The Association of Plasma Levels of miR-146a, miR-27a, miR-34a and miR-149 with Coronary Artery Disease

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Abstract

Background: Coronary artery disease (CAD) is considered to be one of the most pivotal causes of death in the world. Over the past two decades, significant changes occurred in diagnosis, prognosis, and treatment of CAD, which has helped reduce mortality rates. miRNAs are a class of more than 5000 non-encoding RNA molecules (21 to 25 nucleotides across the length) that regulate the complex biological processes. Today, miRNAs are used to study cardiovascular diseases. In the present study, the expression of miR-146a miR-27a miR-149 and miR-34a in plasma suffering from CAD and control group were investigated.

Methods and Results: The present research was performed on 30 men with coronary artery stenosis (CAD) and 30 healthy men as controls. The expression levels of miR-146a, miR-27a, miR-149 and miR-34a in the plasma of patients with CAD and control group were measured using real-time PCR. Also, the correlation between the expression of circulating miRNAs levels and biochemical LDL-C, HDL-C, BMI, and cholesterol were evaluated. The expression of miR-27a in plasma of CAD group was higher than control group (p=0.020). The expression of miR-146a was downregulated in CAD patients compared to of non-CAD group (p=0. 026). However, the expression of miR-34a, miR-149 in the plasma of CAD patients were not significantly difference with the control group. In addition to, a direct correlation was found between the expression of miR-146a and HDL-c, the expression of miR-27a and LDL-C and the expression of miR-34a and total cholesterol. Also, the negative correlation between expressions of miR-149 with BMI was reported.

Conclusion. The obtained results demonstrated that miRNAs was closely related to biochemical factors and it points out the fact that miRNAs can be applied as a potential strategy for diagnosis and treatment of CAD.

Introduction

Coronary artery disease (CAD) is a common issue worldwide [1]. Although, there are many blood markers for diagnosis of CAD, but only a few of these factors are measurable and management [2]. Therefore, use of biomarkers for diagnosis of CAD can help to early and fast treatment. Circulating microRNAs (miRNAs) could be suitable as clinical biomarkers for diagnosis of various disease including many cancers [3], heart failure[4], vascular disorders [5]. MiRNAs are small non-coding RNA molecule with 20–25 nucleotides that regulate cellular functions such as apoptosis, differentiation ,cell growth and proliferation[6]. MiRNAs are detected in body fluids and they have a stable form in plasma and serum of blood. So, due to their stability in body fluids and ease detection, the evaluation of level of miRNAs in body fluids might have a significant role in diagnosis, prognosis and treatment of disease[7]. Numerous miRNAs identified in human body that they release passively and actively into the blood stream[8].

MiR-34a is one of important miRNAs that it dysregulated in several cancers and acts as a tumor suppressor. MiR-34a has a direct relationship with heart diseases as decrease of miR-34a expression

prevented cardiac contractile dysfunction, and reduced apoptosis and fibrosis in myocardial infarction (MI). MiR-146a involved in cardiovascular diseases and the downregulated miR-146a has been associated with cardiac dysfunction through targeting phospholamban[9].

MiR-27 can inhibit adipocyte differentiation and it has closely associated with the obesity and atherosclerosis [10, 11]. MiR-27a affect in oncogenesis, cell growth and adjust the tumor immune response and chemotherapy resistance [12, 13]. MiR-149 induced the differentiation of mouse bone marrow stem cells into cardiac cells in vitro [14].

According to importance of level of miR-146a, miR-27a, miR-34a and miR-149 expression in heart diseases, the present study was aimed to examine the association of level of expression of miR-146a, miR-27a, miR-34a and miR-149 in plasma with coronary artery diseases (CAD). Also, present study was evaluated the correlation of expression of theses miRNAs and biochemical factors in first time.

Methods

Study subjects

Sixty male between the ages of 50-70 years old (30 patients with coronary artery disease and 30 normal subjects) were selected for this study. Patients were diagnosed with CAD through echocardiography and coronary angiogram and with at least more than 50% stenosis.

Patients were categorized into two groups based on the number of coronary artery occlusion of vascular disease (2 VD, 3 VD). After receiving informed consent, the collecting of blood samples were performed as previously described [15].

Sample collection

After evaluation of routine medical history and health examination, 12 ml of blood samples were collected in tubes in tubes coated with EDTA and from the antecubital veins of CAD and non-CAD patients. Samples were centrifuged at 2000 × g for 5 min. The remaining supernatant was centrifuged at 12000 × g for 15 min one more time in order to obtain pure plasma. At last, the plasma was kept in RNase-free tubes at -20°C.

Biochemical and clinical assays

Triglyceride (TG), cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), were measured that in order to 3 ml of blood sample were acquired from participants of both groups. Furthermore family history, medical history, physical examination and drug history were recorded. The weight, height, systolic (SBP) and diastolic (DBP) blood pressure were assessed. Body mass index (BMI) was measured using the formula weight [kg]/ (height) ^{2} [m^2].

RNA isolation and cDNA synthesis

Total RNA was isolated by miRNeasy serum/plasma Kit (QIAGEN GmbH, Hilden, Germany) that uses phenol/ guanidine-based lysis of samples and silica membrane– based purification of total RNA. Poly (A) Polymerase was used to increase length of miRNAs and create a poly (A) tail. cDNA was synthetised using Prime Script RT reagent Kit (TaKaRa) according the manufacturer's instructions [16].

Real-time PCR

The real-time polymerase chain reaction was carried out to determine the level of miRNA expression using SYBR green (Amplicon) on Rotor-Gene Q Sequence Detection System (BIORAD).

The PCR amplification conditions comprised an initial denaturation for 15 min at 95 ºC followed by 40 cycles of a denaturation step at 95 ºC for 20 s and annealing step for 30 s at 60 ºC.

The relative expression level of miRNAs was analyzed with the comparative Ct method ($2^{-\Delta\Delta CT}$) and miR-16 housekeeping gene was utilized to normalize the samples [16]. The primers of this study are presented in Table 1.

Table1. The list of primer sequences for Real time PCR analysis

Primer	Sequence
MiR149a	CTGGCTCCGTGTCTTCACTC
MiR146a	GGGTGAGAACTGAATTCCATGG
MiR34a	GTGGCAGTGTCTTAGCTGGTT
MiR27a	GGGTTCACAGTGGCTAAGTTCT
MiR16	GGGTAGCAGCACGTAAATATTGG

Statistical analysis

All data were presented as mean ± standard deviation. All data was analyzed by Mann-Whitney test and t-test using SPSS software version 18 (SPSS, Inc., Chicago, IL, USA). Kolmogorov–Smirnov test was used for the variables with normal distribution. One-way analysis of variance (ANOVA) and Tukey analysis were exploited for comparing variables between different groups. P-values lower than 0.05 were considered as statistically significant.

Results

The evaluation of demographic & experimental

The demographic variables and experimental characteristics of this study were presented in Table 2. There were no statistically significant differences in age, BMI, and SBP between case and control groups however, a significant difference was observed between DBP, cholesterol and triglyceride ration in both groups ($P \leq 0.05$).

Also, HDL-C ratio was significantly lower in CAD group compared to control group but there was significantly difference in the LDL-C ratio between both groups (P = 0.4).

Among CAD patients 63.33% were diagnosed as smoking while 30% of control group were smoking and there were significant difference between CAD patient and control group (P = 0.01).

Table 2
The experimental characteristics and demographic variables

Parameter	CAD group	Control group	P valve
Age(years)	57.6 ± 20.32	55.30 ± 8.4	0.32
BMI (Kg/m^2)	24.8–30	24.66-27	0.456
SBP (mmHg)	120–130	117.5–125	0.256
DBP (mmHg)	70–90	70–80	0.026
Smoking/ no smoking	19/11	9/21	0.01
Cholesterol (mg/dl)	133–181	119–145	0.03
TG (mg/dl)	96.25–149	85.75-123.75	0.141
LDL-C (mg/dl)			0.4
HDL-C (mg/dl)	25–39	31.5–50.5	0.03

The expression levels of circulating miRNAs in CAD patients and control group

QPCR was exploited to measure plasma miR-146a, miR-27a, miR-34a and miR-149 expressions in both groups (Fig. 1). Analysis by Δct method indicated that there were no significant differences in the miR-34a and miR-149 expression level in patients and control groups ($P \geq 0.05$). However, the expression of miR-27a level was significantly upregulated in CAD patients compared to control group (P = 0.02) and also, the expression of miR-146a level was significantly lower in CAD patients compared to control group (P = 0.026).

The comparison of the expression level of circulating miRNAs in CAD patients with different severity and control group

10 of the patients who suffered severely from CAD, were 2VD and 20 of patients were 3VD. There were no statistically difference in the levels of miR-34a, miR-149, and miR-146a in 2VD, 3VD and control groups. The expression of miR-27a level had no significantly difference between 2VD patients and control group

but plasma levels of miR-27a were found to be significantly increased in 3VD compared to control group (P = 0.03).

The correlation between the expression levels of circulating miRNAs and biochemical factors

The correlation analysis demonstrated that plasma miR-146a level was positively related with HDL-C (r = 0.426) and there was a direct relation between level of miR-27a and LDL-C (r = 0.445). Also, level of cholesterol showed a direct correlation with expression of miR-34a level (r = -0.459). In this assessment was identified that there were an indirect relationship between miR-149 and BMI (r = -0.382).

Discussion

The previous studies were showed that the circulating miRNA as important biomarkers affect on various illnesses, such as cancer, cardiomyopathy, and acute myocardial infarction [17]. In this study, we evaluated the expression of miR-27a, miR146a, miR-149, miR-34a levels in CAD patients. In the current study the correlation of biochemical factors and expression of miR-27a, miR146a, miR-149, miR-34a levels in CAD patients was evaluated for the first time.

We identified the expression of miR-27a was significantly upregulated in patients with angiographic evidence of significant atherosclerosis compared to healthy group. A study assessed expression of many circulating miRNAs in patients after acute myocardial infarction (AMI) and showed that expression of miR-27a level increase in AMI patients and this miRNA has a close association with left ventricular contractility after AMI. So, the results indicate that panels of miRNAs may aid in prognosis after AMI [18].

Another study showed that expression of miRNA-27a, miRNA-451, and miRNA-122 significant downregulated in rats with nonalcoholic fatty liver disease and the downregulation of miR-27a was strongly associated to the production of inflammatory molecules and fatty acid metabolism [19].

MiR-27a inhibits adipocyte formation, downregulated targeting of LDL and increase level of LDL plasma [20] and also, we showed that upregulated of miR-27a increase LDL-C rate in plasma.

Wang et al demonstrated that the increased expression of microRNA-146a protects against myocardial ischemia injury and they identified that miR-146a may act by suppressing of NF-κB and cytokine production [21]. Also, another study indicated that the endothelium derived miR-146a mediates cardioprotection via adjustment of inflammatory mediators in diabetic heart disease and upregulation of miR-146 prevents functional changes and fibrosis in the heart of diabetic mice [22]. Furthermore, we proved that there are an indirect correlation between expression of miR-146a level and CAD.

The researches were established that miR-149 downregulate in CAD patients [23] and AMI [24] whereas other study identified that miR-149 was direct associated with high risk of CAD [25]. We showed that human miR-149 has no significant difference in CAD patients and control group. This differences may related to ethnic and population variations in expression of miRNAs [26, 27].

Han et al the role of many miRNAs were evaluated on human CAD. They showed that miR-34a, miR-21 and miR-23a were upregulated in CAD patients, so, these miRNAs may function as biomarkers of CAD progression and development [28] but in this study no statistically difference was observed in the levels of miR-34a in both control. MiR-34a is targets SIRT1 [29] and SIRT1 involved in mitochondrial biogenesis, regulation of cholesterol, adipose homeostasis, and obesity. Hence, the decreased SIRT1 expression increase cholesterol level in plasma [30, 31]. This study confirmed that level of cholesterol has a positive association with expression of miR-34a.

Conclusion

The current research demonstrated that the expression of miR-27a increased in CAD patients and the expression of miR-146a level reduced in CAD patients, but expression of miR-149 and miR-34a no had significantly difference in both group. Also, there were a significantly correlation between the expression of miR-27a, miR146a, miR-149, miR-34a levels of and biochemical factors. Finding of information about these miRNAs can help as a possible therapeutic target to reduce inflammation and side effects of CAD.

Declarations

Acknowledgements

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Ethics declarations

Conflict of interest: The authors declare no conflict of intersst

Authors' Contributions

Dr.Mirzaei conceived and designed the evaluation and drafted the manuscript. **Dr.Hosseini** participated in designing the evaluation, performed parts of the statistical analysis and helped to draft the manuscript. **Sara Hoseinpoor** re-evaluated the data, revised the manuscript, and collected the clinical data and Study supervision. **Farshad Safari.**, interpreted them and revised the manuscript. **Bahman Khalvati**. Re-analyzed the clinical, material support and statistical data. All authors read and approved the final manuscript

Ethics approval this study was approved by Iran National Committee for Ethics in Biomedical Research (IR.YUMS.REC.1396.121).

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Figures

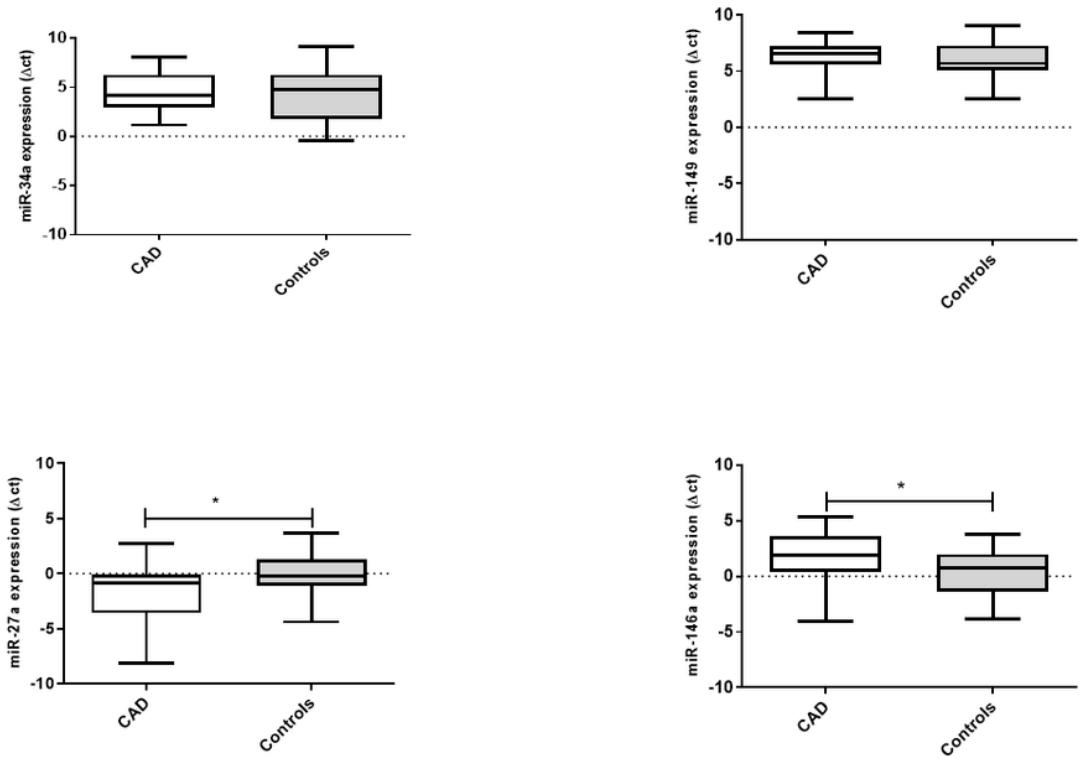

Figure 1

Comparison of expression of miR-34a and miR-149, miR-27a and miR-146a markers in CAD patient and control group by real time RT-PCR (mean ± SEM, *: $p < 0.05$ compared to control groups)

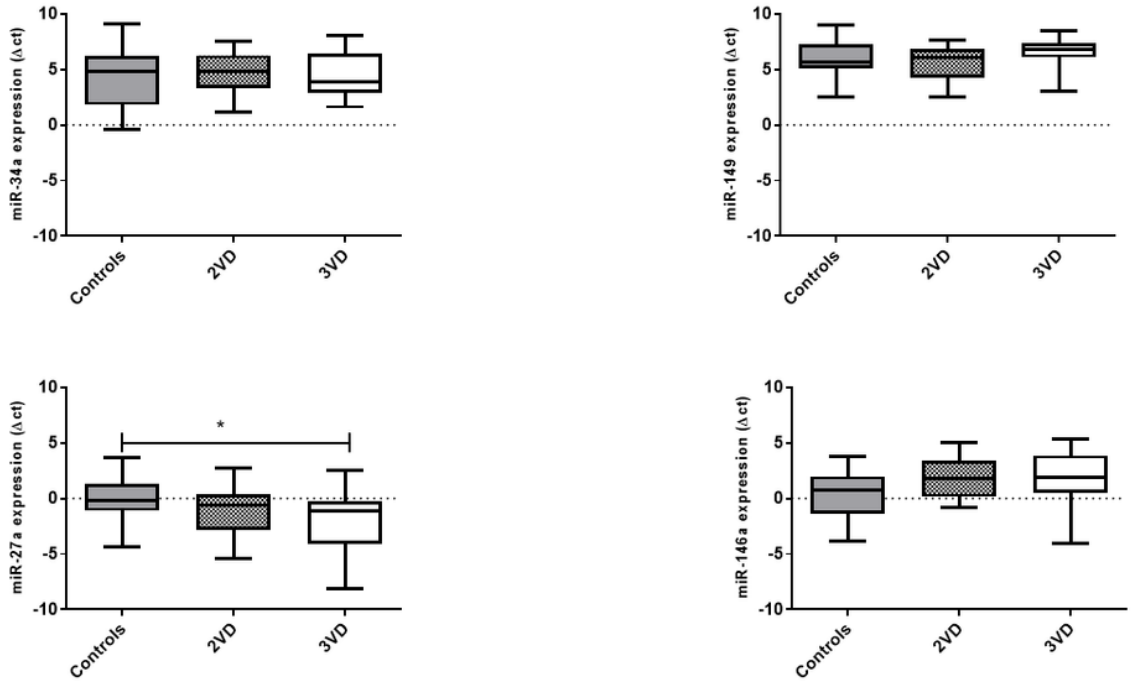

Figure 2

Comparison of expression of miR-34a and miR-149, miR-27a and miR-146a markers in CAD patient 2VD, 3VD and control group by real time RT-PCR (mean ± SEM, *: $p < 0.05$ compared to control groups)