Cor et Vasa

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/crvasa

Původní sdělení | Original research article

Elevated levels of miR-499 protect ischemic myocardium against uric acid in patients undergoing off-pump CABG

Fatemeh Pourrajab[a,b], Marzieh Sharifi[a], Seyedhossein Hekmatimoghaddam[c], Masoud Khanaghaei[a], Mahdi Rahaie[d]

[a] *International Campus of Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
[b] *Department of Clinical Biochemistry and Molecular Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
[c] *Department of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
[d] *Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran*

ARTICLE INFO

Article history:
Received: 13. 10. 2015
Received in revised form: 6. 1. 2016
Accepted: 7. 1. 2016
Available online: 9. 2. 2016

Klíčová slova:
Ischemie
Kyselina močová
Mechanismus
miR-499

SOUHRN

Cíl: Tato studie se zabývala rozdíly v expresi miR-499 koronárním bypassu (coronary artery bypass grafting, CABG) bez mimotělního oběhu (off-pump) a hodnotila vztah mezi expresí miR-499 na jedné straně a ejekční frakcí levé komory (%) a koncentrací kyseliny močové (uric acid, **UA)** na straně druhé.
Metody a výsledky: Bylo vybráno 70 pacientů indikovaných k CABG a v různých časových bodech u nich byla měřena ejekční frakce levé komory, odebírány krevní vzorky a měřeny hemodynamické údaje. Po CABG bez mimotělního oběhu byla zjištěna statisticky významná korelace mezi hodnotami cirkulujícího srdečního troponinu I (cTnI), UA a miR-499 ($p < 0{,}001$). Pooperační hodnota ejekční frakce byla dále silně ovlivněna předoperační mírou exprese miR-499 ve vztahu ke koncentracím UA ($p < 0{,}005$).
Závěr: Tlakové přetížení a ischemie vyvíjejí oxidační stres (OS) na kardiovaskulární systém. Zvýšená exprese miR-499 v ischemizovaném myokardu jej chrání před OS; současně byla zjištěna nižší ochrana při nižší expresi miR-499 ve vztahu ke koncentracím UA.

Keywords:
Ischemia
Mechanism
MiR-499
Uric acid

ABSTRACT

Objective: This study screens the miR-499 differences in off-pump CABG and evaluates the relationship of miR-499 with heart ejection fraction (%) and uric acid (UA) levels.
Methods and results: 70 candidates undergoing CABG were selected and left ventricular EF, blood samples and hemodynamic data were taken at different time points. Statistically significant correlation was found between circulating levels of cardiac troponin I (cTnI), UA and miR-499, after off-pump CABG ($p < 0.001$). Furthermore, post-operative EF was strongly affected by pre-operative miR-499 expression levels vs. UA ($p < 0.005$).
Conclusion: Pressure overload and ischemia result in oxidative stress (OS) on the cardiovascular system. The elevated levels of miR-499 expression in ischemic myocardium protect it from OS, while declining protective effects was observed in decreased levels of miR-499 in UA concentrations.

Address: Dr. Fatemeh Pourrajab, Ph.D., Department of Clinical Biochemistry and Molecular Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran, P.O. 8915173149; e-mail: mina_poorrajab@yahoo.com
DOI: 10.1016/j.crvasa.2016.01.002

Please cite this article as: F. Pourrajab, et al., Elevated levels of miR-499 protect ischemic myocardium against uric acid in patients undergoing off-pump CABG, Cor et Vasa 58 (2016) e600–e608 as published in the online version of *Cor et Vasa* available at http://www.sciencedirect.com/science/article/pii/S0010865016000047

Introduction

Studies have demonstrated that there are significant expression profile changes in a bunch of miRNAs in both ischemic/normal heart tissues and peripheral blood. Some miRNAs perform bold biological functions of protecting or damaging cardiac cells in myocardial ischemia through different pathways [1–4].

Evidence has indicated that miR-499 is produced almost exclusively in the heart. Plasma miR-499 concentrations are increased in all individuals with acute myocardial infarction but were below the limit of detection for all individuals in the other patient groups [2–4].

Also, in patients with heart failure, from plasma levels of heart-associated (miR-1, -133a, -208b, and -499) miRNAs, only miR-499 was significantly elevated (2-fold), whereas no significant changes in selected miRNAs were observed [5].

Remarkably, plasma levels of these miRNAs are reported to be not affected by a wide range of clinical confounders, including age, sex, body mass index, kidney function, systolic blood pressure, and white blood cell count [4,5].

On the other side, UA has been associated with adverse cardiovascular events in several settings. Its utility in patients undergoing surgical revascularization has, however, been associated with poorer survival after CABG [6–9]. Elevated serum UA levels have relationship with inflammatory markers and have deleterious effects on the endothelium [10]. Ischemia/reperfusion injuries can cause OS and can be monitored by using UA as a parameter of OS. During ischemia, there is a cascade of reactions due to a decrease in tissue blood flow, which results in increasing UA expression [11,12]. UA concentrations in patients undergoing CABG are a measure for ischemia/reperfusion injury [13].

We hypothesized that there is a relationship between UA and miR-499 levels with cardiac function in patients with coronary severe ischemia undergoing off-pump CABG, compared to their baseline. To testify this hypothesis, we analyzed the expression of miR-499 by using quantitative RT-PCR on samples at different time points in CABG candidates and then the clinical relevance of miR-499 levels with myocardial biochemical markers and hemodynamic changes were evaluated [1].

Material and methods

Patients

In our procedure, 70 patients, aged 40–79 years, were elective for undergoing off-pump CABG surgery and were consecutively enrolled in a prospective observational setting. No exclusion criteria other than redo of CABG and a minimum of three anastomoses were defined. The study was conducted according to the recommendations by the Declaration of Helsinki on Biomedical Research Involving Human Subjects and also the study protocol was approved by the Medical Ethics Committee of Shahid Sadouqhi University of Medical Sciences. Written informed consent was obtained from each individual [11,13].

Clinical samples

Baseline clinical data, including medical history, cardiac risk factors, operative details, New York Heart Association functional class, and the European System for Cardiac Operative Risk Evaluation (EuroSCORE), were collected prospectively by an experienced full-time data collector [9,11]. Blood samples were collected before CABG surgery and post-surgery, for determining the biochemical markers. All samples were immediately centrifuged for 10 min at 3 000 × g, and stored at –80 °C until analysis. Serum enzyme activities were measured at 37 °C [14,15].

Biochemical markers

CTnI levels as well as CK-MB activities in the serum were measured before and after operation. CTnI was assayed with the Stratus II system (Dade Behring, Marburg, Germany) according to the manufacturer's instructions. Activities of CK and CK-MB were measured with an immunoinhibitory assay at 37 °C, by means of an N-acetylcysteine-activated system (CK NAC-Method, Roche Diagnostics, Mannheim, Germany) [14]. UA was measured by using the same sample and ADVIA 1650 General Chemistry Analyzer (Siemens Diagnostics Solutions, Tarrytown, NY) [9].

Anesthetic technique

Premedication consisted of temazepam (10 mg), given orally 2–3 h before the procedure started. Anesthesia consisted of a balanced opiate-based general anesthesia technique. Induction took place by means of infusion of propofol (1.5–2 mg/kg), pancuronium (0.1 mg/kg) and fentanyl (7 mg/kg). Anesthesia was maintained with nitrous oxide in oxygen and continuous propofol infusion (10–20 ml/h), remifentanil (0.25–1 mg/kg) and pancuronium as required. Hypertension was treated with vasodilators (nitroglycerin and nitroprusside). A mean arterial pressure of 60 mmHg or higher and a heart rate less than 70 beats per min was maintained. Heparin was administered at 150 IU/kg. After all anastomoses were completed, heparin was neutralized with protamine chloride 120 IU/150 IU. All patients received standardized postoperative care [32].

Surgical procedure

Median sternotomy and harvesting of the internal mammary artery were followed by full exposure of the coronary artery branches to be revascularised. The revascularization was performed on the beating heart using the Medtronic Octopus device (MedtronicW, Minneapolis, USA), while using intraluminary shunt during procedure to decrease the acute ischemic insult for myocardium. Most patients had at least one arterial graft (left internal thoracic artery, LITA). The left anterior descending (LAD) was revascularized first in all patients using the LITA. The left-sided grafts were performed followed by the right-sided grafts. Patients were fully heparinized at completion of harvesting of the conduits. Heparinized state was reversed using protamine and closure performed as routine [32].

Echocardiographic study to assess global LV function

Patients underwent a total of 4 contrast-enhanced echocardiographic studies; before, 1 day after, 4 days after, and 1 month after operation, according to the references 15. Global LV function and 5 anatomic regions (anterior, apex, lateral, inferior, and septum) were assessed with respect to

regional LV function before and after contrast enhancement using the semiquantitative methods recommended by the American Society of Echocardiography [15].

RNA extraction

Total RNA was extracted from 100 µL of plasma, using the mirVana PARIS kit (Ambion, Warrington, United Kingdom) according to the manufacturer's instructions and without enrichment for small RNAs and subsequently eluted in 50 µL nuclease-free water. Subsequently, potential genomic DNA contamination was eliminated using DNA--free kit (Ambion) [4,5].

cDNA synthesis and quantitative RT-PCR

RNA (15 µL) was used per 20-µL reaction to generate cDNA using the miScript kit (Qiagen, Venlo, The Netherlands), which is designed to specifically detect mature microRNAs. The 20-µL reaction mix was then diluted × 4 in nuclease-free water, and 2 µL of cDNA was added per quantitative reverse transcriptase-polymerase chain reaction, using BR SYBR-green supermix for IQ (Quanta Biosciences, Amsterdam, Netherlands) in a MyIQ iCycler (Bio-Rad, Veenendal, Netherlands) device [4,5].

All reactions were run in triplicate and miRNA expressions were normalized to small RNA U6, with the similar efficiency of miRNAs. Data of qRT-PCR were demonstrated by nominal CT value (normalized to U6), and fold changes were calculated by $2^{|\Delta\Delta CT|}$. Higher nominal CT value means lower microRNA expression level [1,16].

Statistical analysis

All values are presented as mean ± standard deviation. Results of cardiac enzymes, hemodynamic parameter changes, and qRT-PCR results between time intervals were analyzed by paired t-test. Spearman correlation coefficients were used to examine the relationship between miR-499 and cardiac enzymes levels. $p < 0.05$ was considered statistically significant [1,16].

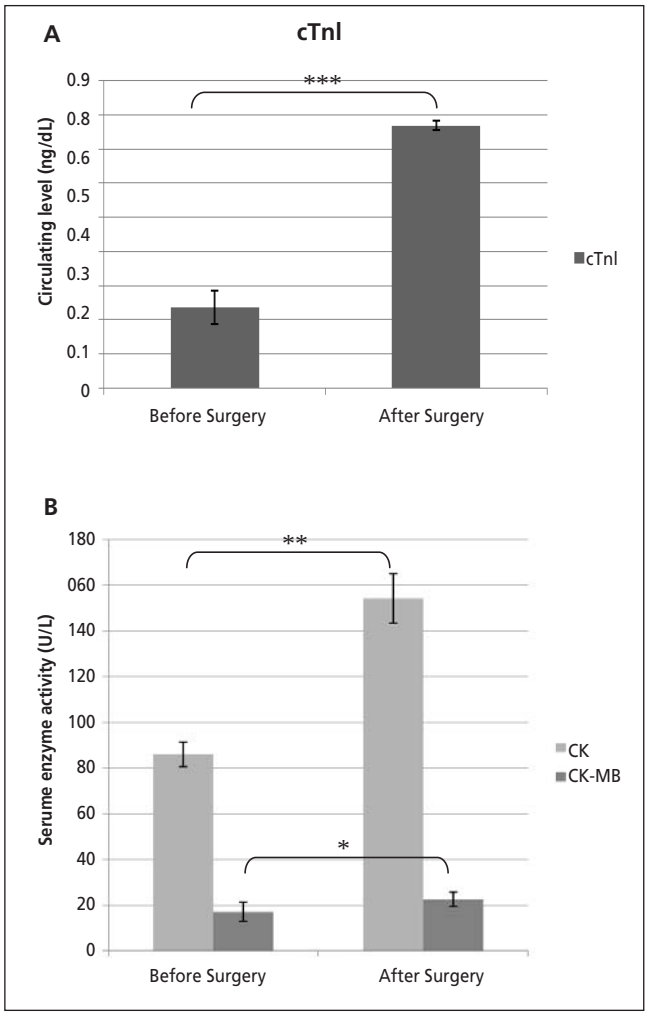

Fig. 1 – Comparison of release patterns of cardiac troponin I (A), CK and CK-MB (B), before (on day 0), and after coronary artery bypass surgery (on day 4 post-surgery). Results are given as median and local 95% confidence levels based on the median. Error bars represent standard deviation of the mean (SD). * p < 0.05; ** p < 0.01; *** p < 0.005; Significantly different after surgery.

Results

Biomarkers of cardiac injury changed during off-pump CABG

None of the patients had increased levels of cTnI as well as CKs activity, preoperatively. Serum concentrations of cTnI in all patients included in the study were low and about the normal range preoperatively (mean of 0.22 ± 0.05 ng/dL). However, circulating levels of cTnI significantly increased after surgery and on day 4 post-operation reached a mean of 0.77 ± 0.01 ng/dL; p < 0.005 (Fig. 1A). Also, none of the patients had increased levels of CK and CK-MB activity (mean of 86 ± 5.25 and 17.20 ± 4.10 U/L, respectively), preoperatively. But following off-pump surgery, plasma CKs activity increased to mean of 154.20 ± 10.87 and 22.66 ± 3.20 U/L, $p < 0.01$ (Fig. 1B).

Expression levels of miR-499 is elevated in myocardium ischemia

Instead of nominal CT value, we used relative expression and fold changes in miR-499 analysis (Fig. 2). The mean expression of miR-499 was examined at two different time points in all patients (Fig. 2A). As shown in Fig. 2B, the expression levels of miR-499 at time point T1 pre-operatively, were considerably higher than time point T2, 4 days post-revascularization surgery (the median of 2.26 $\pm$ 0.830 and 0.95 $\pm$ 0.20; $p < 0.0005$, respectively), with median fold change of 0.42, which shows a reduced level. The considerable higher levels of miR-499 in ischemic condition before revascularization surgery, is indicating that hypoxia would increase the expression of miR-499 in the ischemic myocardium.

MiR-499 expression negatively correlated with myocardium damage

We used the expression levels of miR-499 to analysis the correlation with CKs and cTnI. Pre-surgery in ischemic conditions, the miR-499 levels showed higher expression levels in all subjects and were observed to reduce to considerable lower levels after revascularization surgery. Conversely, it was reversed for myocardial injury biomarkers cTnI and CK-MB. Before surgery in ischemic conditi-

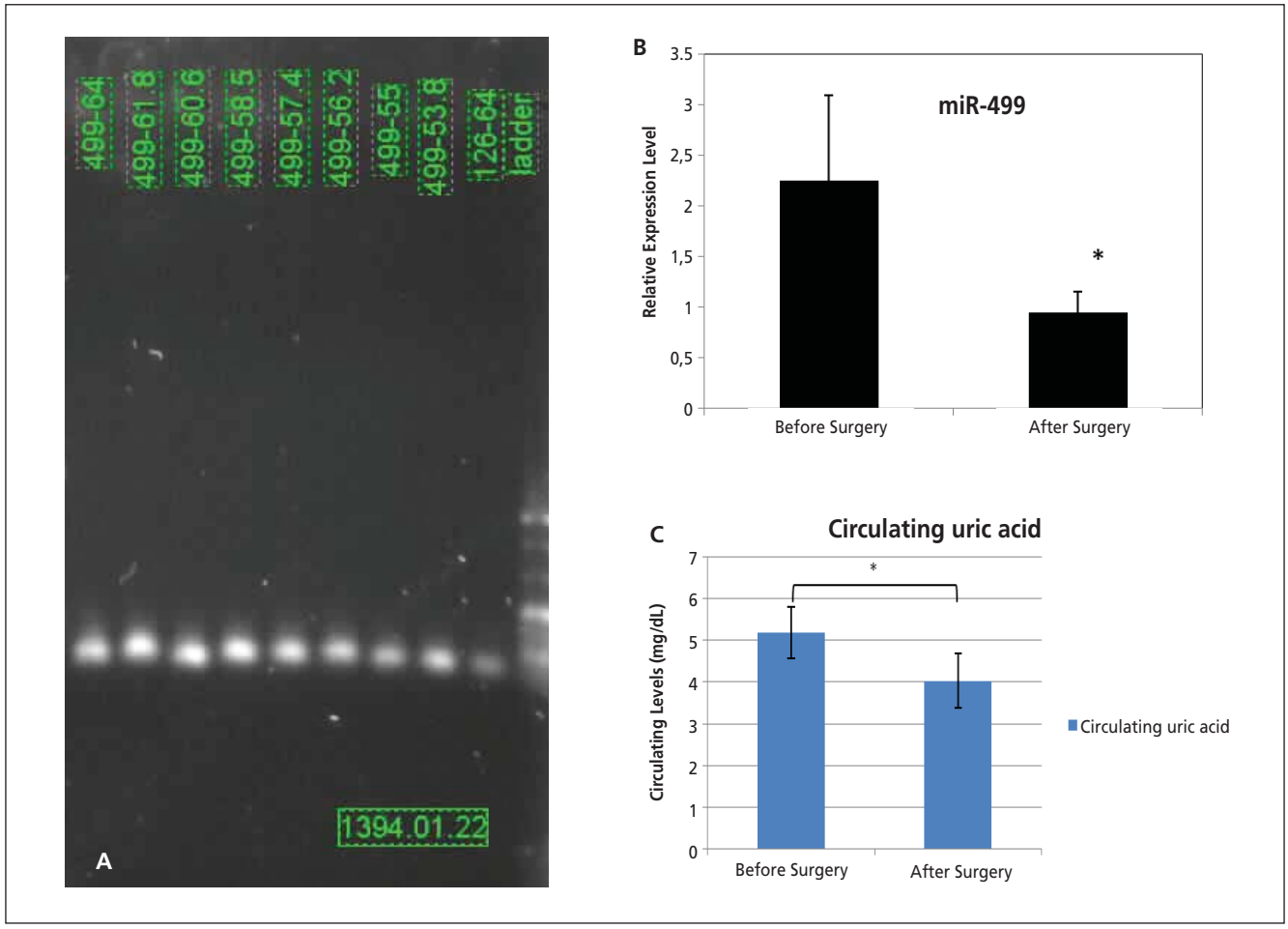

Fig. 2 – Different release patterns of cardiac miR-499 (A and B) and uric acid (C) in patients with coronary ischemia condition candidate for off-pump surgery. Data are given as median and local 95% confidence levels based on the median. The relative expression levels of miR-499 (B), and UA concentration (C) was reduced, post-revascularization surgery in patients with coronary ischemia condition candidate for off--pump surgery. MiRNA levels were transformed into quantities using the formula $2^{-\Delta Ct}$. Error bars represent standard deviation of the mean (SD). * $p < 0.001$; for significant difference between the medians at two time points: on day 0 before and on day 4 after surgery.

ons, the cTnI and CK-MB levels showed no considerable levels in all subjects and were observed to be increased considerably, after revascularization surgery. In post-revascularization surgery, the reduced levels of miR-499 in the blood were not in a correlation with elevated levels of CK (from 86 ± 5.25 to 154.20± 10.87, $p < 0.01$ U/L) and CK-MB (from 17.20 ± 4.10 to 22.66 ± 3.20, $p < 0.05$ U/L), reflecting that the elevated expression levels of miR-499 is not exactly due to myocardium injury but due to myocardium ischemia.

In addition, there was a negative correlation between the miR-499 levels with CK-MB and CK values in the blood, in all subjects before surgery in ischemic conditions ($r \sim -0.45$, $p \sim 0.01$ and $r \sim -0.34$, $p \sim 0.009$, respectively) (Fig. 3A), indicating that the higher miR-499 expression in the ischemic myocardium is related to the lower CKs release from the damaged myocardium into the blood.

Circulating levels of miR-499 specially correlated with cardiac-injury biomarker cTnI

In all subjects, the plasma levels of miR-499 positively correlated with cTnI levels, at time point T2, 4 days post-revascularization surgery ($r \sim 0.5$, $p < 0.001$), indicating that the higher level of cTnI in the blood, the elevated release of miR-499 into the blood, confirming that the blood levels of miR-499 might reflect the degree of myocardial damage (Figure 3A).

However, at day 0 before surgery the elevated levels of miR-499 in the blood were not in any correlation with cTnI, while miR-499 reduced levels in the blood post-surgery, positively correlated with elevated cTnI in peripheral blood.

UA circulating levels specifically related to myocardium damage grade

All of the patients had increased levels of UA and miR-499 preoperatively, which post-operatively reduced significantly (from 5.10 ± 1.20 to 4.0 ± 1.20 mg/dL, $p < 0.004$) (Fig. 2C). In all patients, preoperatively circulating levels of cTnI and CKs were however low, and in about the normal range which increased significantly following surgery.

Circulating levels of UA were in positive correlation with CKs ($r \sim 0.49$, $p < 0.005$ and $r \sim 0.12$, $p < 0.01$) (Figure 3A), and with cTnI ($r \sim 0.14$, $p < 0.005$), (Figure 3B), indicating that the higher level of UA is associated with the increased grade of myocardium damage.

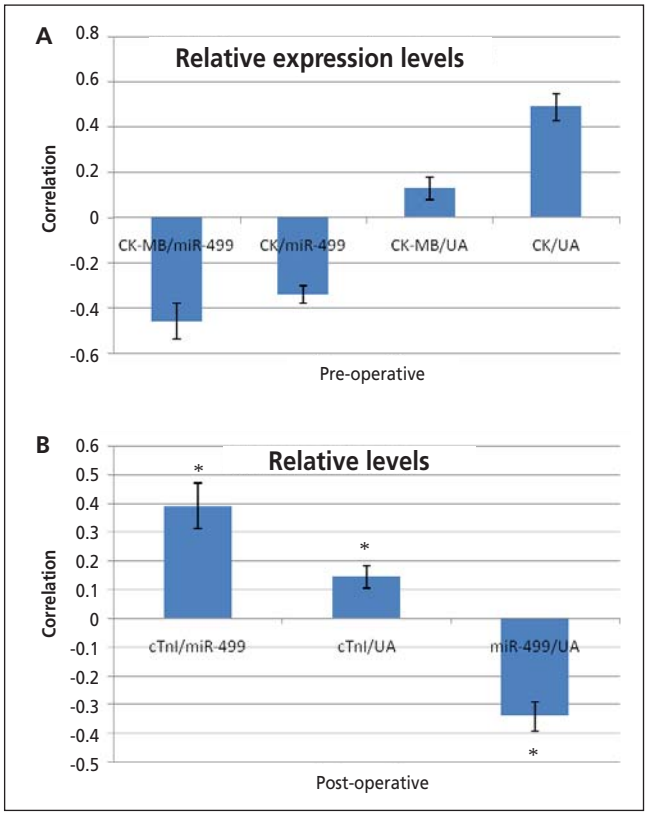

Fig. 3 – The relative circulating levels of miR-499 and UA versus CKs (A) and cTnI (B) were assessed. (A) UA associated highly and positively with CKs, whereas (B) miR-499 post-operatively correlated with cTnI, in candidates for off-pump surgery. Error bars represent standard deviation of the mean (SD). $p < 0.01$, * $p < 0.001$; for significant correlation between the mean values of variables.

Whereas, UA concentrations showed negative correlation with cardiac-enriched miR-499 ($r \sim -0.36$, $p < 0.001$) (Figure 3B), indicating that the higher level of UA is related to the lower expression of miR-499.

Our observations were consistent with the known properties of UA to act as a pro-oxidant agent, depending on its serum concentration, cellular location, and milieu. UA reactions with oxidants may produce other radicals that propagate radical chain reaction and oxidative damage to cells [8].

Increased levels of miR-499 positively related to left ventricle EF vs. UA

The EF, miR-499 expression levels and serum UA concentrations at two time points T1 (on day 0 pre-operative, and T2 (on day 4 post-operative), were measured and compared using paired student t-test.

Post-surgery, EF was significantly increased from 44.50 $\pm$ 8.60 to 50 $\pm$ 8.70, $p < 0.01$. At all time points, the EF was positively correlated with the expression levels of miR-499 ($r = 0.58$, $p < 0.005$) (Fig. 4A), indicating that the higher contractility power of the heart is related to myocardium miR-499 expression.

In contrast, there was a considerable negative relation between EF and UA ($r \sim -0.61$, $p < 0.005$) (Figure 4A). The elevated levels of miR-499 in ischemic myocardium protect it from OS, while declining protective effects was observed in decreased levels of miR-499 in UA concentrations [1].

Discussion

Ischemic heart disease (IHD) is characterized by reduced blood supply to the heart caused by coronary artery disease (CAD), i.e. atherosclerosis in the coronary arteries. IHD is a leading cause of morbidity and mortality worldwide. IHD patients often qualify for revascularization by coronary artery bypass graft (CABG) surgery [17]. During ischemia, there is a decrease in adenosine triphosphate production due to a decrease in tissue blood flow. By decreasing the cell's energy, the cell is no longer able to maintain normal ion gradients across the cell membrane, which leads to swelling and edema with the release of chemotactic factors. Additionally, calcium dependable proteases become activated and convert xanthine dehydrogenase into xanthine-oxidase which plays an important role in the catabolism of purine bases by successively oxidizing hypoxanthine into xanthine, and finally, into UA

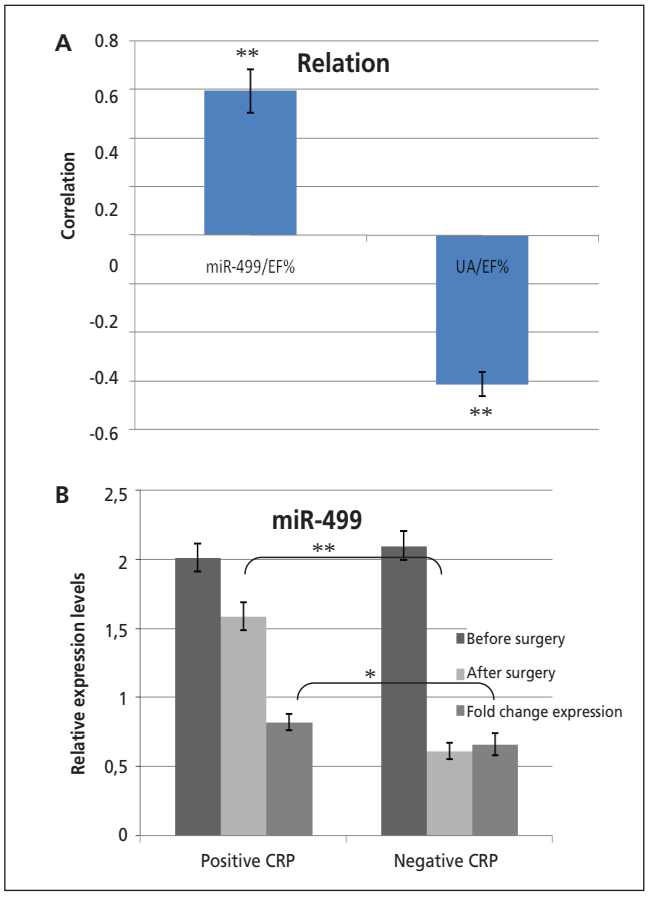

Fig. 4 – The relativity between expression levels of miR-499 and UA with ventricle function (A), and with CRP (B). The cardiac-enriched miR-499 positively affects ventricle contractility, whereas UA levels showed negative impact on ventricle function, in patients with myocardium ischemia undergoing off-pump surgery.
However, CRP showed a good correlation with miR-499 expression levels. Error bars represent standard deviation of the mean (SD). $p < 0.05$, * $p < 0.01$, ** $p < 0.005$; for significant correlation and differences between the mean values of variables.

[11,12]. Increased UA reflects the rate of cell turnover, i.e., degradation of nuclear structures and increased xanthine--oxydase activity and OS, which itself may be followed by inflammatory processes or cerebrovascular stroke [6,13]. Accordingly, ischemia/reperfusion injury is associated with UA in peripheral blood before, during and after surgery in patients who underwent on- and off-pump surgery. Its concentration will increase during ischemia/reperfusion injury [13]. The adverse effects of CABG can be monitored using UA as an oxidative parameter of ischemia/reperfusion injury [11]. Increased activation of the renin-angiotensin system, reduction in renal function and decreased bioavailability of nitric oxide are among the mechanisms responsible for the increased levels of UA [8,13]. Whereas, evidence and also our data show that miR-499 expression increases during myocardial ischemia and are associated with acute myocardial infarction [1,2].

Data indicate that UA levels are related to atherosclerotic burden and induce platelet lysis and increase adhesiveness [9]. UA levels are strongly associated with elevations of inflammatory markers, including the leukocyte and neutrophil count, C-reactive protein (CRP), and pro-inflammatory cytokines [9,18,19]. In addition an increased level of UA is an independent predictor for mortality in coronary heart disease, heart failure and stroke [20].

UA is associated with an increased risk of CHD, and in CABG, can be monitored as a parameter of ischemia/reperfusion injury [7,11].

UA levels are related to subclinical measures of cardiovascular disease, and are associated with an increased risk of several diseases [9,18–20] which is concentration depended and is also reflected in different cellular responses [25–27].

Our observations were consistent with the known properties of UA to act as a pro-oxidant agent, in reactions with oxidants which produce other radicals that propagate radical chain reaction and oxidative damage to cells. Then, adverse effects of CABG can be monitored using UA as an oxidative parameter of ischemia/reperfusion injury [8,11]. Our study provides evidence that elevated UA during myocardial ischemia would decrease post-revascularization in off-pump CABG.

Obviously, UA promote a variety of signaling pathways in a concentration-dependent manner [9,18–20]. UA promotes cellular pathways of protein production and degradation; the ubiquitin–proteasome system and eIF4 signaling **[20]**. UA down regulates GH intracellular signaling; JAK2/Stat5 and leads to low circulating levels of insulin-like growth factor-1, which are associated with reduced insulin-sensitivity and predict development of cardiovascular diseases [23]. UA influences cellular metabolism, especially nitric oxide (NO) signaling and production of reactive oxygen species (ROS), which play a major role in the regulation of cellular function. Furthermore, UA may contribute to pathogenesis of vascular disease by hampering regeneration of endothelial cells and impairing NO production [20,24]. Indeed, UA induces Na+/Ca^{2+} exchanger-mediated mitochondrial calcium overload and mitochondrial dysfunction. Mitochondrial calcium overload increases production of ROS, particularly superoxide radical (SR), accompanied by an inhibition of NO generation, development and progression of atherosclerotic cardiovascular disease [9,18,21,23]. In cardiovascular system, OS is due to increased production of SR by NADPH oxidase and through the induction of N-methyl-D-aspartate receptor-1 (NMDAR1), which leads to a mitochondrial-Ca^{2+} overload and generation of ROS. Suppression of NMDAR1 reduces OS, restores contractility in cardiomyocytes, and ameliorates matrix metalloproteinase-9 (MMP9)-mediated cardiac sudden death by preventing MMP9 activation. A decrease in HDAC1 and an increase in H3K9 acetylation and DNA methylation are associated with heart-chromatin remodeling and causative agents of OS and cardiac--specific miRNAs expression [25].

Recently, attention has being more focused towards the miRNAs risks for CVD. Remarkably, plasma microRNA levels are not affected by clinical confounders, including age, sex, BMI, kidney function, SBP, and WBC count [2–4].

Cardiac-enriched miR-499 is encoded by β-myosin heavy chain geneand shows a pivotal role in inhibiting cardiomyocyte apoptosis [1,2]. In patients with ACS or CHF, plasma elevated levels of miR-499 have been associated with higher risk of death. Since, the admission levels of circulating miR-499 have anti-angiogenic effects via suppressing the secretion of vascular endothelial growth factor (VEGF) [26,27]. Accordingly, miR-499 targets α- and β-isoforms of the calcineurin catalytic subunit (CnAα & β), and dynamin-related protein-1 (Drp1) of mitochondria [1]. CnAα, is related to the nuclear factor of activated T cell (NFAT) pathway. The CnAα/NFAT causes progression of cell-cycle and inhibition of apoptosis, beside regulating the Wnt signaling pathway which promotes cell proliferation [27]. CnA/NFAT activates hypoxia-inducible factor 1-α (HIF1-α) which results in the secretion of VEGF from cells [27,28].

Revascularization off-pump surgery protects myocardium much more against ischemia and apoptosis, and according to our results causes a decrease in elevated miR-499 expression in the myocardium [12,13,26]. Interestingly, the effect of miR-499 is stronger under hypoxia due to enhanced uptake of the miR-499 under the condition of hypoxia [27].

MiR-499 is considered to have the potential to carry out a dual effect. Plasma elevated levels of miR-499 under hypoxia would have synergistic anti-angiogenic effects, but up-regulated miR-499 in ischemic cardiac milieu and in BM-MSCs increases the expression of cardiac-specific genes, such as *NKx2.5, GATA4, MEF2C*, and cTnI and activates the Wnt signaling pathway [27,28]. Moreover, miR-499 regulate a number of cardiac ion channels in the human atria, including the L-type Ca^{2+} channel (down-regulated), the transient outward K^+ channel (down-regulated), the strong inward rectifier K^+ channel (up-regulated), the acetylcholine-activated K^+ channel (up-regulated), and the ultra rapid delayed rectifier K^+ channel (down-regulated). Then, over-expressed miR-499 can cause profound changes in these channels and lead to shortening of the atrial action potential and atrial refractoriness, promoting arrhythmogenesis by reentry. In addition, profound down--regulation of the L-type Ca^{2+} current results in the loss of rate adaptation, which is a prominent feature in the electrical remodeling of myocardium and is strongly associated with atrial fibrillation (AF) [29].

The plasma levels of miR-499 have been reported to be associated with acute myocardial infarction (AMI) [1,2].

But, data shows that miR-499 release is not exclusive for AMI and may occur in ischemia and ejection fraction [2–4]. Our study provides evidence that miR-499 expression was profoundly changed during myocardial ischemia and revascularization by off-pump CABG procedure which are found in blood samples.

A series of mechanisms, including ischemia and OS can trigger apoptosis in myocardium that can be related to UA and miR-499 [9,11–13].

In cardiovascular system, OS cause a decrease in HDAC1 and an increase in the expression of NMDAR1, DNMT1 which is associated with low levels of miR-499 and miR-133 in cardiomyocytes. A decrease in HDAC1 and an increase in H3K9 acetylation and DNA methylation are suggestive of chromatin remodeling. Under pathological conditions, low levels of miR-499 in combination to miR-133 are associated with cardiovascular remodeling through increasing levels of NMDAR1, DNMT1, and MMP-9 [25].

Data evoke the potential functions for miR-499, which modulate cardiomyocyte performance by shifting the balance between slow and fast muscle fiber gene programs, besides demonstrating a functional role for circulating miR-499 through affecting distant gene expression in vasculature [4,5].

The present study afforded to address cardiac miR-499 in a clinical relevant off-pump CABG procedure. There was a moderate elevation in the plasma levels of miR-499 and a negative correlation with elevated levels of UA and cardiac-injury biomarkers, which supports that miR-499 is in association with myocardium protection in hypoxic conditions. Some reports are inconsistent with our results and have reported that miR-499 is up-regulated in myocardial ischemia and its severe elevation in plasma is associated with the severity of cardiomyocytes damage [1,2].

Interestingly, it has been also reported that after injury, miRNAs elevated in plasma were consistently down-regulated in corresponding organ suggesting the intriguing possibility of a cellular survival mechanism in which, during stress, undesirable miRNAs are actively excreted [1–4]. Our data generally support these conclusions and extend on their findings by identifying miR-499 as far more sensitive markers for myocardial damage not only in AMI but also in ischemia/reperfusion condition [4]. However, the detailed mechanism between miRNAs, ischemia and heart injury are not fully understood. Importantly, CRP as a risk indicator for atrial fibrillation after myocardial revascularization and positively associated with UA [30,31], causes in our study an up-regulation of miR-499 (Fig. 4B). In our results (Fig. 4B), patients with high baseline CRP levels, had an elevated levels of miR-499 and may be predisposed at higher risk of having postoperative AF in CABG surgery [25,26,29–31]. Postoperative AF occurs frequently after CABG and represents one of the most common complications of surgical myocardial revascularization. CRP ligation on the membrane of ischemic cells activates phospholipase A2 whose products would contribute to membrane dysfunction by inhibiting the exchange of NA^{+}/Ca^{2+} ion channels in sarcolemma and thus lead to the development of arrhythmia [30,31].

However, our results show that elevated levels of miR-499 start to become normalized after revascularization by off--pump CABG. Such phenomenon might origin from the protection effect of the procedure. According to a report, at 90 minutes after reperfusion, the miR-499 up-regulated levels would decrease significantly compared to the baseline.

We verified that miR-499 expression levels were significantly up-regulated in ischemic conditions and strongly associated with EF and reversely correlated with UA. In the present study, we observed that the lower myocardium expression of miR-499 was associated with poorer cardiac EF during ischemia, in opposite to UA. However, the possible benefit of combined assessment of UA, CRP and miR-499 levels for risk stratification of patients with CAD has not been previously evaluated. To the best of our knowledge, the present study is confirming that the blood level of miR-499 might reflect the degree of cardiomyocyte damage and is the first study demonstrating that miR-499 assessment has an impact similar to that of UA/CRP for prediction of major cardiac events in patients with CAD.

The revascularization surgery was observed to exclusively decrease the expression of UA. Our results inconsistent with other studies suppose that miR-499 might be a potential therapeutic target of myocardial protection during hypoxia or in ischemia before CABG surgery [16,17,25–28].

The molecular mechanism regulating miR-499 expression in cardiac ischemia/reperfusion can be interpreted from those control chromatin remodeling by cardiac-specific miRNAs such as up-regulated miR-133 which produces opposing effects on apoptosis by targeting heat shock protein 60/70 and caspase-9 in cardiomyocytes [1,2,25].

One explanation of these results is possible involvement of UA and CRP in cardiovascular remodeling [6,25] and in increased miR-499 which may reflect the rate of cardiac cell turnover and increased OS in atherosclerotic inflammatory processes [1–4,20,24]. Though the detailed mechanism is not well understood, UA is related to ischemia and reflects the rate of cell turnover and degradation of nuclear structures, in cardiovascular system [11,12]. UA was shown to be related to OS and increase ROS which inhibits nitric oxide-dependent regulation of cardiac O2 consumption. UA leads to an influx of Ca^{2+} and further generation of ROS. During OS, cardiac-specific miR-499 is up-regulated in combination with miR-133 to reducing ROS production, to restore contractility in cardiomyocytes and ameliorate heart chromatin remodeling. On the other hand, miR-499 expression in OS has been associated with HDAC1, H3K9 acetylation and DNA methylation, suggestive of heart-chromatin remodeling and increased OS. Increased level of DNMT1 and decreased level of HDAC1 has been associated with reduced levels of miR-499, in cardiac OS and is linked to cardiovascular remodeling [9,18,21,25]. OS leads to up-regulation of cardiac-specific miR-499 and miR-133 which decrease *DNMTs* gene expression, H3K9 acetylation and DNA methylation causative of chromatin remodeling in chronic ischemia. Our present study suggests a mechanism by miR-499 as a potential therapeutic biomarker in myocardial protection in open heart surgery. Pressure overload and ischemia result in OS on the cardiovascular system. The elevated levels of miR-499 and miR-133 in ischemic myocardium protect it from OS, while declining protective effects was observed in decreased levels of miR-499 in UA concentrations.

Conclusions

The present findings confirmed the hypothesis that miR-499 expression level can be influenced by myocardial ischemia. Our results confirm previous reports for extend and concept of miR-499. It shows that the elevated levels of miR-499 are related to ischemia and correlated with myocardial damage.

In addition, LV dysfunction dose correlate oppositely with the extent of miR-499 and UA in this condition, suggesting that the pathophysiologic mechanism for these measures is related.

Higher miR-499 levels vs. UA correlate to better cardiac performance through more EF and poor hemodynamic stress, in ischemia. Although the mechanism remains to become exactly clear, but under pathological conditions, up-regulated miR-499 regulate the expression of a number of cardiac Ca^{2+}/K^{+} ion channels. Data also evoke the potential functions of miR-499 in improving cardiomyocyte performance by shifting the balance between slow and fast muscle fiber gene programs, besides affecting distant gene expression in cardiovascular system [4,5]. Moreover, under pathological conditions miR-499 in combination with miR-133 regulate a number of cardiac-chromatin remodeling factors involved in OS-related cardiovascular remodeling.

Conflict of interest
No conflict of interest.

Funding body
None.

Ethical statement
I declare, on behalf of all authors that the research was conducted according to Declaration of Helsinki.

Informed consent
I declare, on behalf of all authors that informed consent was obtained from all patients participating in this study.

References

[1] H. Qin, G. Chen, M. Liang, et al., The altered expression profile of microRNAs in cardiopulmonary bypass canine models and the effects of miR-499 on myocardial ischemic reperfusion injury, Journal of Translational Medicine 11 (154) (2013) 1–9.
[2] T. Adachi, M. Nakanishi, Y. Otsuka, et al., Plasma microRNA 499 as a biomarker of acute myocardial infarction, Clinical Chemistry 56 (2010) 1183–1185.
[3] F. Olivieri, R. Antonicelli, L. Spazzafumo, et al., Admission levels of circulating miR-499-5p and risk of death in elderly patients after acute non-ST elevation myocardial infarction, International Journal of Cardiology 172 (2014) e276–e278.
[4] M.F. Corsten, R. Dennert, S. Jochems, et al., Circulating microRNA-208b and microRNA-499 reflect myocardial damage in cardiovascular disease, Circulation: Cardiovascular Genetics 3 (2010) 499–506.
[5] Y. Fukushima, M. Nakanishi, H. Nonogi, et al., Assessment of plasma miRNAs in congestive heart failure, Circulation Journal 75 (2011) 336–340.
[6] Y. Brodov, S. Behar, I. Goldenberg, et al., Usefulness of combining serum uric acid and C-reactive protein for risk stratification of patients with coronary artery disease (Bezafibrate Infarction Prevention [BIP] Study), American Journal of Cardiology 104 (2009) 194–198.
[7] M. Dunkelgrun, G.M. Welten, D. Goei, et al., Association between serum uric acid and perioperative and late cardiovascular outcome in patients with suspected or definite coronary artery disease undergoing elective vascular surgery, American Journal of Cardiology 102 (2008) 797–801.
[8] V. Lapsia, R.J. Johnson, B. Dass, et al., Elevated uric acid increases the risk for acute kidney injury, American Journal of Medicine 125 (2012) 302.e9–302.e17.
[9] G.S. Hillis, B.H. Cuthbertson, P.H. Gibson, et al., Uric acid levels and outcome from coronary artery bypass grafting, Journal of Thoracic and Cardiovascular Surgery 138 (2009) 200–205.
[10] D.Y. Şahin, M. Gur, Z. Elbasan, et al., Predictors of preinterventional patency of infarct-related artery in patients with ST-segment elevation myocardial infarction: importance of neutrophil to lymphocyte ratio and uric acid level, Experimental and Clinical Cardiology 18 (2013) e77–e81.
[11] W.B.M. Gerritsena, W.J.P. Boven, A.H.G. Driessen, et al., Off- pump versus on-pump coronary artery bypass grafting: oxidative stress and renal function, European Journal of Cardio-Thoracic Surgery 20 (2001) 923–929.
[12] A. Gonenc, A. Hacsevki, H.R. Griffiths, et al., Free radical reaction products and antioxidant capacity in beating heart coronary artery surgery compared to conventional bypass, Biochemistry 76 (2011) 677–685.
[13] W.B. Gerritsen, W.P. Boven, D.S. Boss, et al., Malondialdehyde in plasma, a biomarker of global oxidative stress during mini-CABG compared to on- and off-pump CABG surgery: a pilot study, Interactive Cardiovascular and Thoracic Surgery 5 (2006) 27–31.
[14] A.A. Peivandi, M. Dahm, U.T. Opfermann, et al., Comparison of cardiac troponin I versus T and creatine kinase MB after coronary artery bypass grafting in patients with and without perioperative myocardial infarction, Herz 29 (2004) 658–664.
[15] P.P. Chang, M.S. Sussman, J.V. Conte, et al., Postoperative ventricular function and cardiac enzymes after on-pump versus off-pump CABG surgery, American Journal of Cardiology 89 (2002) 1107–1110.
[16] P.W. van Boven, B.W. Gerritsen, H.A. Driessen, et al., Myocardial oxidative stress, and cell injury comparing three different techniques for coronary artery bypass grafting, European Journal of Cardio-Thoracic Surgery 34 (2008) 969–975.
[17] F. Olivieri, R. Antonicelli, M. Lorenzi, et al., Diagnostic potential of circulating miR-499-5p in elderly patients with acute non ST-elevation myocardial infarction, International Journal of Cardiology 167 (2013) 531–536.
[18] M. Caputo, J. Saif, C. Rajakaruna, et al., MicroRNAs in vascular tissue engineering and 3 post-ischemic neovascularization, Advanced Drug Delivery Reviews (2015), http://dx.doi.org/10.1016/j.addr.2015.05.003.
[19] A. Shankar, B.E.K. Klein, F.J. Nieto, R. Klein, Association between serum uric acid level and peripheral arterial disease, Atherosclerosis 196 (2008) 749–755.
[20] Y. Tavil, M. Gungor-Kaya, S. Ozhan-Oktar, et al., Uric acid level and its association with carotid intima-media thickness in patients with hypertension, Atherosclerosis 197 (2008) 159–163.
[21] A. Oberbach, J. Neuhaus, N. Jehmlich, et al., A global proteome approach in uric acid stimulated human aortic endothelial cells revealed regulation of multiple major cellular pathways, International Journal of Cardiology 176 (2014) 746–752.
[22] P. Chaturvedi, A. Kalani, S. Givvimani, et al., Differential regulation of DNA methylation versus histone acetylation in cardiomyocytes during HHcy in vitro and in vivo: an epigenetic mechanism, Physiological Genomics 46 (2014) 245–255.
[23] H. Ando, T. Asai, H. Koide, et al., Advanced cancer therapy by integrative antitumor actions via systemic administration of miR-499, Journal of Controlled Release 181 (2014) 32–39.
[24] G. Sesti, M.L. Hribal, T. Procopio, et al., Low circulating insulin-like growth factor-1 levels are associated with high serum uric acid in nondiabetic adult subjects, Nutrition, Metabolism and Cardiovascular Diseases 24 (2014) 1365–1372.
[25] J.H. Park, Y.M. Jin, S. Hwang, et al., Uric acid attenuates nitric oxide production by decreasing the interaction between endothelial nitric oxide synthase and calmodulin in human umbilical vein endothelial cells: a mechanism for uric acid-induced cardiovascular disease development, Nitric Oxide 32 (2013) 36–42.
[26] H. Quana, Q. Kaa, F. Zhea, et al., Hyperuricemia induces endothelial dysfunction via mitochondrial Na^{+}/Ca^{2+} exchanger-mediated mitochondrial calcium overload, Cell Calcium 51 (2012) 402–410.
[27] L. Zhang, J. Liu, F. Liu, et al., miR-499 induces cardiac differentiation of rat mesenchymal stem cells through Wnt/b-catenin signaling pathway, Biochemical and Biophysical Research Communications 420 (2012) 875–881.
[28] T. Ling, X. Wang, Q. Chai, et al., Regulation of the SK3 channel by microRNA-499. Potential role in atrial fibrillation, Heart Rhythm 10 (2013) 1001–1009.
[29] B. Lo, R. Fijnheer, A.P. Nierich, et al., C-reactive protein is a risk indicator for atrial fibrillation after myocardial revascularization, Annals of Thoracic Surgery 79 (2005) 1530–1535.
[30] K. Ishida, F. Kimura, M. Imamaki, et al., Relation of inflammatory cytokines to atrial fibrillation after off-pump coronary artery bypass grafting, European Journal of Cardio-Thoracic Surgery 29 (2006) 501–505.
[31] F. Perticone, R. Maio, J.E. Tassone, et al., Interaction between uric acid and endothelial dysfunction predicts new onset of diabetes in hypertensive patients, International Journal of Cardiology 167 (2013) 232–236.