ABSTRACT

Background and objectives: Coronary artery disease (CAD) refers to stenosis or obstruction of coronary artery due to atherosclerosis or clotting. The aim of this study was to evaluate possible association of serum miRNA-197 (miR-197) expression as a biomarker for CAD diagnosis.

Methods: In this study, 100 patients with CAD who had angiography and vascular transplantation were selected. Expression of miR-197 was evaluated using real-time RT-PCR technique and the SYBR Green method. The Pearson's correlation coefficient was used to determine relationship of miR-197 expression and severity of coronary artery disease. The t-test was used to determine significance of expression of miR-197 in the study groups. All statistical analyses were carried out in SPSS 16 and at significance of 0.05.

Results: The results showed a direct relationship between miR-197 expression and CAD severity. The relative expression of miR-197 in the CAD patients was significantly higher than that in control subjects (P<0.004).

Conclusion: It seems that miR-197 can be considered as an indicator of coronary endothelial cell function. This microRNA could be used as a biomarker for CAD prognosis and treatment progression.

Keywords: MIRN197 microRNA, human, Coronary Artery Disease, U6 small nuclear RNA.
INTRODUCTION
Coronary artery disease (CAD) refers to narrowing or obstruction of all or part of coronary arteries due to atherosclerosis or clotting (1, 2). There are several ways to diagnose, detect, track and control cardiovascular disease. In this regard, anticoagulants therapy, percutaneous coronary intervention (PCI) and coronary stenting, coronary artery bypass graft (CABG) and transplantation of parts of the saphenous vein are considered as possible treatment. However, these interventions are invasive, dangerous and costly (3, 4). Therefore, it seems necessary to seek new methods for identifying susceptible individuals with stenosis. In this regard, a study was conducted on miRNAs (miRNAs), an important family in regulating the expression of genes (5). MiRNAs are non-coding RNAs with a length of 19-24 nucleotides that regulate the expression of target cell mRNAs, are therefore involved in various cellular bioassays, including differentiation, proliferation, apoptosis, metabolism, regulation of gene expression and even neoplasia (6, 7). Several studies have been conducted on microRNA-197 (miR-197) and its function in cardiac disorders. It has been shown that miR-197 levels increase in the blood of CABG patients with severe symptoms of coronary artery stenosis, which ultimately leads to death of the patient. In addition, miR-197 levels are significantly increased in coronary artery inflammatory reaction and platelet activation (8).
This interleukin can increase the expression of miR-197 by binding STAT3 phosphoric to the miR-197 sequence promoter, as well as the IL-22 receptor itself, a direct target for miR-197. Consequently, miR-197 controls the IL-22 inflammatory signal (9). Another study demonstrated that there is a bilateral regulatory relationship between miR-197 and IL16 / STAT3 inflammatory signals in the cell (10). Moreover, increased expression of miR-197 not only induces cellular proliferation, but also prevents programmed cell death (11).
Another study showed that miR-197 and miR-146b are upregulated in end-stage pulmonary arterial hypertension and pressure-overloaded induced right ventricular heart failure (12).
It has been shown that serum exosomal miR-197 represents a candidate diagnostic biomarker to distinguish Kawasaki disease patients from other febrile patients as well as from healthy individuals in a single pass, with a minimal rate of false positives and negatives (13). Cardiometabolic risk factors are heritable and cluster in individuals. A study reported that these risk factors are associated with multiple shared and unique mRNA and miRNA features (14). The molecular and regulatory function of miR-197 in various neoplasia has been extensively studied (15-17). In this study, we evaluate relationship of miR-197 expression level with CABG and associated risk factors in CAD patients and healthy subjects. The results of this study could determine the potential of this miRNA as a biomarker for diagnosis, progression and prognosis CAD.

MATERIALS AND METHODS
This research was conducted on patients who had been referred for angiography (PCI) and CABG at the Imam Ali Cardiovascular Hospital in Kermanshah (Iran) between February 2018 and December 2019. This cross sectional study was performed on 100 CAD patients (83 males and 17 females) with coronary artery stenosis and varying degrees of vascular involvement and 30 (16 males and 14 females) control individuals without coronary artery stenosis.
About 5 ml of blood were collected from all CAD+ and control patients in nuclease-free tubes containing sterile anticoagulant ethylenediamine tetraaesthetic acid. The samples were immediately stored at -80 °C until testing. In addition, demographic characteristics of the patients were recorded.
Extraction of miRNAs from peripheral blood sample was carried out using Favorgen miRNA isolation kit (Cat No: FAMIK001, Biotech Corporation, Taiwan). The purity and concentration of the extracted miRNAs were evaluated by measuring absorbance (Nanodrop spectrophotometer, USA) at 260 nm and 280 nm, respectively. The extracted miRNA samples were used for cDNA synthesis using the following primers: Forward: 5'-TGA TGA CCC CAG GTA ACT CT-3' & Reverse: 5'-GCG AGC ACA GAA TTA ATA-3' and the U6 snRNA primer as housekeeping gene include Forward: 5'-CTC GCT TCG GCA GCA CA-3' & Reverse Sequence: 5'-TGG TGT CGT GGA GT-3'. Synthesis of cDNA from microRNA was performed in two steps.
The first step involved adding poly A tail to the 3´ end of miRNAs by a poly-A polymerase enzyme. The polyadenylation reaction solution (20 μl) consisted of 10X buffer, poly A enzyme, ATP and RNA. The reaction mixture was then incubated at 37°C for 10 minutes. Next, cDNA synthesis was performed using two primers: one reverse primer oligo dT-VN as primer adapter (Qiagen, Germany) and another direct primer as specific primer similar to the miRNA sequence (Qiagen, Germany). For this purpose, the dNTP mixture, (M-MLV) RT enzyme, RNase inhibitor, DEPC water and the corresponding buffer were used. The reaction was carried out at 42°C for 60 minutes. Next, quantitative real-time PCR was carried out in StepOne PCR system (AB Applied Biosystems, USA) to measure miRNA expression level using two primers. The forward primer was similar to the specific sequence of miR-197 and the reverse primer complementary of the unique oligo dT-VN primer. In order to normalize the assay at each stage, U6 snRNA was used. The PCR reaction mixture included 10μl SYBR Green Master Mix consisting of Tag DNA Polymerase, MgCl₂, dNTP, dUTP and buffer, 1μl of each primer, 8μl of cDNA (diluted) and 0.4 μl of fluorescence dye. The experiment was performed in duplicate. Cycling conditions were as follows: 10 minutes at 95 °C, 40 cycles of 10 seconds at 95 °C, 60 seconds at 57 °C, and 30 seconds at 72 °C.

For each sample, CT, ΔCT, ΔΔCT, fold change (2ΔΔCT), melting curve and mean PCR efficiency were determined. Finally, based on the Livak formula, the expression ratio of miR-197 in the samples was determined in comparison with the control sample.

The Pearson’s correlation coefficient was used to determine relationship of miR-197 expression and severity of coronary artery disease. The t-test was used to determine significance of expression of miR-197 in the study groups. All statistical analyses were carried out in SPSS 16 and at significance of 0.05.

RESULTS
The level of miR-197 expression in CAD patients was significantly higher than in healthy subjects (P=0.02) (Table 1).

<table>
<thead>
<tr>
<th>Type of coronary artery disease</th>
<th>Expression level of miR-206</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1CAD</td>
<td>1.08</td>
<td>&lt;0.059</td>
</tr>
<tr>
<td>2CAD</td>
<td>1.64</td>
<td>&lt;0.012</td>
</tr>
<tr>
<td>3CAD</td>
<td>2.14</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>MCAD</td>
<td>2.76</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td>Healthy control</td>
<td>0.78</td>
<td>-</td>
</tr>
</tbody>
</table>

1CAD: stenosis in one coronary artery, 2CAD: stenosis in two coronary arteries, 3CAD: stenosis in three coronary arteries, MCAD: stenosis in the main coronary arteries.

As shown in table 2, none of the studied variables differed significantly between CAD patients and control subjects.

<table>
<thead>
<tr>
<th>Factors</th>
<th>CAD⁺</th>
<th>CAD⁻</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>87/13</td>
<td>16/14</td>
<td>-</td>
</tr>
<tr>
<td>Age (year)</td>
<td>57±9</td>
<td>55±8</td>
<td>0.364</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.78±3.45</td>
<td>27.45±2.09</td>
<td>0.228</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>190±45</td>
<td>153±22</td>
<td>0.407</td>
</tr>
<tr>
<td>Low-density lipoprotein (mg/dl)</td>
<td>129±31</td>
<td>100±13</td>
<td>0.733</td>
</tr>
<tr>
<td>High-density lipoprotein (mg/dl)</td>
<td>33±8</td>
<td>30±2</td>
<td>0.278</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>188±49</td>
<td>145±18</td>
<td>0.320</td>
</tr>
</tbody>
</table>

Table 1- Level of miR-197 expression in samples taken from CAD patients

Table 2- Comparison of mean values of demographic and biochemical parameters between CAD patients and control subjects
DISCUSSION
Over the past few years, research has been focused on the role of miRNAs in various diseases (18). The purpose of this study was to investigate the expression of miR-197 in patients with CAD. Based on the results, the level of miR-197 expression differed significantly between CAD patients and healthy controls. Moreover, the miR-197 expression differed significantly based on the number of arteries affected by the disease. In other words, there was a direct correlation between the number of affected arteries in CAD and level of miR-197 expression. We found no significant between CAD patients and control subjects in terms of risk factors of cardiovascular disease including total cholesterol, low-density lipoprotein, high density lipoprotein, triglycerides, body mass index and age. The increased expression of miR-197 in patients with CAD may well reflect the change in the endothelial function of coronary arteries.

CONCLUSION
Based on the results, miR-197 can be considered as a potential biomarker for detecting and tracking the status of CAD. In addition, the overexpression of miR-197 may be an important indication for CAD before any clinical-pathological symptoms appear in the patients.

ACKNOWLEDGMENTS
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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

REFERENCES


