Medical Laboratory Journal
Online ISSN: 2538-4449
Golestan University of Medical Sciences

Original Article

Development of a New Framework for Health Assessment in Patients with by using miRNA-197 in Adults Coronary Artery Disease

Shahram Zehtabian
(PhD Candidate) Department of Biology, Faculty of Biological Science, Islamic Azad University, North Tehran Branch, Tehran, Iran

Reza Alibakhshi
(PhD) Department of Medical Genetics, Kermanshah University of Medical Sciences, Kermanshah, Iran

Seyed Yousef Seyedena
(PhD) Department of Biology, Faculty of Biological science, Islamic Azad University, North Tehran Branch, Tehran, Iran

Ali-Reza Rai
(PhD) Research & Educational Center, Imam Ali Cardiovascular Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran

Corresponding author: Reza Alibakhshi
Email: ralibakhshiy@gmail.com
Tel: 08334274618
Address: Department of Medical genetics, Kermanshah University of Medical Sciences, Iran

Received: 2020/06/24
Revised: 2020/09/09
Accepted: 2020/09/19

DOI: 10.29252/mlj.15.5.27

ABSTRAC

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 microRNAs (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 tetraesthetic 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, $MgCl_2$, 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 ($2^{-\Delta\Delta CT}$), 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 1- Level of miR-197 expression in samples taken from CAD patients

Type of coronary artery disease	Expression level of miR-206	P-Value
1CAD	1.08	P<0.059
2CAD	1.64	P<0.012
3CAD	2.14	P< 0.005
MCAD	2.76	P< 0.004
Healthy control	0.78	_

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 2- Comparison of mean values of demographic and biochemical parameters between CAD patients and control subjects

Factors	CAD^+	CAD^-	P-value
Sex (male/female)	87/13	16/14	-
Age (year)	57±9	55±8	0.364
Body mass index (kg/m^2)	27.78±3.45	27.45±2.09	0.228
Total cholesterol (mg/dl)	190±45	153±22	0.407
Low-density lipoprotein (mg/dl)	129±31	100±13	0.733
High-density lipoprotein (mg/dl)	33±3	30±2	0.278
Triglycerides (mg/dl)	188±49	145±18	0.320

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

We would like to express our gratitude to all those who assisted us in this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Kanuri SH, Ipe J, Kassab K, Gao H. *Next generation MicroRNA sequencing to identify coronary artery disease patients at risk of recurrent myocardial infarction*. Atherosclerosis. 2018 Oct 3;278:232-239. doi: 10.1016/0002-9149(63)90064-X. [View at Publisher] [DOI:10.1016/0002-9149(63)90064-X] [PubMed] [Google Scholar]

2. Maleki A, Ghanavati R, Montazeri M, Forughi S, Nabatchi B. *Prevalence of Coronary Artery Disease and the Associated Risk Factors in the Adult Population of Borujerd City, Iran. J Tehran Heart Cent*. 2019 ;14(1):1-5. doi: 10.1016/0002-9343(77)90423-5. [View at Publisher] [DOI:10.1016/0002-9343(77)90423-5] [PubMed] [Google Scholar]

3. Christian Albus, Jörg Barkhausen. *The Diagnosis of Chronic Coronary Heart Disease*. Dtsch Arztebl Int. 2017 ; 114(42): 712-719.doi: 10.1161/01.CIR.67.1.134. [DOI:10.1161/01.CIR.67.1.134] [PubMed] [Google Scholar]

4. Albus C, Barkhausen J, Fleck E, Haasenritter J, Lindner O, Silber S. *The Diagnosis of Chronic Coronary Heart Disease.* Dtsch Arztebl Int. 2017 20;114(42):712-719. [DOI:10.1161/CIRCULATIONAHA.112.130153] [PubMed] [Google Scholar]

5. Caterina Catalanotto, Carlo Cogoni. *MicroRNA in Control of Gene Expression: An Overview of Nuclear Functions.* Int J Mol Sci. 2016 ; 17(10): 1712. doi: 10.1093/omcr/omw036. [View at Publisher] [DOI:10.1093/omcr/omw036] [PubMed] [Google Scholar]

6. Liu H, Lei C, He Q, Pan Z, Xiao D, Tao Y. Nuclear *functions of mammalian MicroRNAs in gene regulation, immunity and cancer*. Mol Cancer. 2018 22;17(1):64. [View at Publisher] [DOI:10.2353/ajpath.2006.050646] [PubMed] [Google Scholar]

7. *Gene expression regulation: lessons from noncoding RNAs.* RNA. 2015 ;21(4):695-6. [DOI:10.1055/s-2005-872844] [PubMed] [Google Scholar]

8. Schulte C, Molz S, Appelbaum S, Karakas M, Ojeda F, Lau DM, et al. *miRNA-197 and miRNA-223 Predict Cardiovascular Death in a Cohort of Patients with Symptomatic Coronary Artery Disease*. PLoS One. 2015 31;10(12) [DOI:10.1242/jcs.184770] [PubMed] [Google Scholar]

9. Lerman G, Sharon M, Leibowitz-Amit R, Sidi Y, Avni D. *The crosstalk between IL-22 signaling and miR-197 in human keratinocytes*. PLoS One. 2014 10;9(9):e107467. [DOI:10.1097/GIM.0b013e31820ad795] [PubMed] [Google Scholar]

10. Wang H, Su X, Yang M, Chen T, Hou J, Li N, Cao X. *Reciprocal control of miR-197 and IL-6/STAT3 pathway reveals miR-197 as potential therapeutic target for hepatocellular carcinoma*. Oncoimmunology. 2015 4;4(10):e1031440. [DOI:10.1001/jama.2009.371] [PubMed] [Google Scholar]

11. Wang H, Su X, Yang M, Chen T, Hou J, Li N, et al. *Reciprocal control of miR-197 and IL-6/STAT3 pathway reveals miR-197 as potential therapeutic target for hepatocellular carcinoma*. Oncoimmunology. 2015 4;4(10) [View at Publisher] [DOI:10.1002/mus.23517] [PubMed] [Google Scholar]

12. Legchenko E, Chouvarine P, Borchert P, Fernandez-Gonzalez A. *PPARγ agonist pioglitazone reverses pulmonary hypertension and prevents right heart failure via fatty acid oxidation*. Sci Transl Med. 2018 25;10(438). [DOI:10.14503/THIJ-13-3896] [PubMed] [Google Scholar]

13. Jia HL, Liu CW, Zhang L, Xu WJ, Gao XJ, Bai J, Xu YF, Xu MG, Zhang G. *Sets of serum exosomal microRNAs as candidate diagnostic biomarkers for Kawasaki disease.* Sci Rep. 2017 20;7:44706. [View at Publisher] [DOI:10.4103/0189-7969.187732] [PubMed] [Google Scholar]

14. McManus DD, Rong J, Huan T, Lacey S, Tanriverdi K, Munson PJ, et al. *Messenger RNA and MicroRNA transcriptomic signatures of cardiometabolic risk factors*. BMC Genomics. 2017 8;18(1):139. [View at Publisher] [DOI:10.14503/THIJ-15-5450] [PubMed] [Google Scholar]

15. Tang T, Cheng Y, She Q, Jiang Y, Chen Y, Yang W, et al. *Long non-coding RNA TUG1 sponges miR-197 to enhance cisplatin sensitivity in triple negative breast cancer*. Biomed Pharmacother. 2018 ;107:338-346. [View at Publisher] [DOI:10.1093/eurheartj/ehi471] [PubMed] [Google Scholar]

16. Lu X, Liu Z, Ning X, Huang L, Jiang B. *The Long Noncoding RNA HOTAIR Promotes Colorectal Cancer Progression by Sponging miR-197*. Oncol Res. 2018 10;26(3):473-481. [DOI:10.4244/EIJV8I1A20] [PubMed] [Google Scholar]

17. Zhang Y, Huang S, Li P, Chen Q, Li Y, Zhou Y, et al. *Pancreatic cancer-derived exosomes suppress the production of GIP and GLP-1 from STC-1 cells in vitro by down-regulating the PCSK1/3*. Cancer Lett. 2018 1;431:190-200. [View at Publisher] [DOI:10.1016/j.canlet.2018.05.027] [PubMed] [Google Scholar]

18. Orlicka-Płocka M, Gurda D, Fedoruk-Wyszomirska A, Smolarek I, Wyszko E. *Circulating microRNAs in Cardiovascular Diseases.* Acta Biochim Pol. 2016;63(4):725-729. [View at Publisher] [DOI:10.1016/j.jjcc.2015.09.009] [PubMed] [Google Scholar]

19. Tien WP, Lim G, Yeo G, Chiang SN, Chong CS, Ng LC, et al. *SYBR green-based one step quantitative real-time polymerase chain reaction assay for the detection of Zika virus in field-caught mosquitoes*. Parasit Vectors. 2017 19;10(1):427. [View at Publisher] [DOI:10.1002/ajmg.a.38320] [PubMed] [Google Scholar]

20. Romeiro MF, Souza WM, Tolardo AL, Vieira LC, Colombo TE, Aquino VH, et al. *Evaluation and optimization of SYBR Green real-time reverse transcription polymerase chain reaction as a tool for diagnosis of the Flavivirus genus in Brazil.* Rev Soc Bras Med Trop. 2016 ;49(3):279-85. [View at Publisher] [DOI:10.1016/j.spen.2006.06.004] [PubMed] [Google Scholar]

How to Cite:
Zehtabian SH, Alibakhshi R, Seyedena SY, Rai AR[Development of a New Framework for Health Assessment in Patients with Coronary Artery Disease by using miRNA-197 in Adults]. mljgoums. 2021; 15(5): 27-31 DOI: 10.29252/mlj.15.5.27