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 the diagnosis, prognosis, and treatment of CAD, which has helped reduce mortality rates. microRNAs (miRs) are a class of more than 5000 non-encoding RNA molecules (21–25 nucleotides across the length) that regulate 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 the control group were investigated.

Methods and results The present research was performed on 30 men with 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 the control group were measured using real-time PCR. Also, the correlation between the expression of circulating miRs levels and biochemical LDL-C, HDL-C, BMI, and total cholesterol was evaluated. The expression of miR-27a in the plasma of the CAD group was higher than in the control group (p = 0.020). The expression of miR-146a was downregulated in CAD patients compared to normal subjects (p = 0.026). However, the expression of miR-34a, miR-149 in the plasma of CAD patients was not significantly different 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 miRs were 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.

Keywords miR-146a, miR-27a, miR-34a and miR-149 · Coronary artery disease

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

miR-34a is one of the important miRs that is dysregulated in several cancers and acts as a tumor suppressor. miR-34a has a direct relationship with heart diseases as a decrease of miR-34a expression prevented cardiac contractile dysfunction, and reduced apoptosis and fibrosis in myocardial infarction (MI) [6]. miR-146a is involved in cardiovascular diseases and the downregulated miR-146a has been associated with cardiac dysfunction through targeting phospholamban [7].

miR-27 can inhibit adipocyte differentiation and it has closely associated with obesity and atherosclerosis [8, 9]. miR-27a affects oncogenesis, cell growth, and adjust the tumor immune response and chemotherapy resistance [10, 11]. miR-149 induced the differentiation of mouse bone marrow stem cells into cardiac cells in vitro [12]. The overall data about investigated miRs are summarized in Table 1.

According to the importance of the 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 CAD. Also, the present study was evaluated the correlation of the expression of these miRs and biochemical factors in the first time.

### Methods

#### Study subjects

Sixty males between the ages of 50–70 years old (30 patients diagnosed with CAD and 30 volunteer normal subjects without any evidence or history of CAD) 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 was performed as previously described [26]. The SYNTAX SCORE (SS) was determined by an experienced cardiologist who was blinded to the laboratory and clinical data of patients. Patients were divided into three groups according to the SS levels as follows: high SS > 32, 22 < intermediate SS ≤ 32, and low SS ≤ 22.

#### 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

| miR   | Target          | Function                                      | References                       |
|-------|-----------------|-----------------------------------------------|----------------------------------|
| 34a   | SIRT1           | Persist infection of T cells                  | Fomison-Nurse [13]              |
|       | FOXP3           | The elevation of Th17 cells and inflammation  | Taheri [14]                     |
|       | IL-21           | Initiation of Th17-induced inflammatory responses | Taheri                      |
| 27a   | PPARγ           | Adipocyte differentiation                      | Shirasaki [15]                  |
|       | FASN            | Lipid synthesis                               | Shirasaki                       |
|       | ATAD3a          | Cholesterol metabolism                        | Bao [16]                        |
|       | TGFBR1          | Can modulate expression INFγ and TNFα         | Zhang [17]                      |
|       | Mef2c           | Early cardiac development                     | Chinchilla [18]                 |
|       | ABCA1           | Transferring lipids to apolipoprotein A–I     | Liu [19]                        |
|       | NF-κB           | Inflammation and apoptosis                     | Liu                              |
|       | Hoxa 10         | Regulation of embryonic development           | Cao [20]                        |
|       | Atg7            | Autophagy and apoptosis of cardiomyocyte      | Zhang [21]                      |
| 146a  | TLR4            | Induce pro-inflammatory cytokine production   | Roldán [22]                     |
|       | TRAF6           | Induce pro-inflammatory cytokine production   | Jin [23]                        |
|       | IRAK1           | Induce pro-inflammatory cytokine production   | Jin [23]                        |
|       | TNF-α           | Induce pro-inflammatory cytokine production   | Jeon [24]                       |
| 149a  | Dab2            | Mediated the cardiac differentiation          | LU [25]                         |
|       | MTHFR           | Involved in conversion of remethylation to methionine | Jeon [24]  |
CAD and normal subjects. Samples were centrifuged at 2000×g for 5 min. The remaining supernatant was centrifuged at 12,000×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), total 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 (m²).

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 the length of miRs and create a poly (A) tail. cDNA was synthesized using Prime Script RT reagent Kit (TaKaRa) according to the manufacturer’s instructions [27].

Real-time PCR

The real-time polymerase chain reaction was carried out to determine the level of miR expression using SYBR green (Amplicon) on the Rotor-Gene Q Sequence Detection System (BIORAD). (Primer sequence were designed blast software). (Table 2).

| Table 3 | The experimental characteristics and demographic variables |
|---------|-----------------|------------------|------------------|
| Parameter | CAD group | Control group | p value |
| Age(years) | 57.6 ± 20.32 | 55.30 ± 8.4 | 0.32 |
| BMI (kg/m²) | 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) | 96–149 | 85–123 | 0.141 |
| LDL-C (mg/dl) | 110–130 | 88–176 | 0.4 |
| HDL-C (mg/dl) | 25–39 | 31.5–50.5 | 0.03 |

The significance of the bold values reflected in Table 3 was highlighted. Summary of mir-34a, miR-27a, miR-146a, and miR-149a target genes and function in cardiovascular related disorder
Fig. 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)

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

The evaluation of demographic & experimental

The demographic variables and experimental characteristics of this study were presented in Table 3. There were no statistically significant differences in age, BMI, and SBP between case and control groups however, a significant difference was observed between DBP, total cholesterol, and triglyceride in both groups (p ≤ 0.05).

Also, the HDL-C was significantly lower in the CAD group that measured to the control group but there was no a significant difference in the LDL-C between both groups (p = 0.4).

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

Syntax scoring revealed that 13%, 33%, and 54% of CAD patients respectively were low, intermediate, and high risk.
The expression levels of circulating miRs 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 the Δct method indicated that there were no significant differences in the miR-34a and miR-149 expression levels in patients and control groups (p ≥ 0.05). However, the expression of the miR-27a level was significantly upregulated in CAD patients compared to the control group (p = 0.02), and also, the expression of the miR-146a level was significantly lower in CAD patients compared to the control group (p = 0.026).

The comparison of the expression level of circulating miRs 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 was no statistical 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 significant difference between 2VD patients and the control group but plasma levels of miR-27a were found to be significantly increased in 3VD compared to the control group (p = 0.03) (Fig. 2).

The correlation between the expression levels of circulating miRs 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 the level of miR-27a and LDL-C (r = 0.445). Also, the level of cholesterol showed a direct correlation with the expression of miR-34a level (r = −0.459). In this assessment was identified that there was an indirect relationship between miR-149 and BMI (r = −0.382).

Diagnostic Potential of miR-27a

The potential of each miR as a diagnostic biomarker of CAD was assessed by area under the receiver-operating-characteristic (ROC) curve analyses. Only miR-27a revealed a weak predictive value/AUC (0.6–0.7). The other miRs miR-34, miR-146, and miR-149 have shown a useless predictive value/AUC (0.5–0.06).

The ROC curve analysis (Fig. 3a) showed that AUC for miR-27 was 0.67 (0.54–0.81 at 95% CI) with 86.7% (69.28–96.24 at 95% CI) sensitivity and 46.7% (28.3–65.6 at 95% CI) specificity when the relative expression level of miR-133b was at the optimal cut-off point.

Discussion

The previous studies were showed that the circulating miR is an important biomarkers effect on various illnesses, such as cancer, cardiomyopathy, and acute myocardial infarction [29]. In this study, we evaluated the expression of miR-27a, miR146a, miR-149, miR-34a levels in CAD patients from Kohgiluyeh-Boyerahmad province in the South-west of Iran. 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 [30]. In this study, we evaluated the expression of miR-27a, miR146a, miR-149, miR-34a levels in CAD patients from Kohgiluyeh-Boyerahmad province in the South–west of Iran. 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 the healthy group (p = 0.02). A study assessed the expression of many circulating miRs in patients after acute myocardial infarction (AMI) and showed that expression of miR-27a level increased in AMI patients and this miR has a close association with left ventricular contractility after AMI (p = 0.03). So, the results indicate that panels of miRs may aid in prognosis after AMI [30]. Roncarati et al. have shown the miR-27a correlation with left ventricular hypertrophy parameters however they showed that there was no relevance between the expression of plasma level of miR-27a and fibrosis in patients [31]. In a bioinformatics study, Wu et al. showed that miR-27a and its targets potentially associated with myocardial infarction [32]. In another study Xue et al. investigated the prediction
value of some candidate miRNAs including miR-27a. ROC analysis indicated that miR-26a-1, miR-146a, and miR-199a-1 but not miR-27a showed considerable diagnostic efficiency as diagnostic biomarkers in myocardial infarction [33]. Another study showed that expression of miR-27a, miR-451, and miR-122 was significantly downregulated in rats with nonalcoholic fatty liver disease, and the downregulation of miR-27a was strongly associated with the production of inflammatory molecules and fatty acid metabolism [34].

miR-27a inhibits adipocyte formation, downregulated targeting of LDL, and increase the level of LDL plasma [35] and also, we showed that upregulated miR-27a increase LDL-C rate in plasma.

Other studies investigate the mechanisms and targets of mir-27a in myocardial ischemia-reperfusion injury or hypertrophic cardiomyocyte animal models. All these studies showed that miR-27 related to these conditions [16, 19, 20].

As mentioned earlier Xue et al. showed a predictive value of miR-146a as diagnostic biomarkers in myocardial infarction [34]. Some other studies showed that miR-146a polymorphisms has a considerable effect on cardiovascular events such as fibrillation or coronary artery disease which could be considered as an impact of this micro RNA on heart diseases [22, 36]. 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 NF-κB and cytokine production [37]. 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 [38]. Furthermore, we proved that there is an indirect correlation between expression of miR-146a level and CAD.

The researches were established that miR-149 downregulates in CAD patients [39] and AMI [40] whereas other studies identified that miR-149 was directly associated with a high risk of CAD [41]. We showed that human miR-149 has no significant difference in CAD patients and the control group. These differences may related to ethnic and population variations in the expression of miRNAs [42, 43].

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 [44] in another study Gatsiou et al. found that high miR-34a was independently associated with the presence of CAD [45]. Ghasempour et al. south the plasma level of miR-37a contributed to the stenosis of coronary arteries, this study indicated significant differences between the expression of miR-37a in patients with vessel in-stent restenosis compared to healthy controls [46]. In this study no statistical difference was observed in the levels of miR-34a between CAD patients and normal subjects. miR-34a is targeting SIRT1 [47] and SIRT1 is involved in mitochondrial bio genesis, regulation of cholesterol, adipose homeostasis, and obesity. Hence, the decreased SIRT1 expression increase cholesterol level in plasma [48, 49]. This study confirmed that the level of cholesterol has a positive association with the 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 the expression of miR-149 and miR-34a no had significant difference in both groups. Also, there was a significant correlation between the expression of miR-27a, miR146a, miR-149, miR-34a levels of and biochemical factors. The finding of information about these miRs can help as a possible therapeutic target to reduce inflammation and side effects of CAD.

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Author contributions Dr. AM conceived and designed the evaluation and drafted the manuscript. Dr. EH participated in designing the evaluation, performed parts of the statistical analysis, and helped to draft the manuscript. SH re-evaluated the data, revised the manuscript, and collected the clinical data and Study supervision FS. Interpreted them and revised the manuscript BK. Re-analyzed the clinical, material support, and statistical data. All authors read and approved the final manuscript.

Declarations

Conflict of interest The authors declare no conflict of interest.

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

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