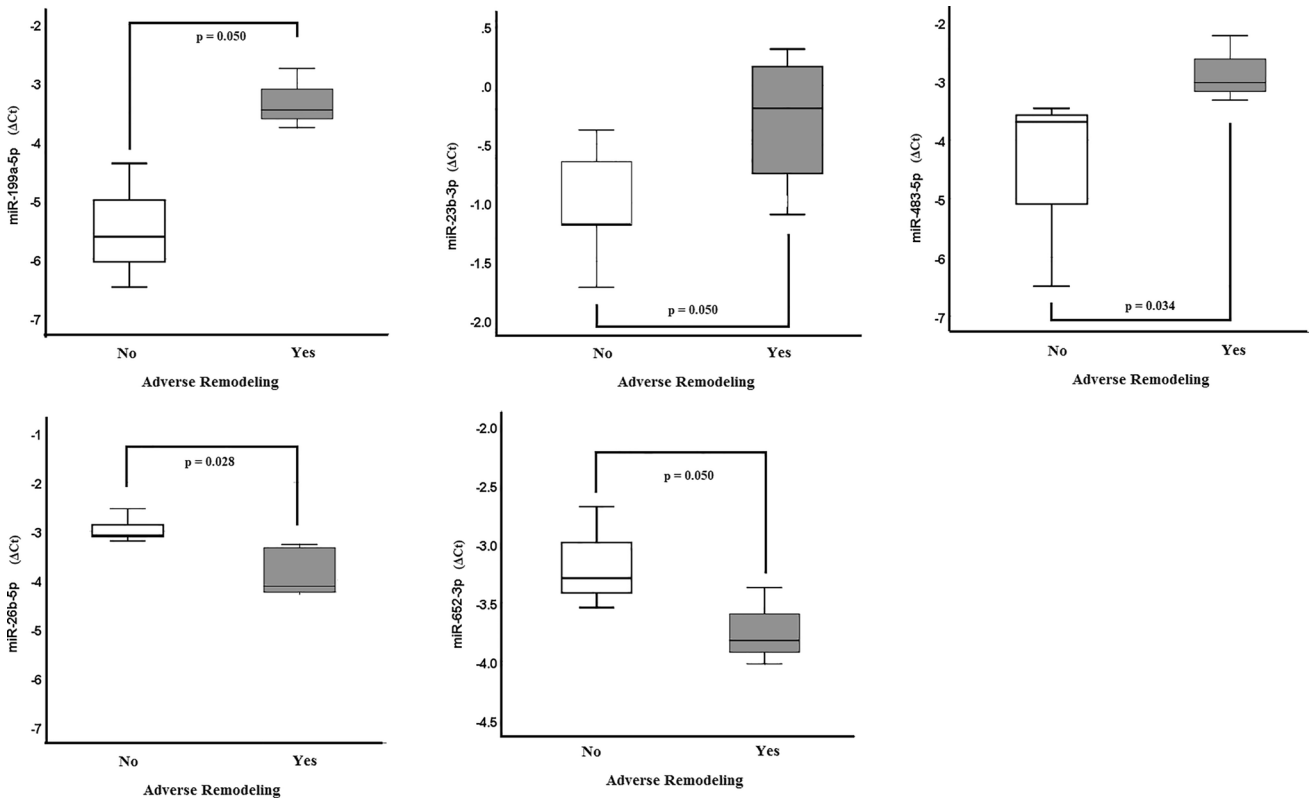

Fig. 2 Comparison the miRNAs (Δ*Ct*) at 1st day of post-MI between adverse left ventricular remodeling compared to reserve left ventricular remodeling

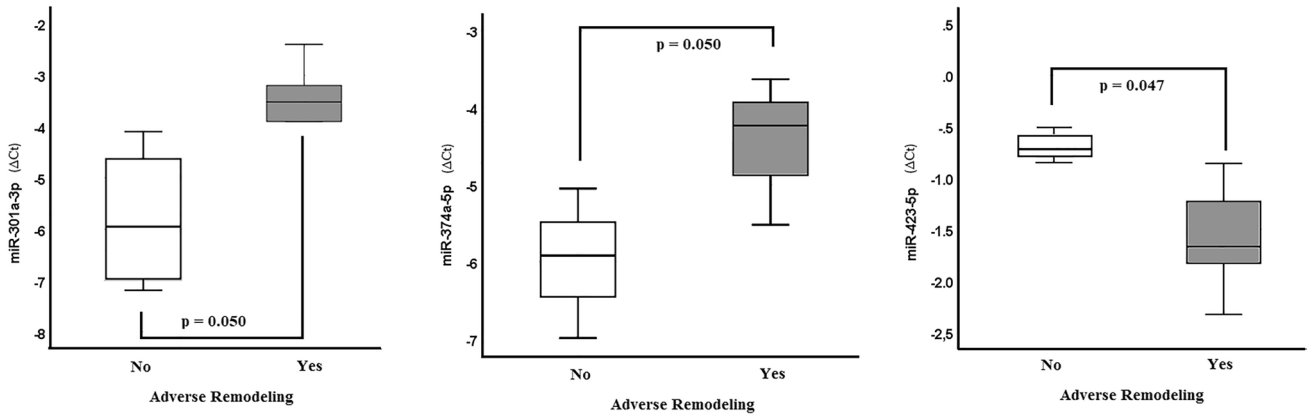

Fig. 3 Comparison the $\log 2^{-\Delta CT}$ of miRNAs at 6th week of post-MI between adverse left ventricular remodeling compared to reserve left ventricular remodeling

Cardiac remodeling can occur weeks to months after reperfusion. Exosomal miRNA is known to show extra stability, even under different storage conditions. Therefore, post-AMI exosome miRNAs may be good biomarkers based on their stability under various storage conditions, with future diagnostic and therapeutic uses. Although clinical indicators or circulating biomarkers are helpful in determining the risk of ALVR after AMI, but they have certain limitations. Increasing evidence indicates that miRNAs expressions may be an important biomarker in detecting high-risk patients post-MI. Besides, miRNAs may be a new individualized targeted therapy on adverse LV remodeling. MiRNA-based therapies will have the ability to modulate multiple target genes or networks involved in the cardiac remodeling process [52]. The regulation of apoptotic pathway could represent a therapeutic approach to treat apoptotic-related cardiac disease [53]. Thus, normalizing miRNA expression in the heart represents a new approach to the pharmacotherapy of heart disease.

There are some important limitations of our study. It was recognized that this clinical study sample was small with limited power for full adjustment of clinical and RNA-based biomarkers. On the other hand, we could not conduct experiments to modulate miRNA levels in native human tissues. However, this was designed as a small-sized preliminary study rather than one to determine a cause-effect relationship. Myocardial healing processes comprise complex interactions that involve many molecules. Hence, eliminating individual variations and interactions are almost impossible. In addition, our entire study population was male. MiRNA transcriptome are associated with significant gender differences that may affect cardiac homeostasis [54, 55].

As a conclusion; it was observed that certain exosomal miRNAs are associated with ALVR after AMI. It was also found that the majority of miRNAs showed significant changes in the early period of AMI, while the remaining ones showed changes over time. The detection of miRNAs with, different roles in various pathways might provide insight for ALVR after AMI. Investigating the roles of miRNAs in modulating various aspects of the LV remodeling process will contribute to identifying the potential implications of miRNA biology in the field of heart failure. However, larger confirmatory studies to investigate the miRNA's of interest are needed and pre-clinical experiments are necessary to understand their mechanistic role in the development of ALVR.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

Declarations

Conflict of interest The authors declare they have no conflicts of interest.

Ethical approval The study was performed in accordance with the Declaration of Helsinki, and approved by the Faculty Of Medicine Non-Drug Clinical Research Ethics Committee of the Ankara Yildirim Beyazit University, on 24 June 2013, under Decision No. 2013/106.

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