



Expression levels of miR-22, miR-30c, miR-145, and miR-519d and their possible associations with inflammatory markers among patients with coronary artery disease

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Original Article

Abstract

BACKGROUND: Coronary artery disease (CAD) is a leading cause of death around the world. Micro-ribonucleic acid (miRNA) can be involved in forming of atherosclerotic plaques, inflammation, cholesterol metabolism, and other mechanisms involved in CAD development. This study aimed to evaluate the expression level of miR-22, miR-30c, miR-145, and miR-519d and their possible association with inflammatory markers among patients with CAD.

METHODS: The expression level of miR-22, miR-30c, miR-145, miR-519d, interleukin 6 (IL-6), and transforming growth factor beta (TGF- β) was determined in peripheral blood mononuclear cells (PBMCs) from 46 patients with CAD and 39 healthy controls using real-time quantitative polymerase chain reaction (qPCR) assay.

RESULTS: 53.8% (n = 21) and 52.2% (n = 24) of controls and cases were men, respectively; the mean age was 59.8 ± 7.4 and 57.0 ± 9.8 years, respectively. The miRNA expression pattern of each group showed significantly different expression profiles. In the CAD patients group, miR-22, miR-30c, and miR-145 were down-regulated compared to the control group. On the opposite, miR-519d was up-regulated in patients with CAD compared to the control group. Our results also showed that the expression levels of IL-6 and TGF- β were up-regulated among patients with CAD compared to the control group. In addition, the expression of miR-145 and miR-519d had a significantly negative and positive correlation with TGF- β and IL-6, respectively.

CONCLUSION: The change in expression levels of miR-22, miR-30c, miR-145, and miR-519d in PBMCs of patients with CAD could be considered as a potential biomarker for CAD.

Keywords: Coronary Artery Diseases; MiRNAs; Peripheral Blood Mononuclear Cell

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Introduction

Coronary artery disease (CAD) is a leading cause of death around the world.¹ In the United States (US), CAD is the most common form of heart disease. . In 2015, 110 million people were diagnosed with CAD, of which 8.9 million died.^{2,3} It accounts for 15.6 percent of all deaths worldwide, making it the leading cause of death.³

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This phenomenon is a complex, chronic, and progressive pathological process characterized by the accumulation of lipid, low-grade inflammation, and fibrous elements in the sub-endothelial of coronary arteries, yielding the formation of atherosclerotic lesions that restrict blood flow to the myocardium, which causes several cardiovascular-related diseases.^{4,5}

The comprehensive data revealed that the inflammatory process and dysfunctional vascular endothelial cells (ECs) are the major pathogenic features of CAD.⁶ It leads to the diapedesis of leukocytes and monocytes into the vessel wall, accumulation of foam cell and fatty streak, migration and proliferation of vascular smooth muscle cells (VSMCs), extracellular matrix remodeling, and plaque formation.⁴ The simultaneous increases in the expression levels of inflammatory cytokines such as interleukin 6 (IL-6)⁷ and an increase in lipoproteins may be associated with atherosclerosis.⁸ Transforming growth factor beta (TGF- β), as a multifunctional cytokine, was increased in the early stage of congestive heart failure (CHF).⁹ Elevated serum levels of the transforming growth factor (TGF) family are potential new biomarkers in heart failure (HF).¹⁰ Eventually, acute plaque rupture causes thrombosis by complications of extensive lesions to cause unstable coronary syndromes, myocardial infarction (MI), and HF.⁴

Early detection of this disease provides early management and intervention and decreases morbidity and mortality. Recent research has shown that micro-ribonucleic acid (miRNA) can be used as a biomarker in various clinical settings.¹¹ MiRNAs are a large family of single-stranded non-coding ribonucleic acids (RNAs) (18-25 nucleotides in length) that regulate gene expression at the post-transcriptional level by inhibiting and degrading messenger RNA (mRNA) translation.¹² At the cellular level, they are implicated in a range of physiological and biochemical processes. MiRNAs may play a role in forming atherosclerotic plaques, inflammation, cholesterol metabolism, and other CAD-related mechanisms.^{1,4} As a result, changes in miRNA expression patterns are thought to be important markers for patients with CAD.¹ This study aimed to evaluate the expression of miR-22, miR-30c, miR-145, and miR-519d and their possible association with inflammatory markers among patients with CAD.

Materials and Methods

Study population: This case-control study included 46

patients with CAD and 39 age-matched healthy individuals as the control group.¹³ The sample size was calculated based on the model $n=2 \times (Z_{1-\alpha/2} + Z_{1-\beta})^2 \delta^2/\Delta^2$ (δ : 0.7, Δ : 0.41) to estimate the minimum number of samples required in each study group (CAD versus control) to obtain an alpha error of 5% (Z_α) (two-tailed test because the results could be bidirectional), 80% power ($Z_{1-\beta}$) to detect approximately double exchange rate transcriptional expression between groups (Δ) and standard deviation (SD) levels (δ).¹⁴

Ethylenediaminetetraacetic acid (EDTA)-coated vacutainer tubes were used to collect about 7 ml of peripheral blood from patients with CAD and healthy controls. Patients with a confirmed diagnosis of CAD were included in this study. All cases were selected from the heart department of Rasoul Akram Hospital located in Tehran, Iran, for five months from April to September 2020, which officially declared that primary percutaneous coronary intervention (PPCI) was conducted 24 hours a day, seven days a week. Data from patients were collected through the hospital information system (HIS). Security and anonymity of data and confidentiality of individual medical records were ensured at all stages. All patients who were referred to the hospital and underwent PPCI were eligible. After performing electrocardiogram (ECG) analysis, catheterization, measurement of laboratory indexes such as creatine kinase-MB (CK-MB), creatine kinase (CK), lactate dehydrogenase (LDH), cardiac troponin I (cTn-I), very-low-density lipoprotein (VLDL), and C-reactive protein (CRP), and finally, review of hospital records by a cardiologist, patients were categorized into two groups: group A comprised patients with CAD, and group B comprised patients without CAD (healthy control). Our control group consisted of people referred to the cardiology department due to chest pain, and after an examination, it was determined that they did not have CAD. A total of 39 individuals were randomly selected according to age and gender. This project was approved by the Ethics Committee of Iran University of Medical Sciences, Tehran.

Isolation of peripheral blood mononuclear cells (PBMCs):

Ficoll-Paque density gradient method was used to isolate PBMC from a 7 ml sample of peripheral blood. There are four steps to this method: (1) drawing a blood sample, (2) centrifuging the plasma, (3) isolating PBMC using Ficoll-Hypaque density gradient centrifugation as a standard procedure, and (4) cleaning the isolated PBMC [three times with phosphate-buffered saline (PBS)] and storing at -20°

C until use.

RNA extraction and quantitative real-time polymerase chain reaction (PCR): To determine IL-6 and TGF- β expression levels, total RNA was extracted using the AccuPrep® Universal RNA Extraction Kit (Bioneer, Daejeon, Korea), according to the manufacturer's instructions. A NanoDrop spectrophotometer was used to determine the quantity and quality of the extracted RNA (Thermo Scientific, Wilmington, USA). According to the protocol, RNA was extracted from PBMCs using the miRNeasy Mini Kit (Qiagen GmbH, Hilden, Germany).

MiRNA expression assay: The miScript II RT Kit (Qiagen GmbH, Hilden, Germany) was used to perform reverse transcription reactions with 5 μ l of total RNA. The miScript SYBR Green PCR Kit (Qiagen GmbH, Hilden, Germany) was used to quantify relevant miRNAs, such as miR-22, miR-30c, miR-145, and miR-519d, according to the manufacturer's protocol. Triplicates of each reaction were performed. U6 was used as a normalization control for relative quantification. Furthermore, the $2^{-\Delta\Delta CT}$ method was used for the analysis of relative gene expression data.

1 μ g of complementary deoxyribonucleic acid (cDNA), 2X Master Mix (Qiagen GmbH, Hilden, Germany), 1 μ M of each primer (Metabion, Munich, Germany), and nuclease-free water up to 20 μ l made up the reaction mixture. The optimal real-time PCR conditions were as follows: a 10-minute denaturation step at 95° C, 40 cycles of 95° C for 15 seconds, and 60° C for 60 seconds, and a final extension step at 72° C for 30 seconds. The sequences of all primers are shown in table 1.

Statistical methods: Continuous data were displayed

as mean \pm SD and median \pm interquartile range (IQR) for normal and non-normal distributions, respectively. Normality was checked using the Shapiro-Wilk test. The statistical comparisons between the study groups were made with a t-test. Categorical data were displayed as number (percent) and were compared using the chi-square test or Fisher's exact test. However, correlation analysis between the miRNA expression level and IL-6 and TGF- β was done by Spearman's correlation coefficient. Furthermore, receiver operating characteristic (ROC) curve analysis was undertaken using expression level for each miRNA in the PBMC specimens from patients with CAD and control individuals by Analyse-it software (Analyse-it Software, Leeds, UK). ROC curve and the area under the curve (AUC) can be used as a diagnostic method to evaluate the specificity and sensitivity of the indicators. All statistical analyses were performed using Stata software (version 14.2, Stata Corporation, College Station, TX, USA), and P-values less than 0.05 were considered statistically significant.

Results

Basic characteristics of study participants: 53.8% (n = 21) and 52.2% (n = 24) of controls and cases were men, respectively; the mean age was 59.8 \pm 7.4 and 57.0 \pm 9.8 years, respectively. The risk factors for CAD were compared between these groups. Hypertension (HTN) (76%), hyperlipidemia (67.4%), hypercholesterolemia (56.5%), and diabetes (43.5%) were the most common risk factors among patients with CAD (P < 0.05). More details are shown in table 2.

Table 1. Primers for micro-ribonucleic acid (miRNA) and inflammatory associated biomarker amplification

Gene	Direction	Sequence of primers	Reference
GAPDH	Forward	5'-AGCCCAGAACATCATCCCTG-3'	41
	Reverse	5'-CACCACCTTCTTGATGTCATC-3'	
U6	Forward	5'-GCTTCGGCAGCACATATACTAAAAT-3'	42
	Reverse	5'-CGCTTCACGAATTTGCGTGTTCAT-3'	
MiR-22	Forward	5'-AAGCUGCCGUUGAAGAACUGU-3'	43
	Reverse	5'-GTGCAGGGTCCGAGGT-3'	
MiR-30c	Forward	5'-GCC GCT GTA AAC ATC CTA CAC T-3'	44
	Reverse	5'-GTG CAG GGT CCG AGG T-3'	
MiR-145	Forward	5'-GTCCAGTTTTCCAGGAATCC-3'	45
	Reverse	5'-CAGTGCAGGGTCCGAGGTAT-3'	
MiR-519d	Forward	5'-CAAAGTGCCTCCCTTT-3'	46
	Reverse	5'-CAGTGCCTGTCGTGGAGT-3'	
IL-6	Forward	5'-GACAGCCACTCACCTCTTCA-3'	47
	Reverse	5'-CATCTTTGGAAGGTTTCAGGTTGT-3'	
TGF- β	Forward	5'-TGGCGTTACCTTGGTAACC-3'	41
	Reverse	5'-GGTGTGAGCCCTTCCAG-3'	

GADPH: Glyceraldehyde-3-phosphate dehydrogenase; IL-6: Interleukin 6; TGF- β : Transforming growth factor beta

Table 2. Participant's characteristics between patients with coronary artery disease (CAD) and healthy controls

Characteristics	CAD (n = 46)	Control (n = 39)	P*
Age (range) (year)	59.8 ± 7.4 (34-79)	57.0 ± 9.8 (32-82)	0.1390
Sex (men)	24 (52.2)	21 (53.8)	0.8780
Hypertension	35 (76.0)	4 (10.2)	0.0001
Hyperlipidemia	31 (67.4)	11 (28.2)	0.0001
Diabetes	20 (43.5)	4 (10.2)	0.0010
Active smoking	11 (24.0)	6 (15.4)	0.3270
Obesity	8 (17.4)	4 (10.2)	0.8860
Hypercholesterolemia	26 (56.5)	8 (20.5)	0.0010

Data are presented as number and percentage or mean ± standard deviation (SD)

*P-value less than 0.05 was statistically significant

Independent T-test and chi-square test were used for comparing groups in continuous and categorical variables

Expression levels of miR-22, miR-30c, miR-145, and miR-519d in PBMC of the subjects: In this study, the expression levels of four miRNAs, including miR-22, miR-30c, miR-145, and miR-519d were quantified in the PBMCs of patients with CAD, and the results were compared with the healthy control group. The miRNA expression pattern of each group showed significantly different expression profiles (Table 3).

In the CAD patients group, the expression levels of miR-22 (-4.583-fold change, $P < 0.0001$), miR-30c (4.671-fold change, $P < 0.0001$), and miR-145 (-6.068-fold change, $P < 0.0001$) were down-regulated compared to the control group. Conversely, miR-519d (3.92-fold change, $P < 0.001$) was up-regulated in patients with CAD compared to the control group. Among patients with CAD, the highest and the lowest expression levels were observed for miR-519d and miR-145, respectively (Figure 1).

Expression levels of IL-6 and TGF- β in serum of subjects: We also investigated the expression levels of two inflammation-associated biomarkers, IL-6 and TGF- β , among CAD and control groups. Our results showed that the expression levels of IL-6 (4.086-fold change, $P < 0.0001$) and TGF- β (3.945-fold change,

$P < 0.0001$) were up-regulated among patients with CAD in comparison to the control group (Table 4). More information about fold changes and statistical data are shown in figure 2.

Correlation between miRNA expression levels with TGF- β and IL-6: The correlation analysis results showed that all miRNAs were significantly correlated with TGF- β , and among them, miR-145 had a significantly negative correlation with TGF- β and IL-6. In contrast, miR-519d was positively correlated with TGF- β and IL-6. More details are provided in table 5.

ROC curve analysis: In ROC curve analysis, we obtained the following AUC values: miR-22: 0.895 [95% confidence interval (CI): 0.823-0.968, sensitivity 80.4%, specificity 84.6%, $P < 0.0001$], miR-30c: 0.873 (95% CI: 0.797-0.949, sensitivity 87.4%, specificity 79.5%, $P < 0.0001$), miR-145: 0.985 (95% CI: 0.969-1.000, sensitivity 91.3%, specificity 94.8%, $P < 0.0001$), and miR-519d: 0.873 (95% CI: 0.800-0.945, sensitivity 78.2%, specificity 79.4%, $P < 0.0001$), respectively (Figure 3). Our results demonstrated that these four miRNAs had a great potential to provide sensitive and specific diagnostic values. More information about ROC curve and AUC is shown in figure 3.

Table 3. Expression of micro-ribonucleic acid (miRNA)

MiRNA	CAD	Control	P*
MiR-22	-4.583 ± 2.407 (median: -4.40, IQR: -3.25)	-0.003 ± 2.573 (median: 0.83, IQR: -2.50)	< 0.0001
MiR-30c	-4.671 ± 3.174 (median: -4.70, IQR: -4.53)	-0.004 ± 2.641 (median: 0.47, IQR: -3.00)	< 0.0001
MiR-145	-6.068 ± 1.917 (median: -5.90, IQR: -2.63)	0.004 ± 2.218 (median: 0.03, IQR: -3.01)	< 0.0001
MiR-519d	3.920 ± 2.393 (median: 4.20, IQR: -3.00)	0.001 ± 2.519 (median: 0.04, IQR: -3.50)	< 0.0001

Data are presented as mean ± standard deviation (SD)

*P-value less than 0.05 was statistically significant

The Mann-Whitney U test was used to compare the expression level of cellular miRNAs between the case and control groups

MiRNA: Micro-ribonucleic acid; CAD: Coronary artery disease; IQR: Interquartile range

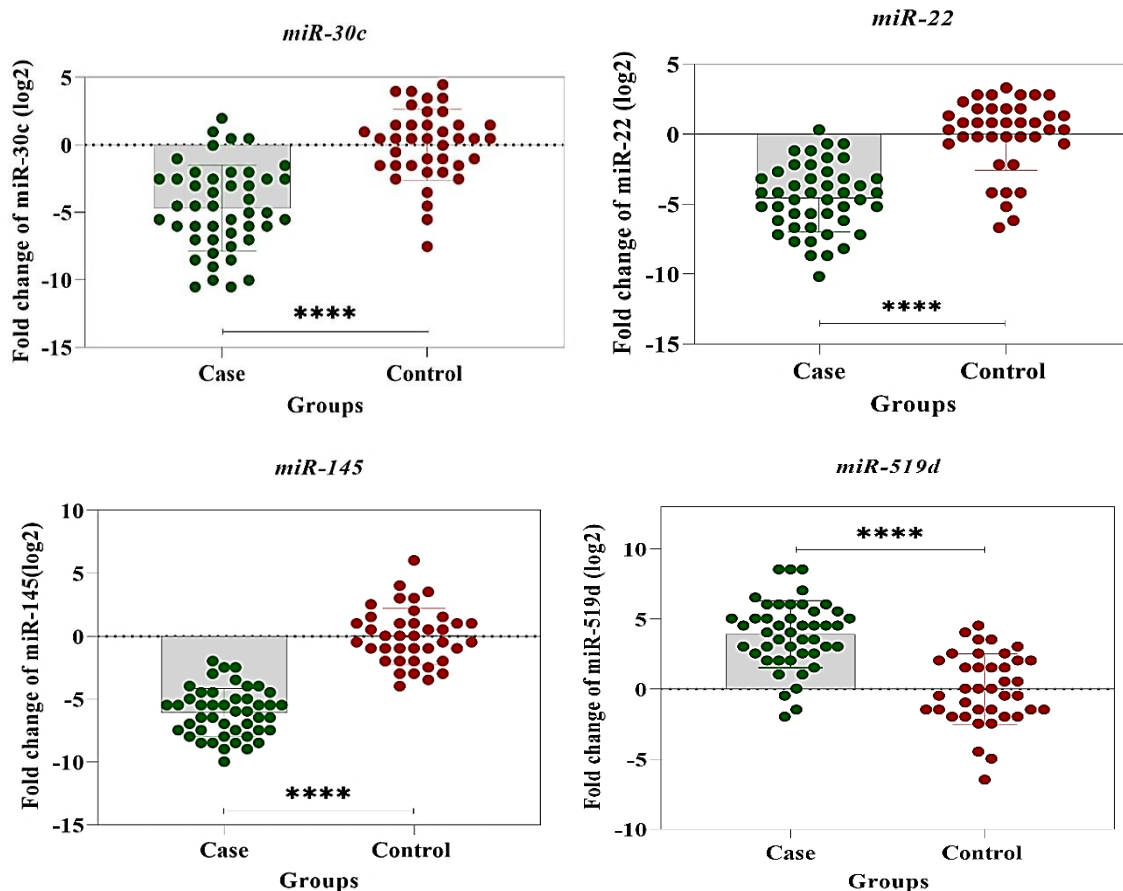


Figure 1. Comparison of micro-ribonucleic acids (miRNAs) (miR-22, miR-30c, miR-145, and miR-519d) expression level between patients with coronary artery disease (CAD) (n = 46) and healthy controls (n = 39); in patients with CAD compared to healthy control, the mean expression level of miR-22 (-4.49-fold), miR-30c (-6.68-fold), and miR-145 (-6-fold) were downregulated, and also the mean expression level of miR-519d (3.92-fold) was upregulated. The expression level of selected cellular miRNAs in peripheral blood mononuclear cells (PBMCs) was measured by quantitative polymerase chain reaction (qPCR). The Mann-Whitney U test and unpaired t-test were used to compare the expression level of cellular miRNAs between the case and control groups (****P ≤ 0.0001).

Discussion

The involvement of miRNAs in a wide range of cellular physiological and pathophysiological processes,¹⁵ especially in lipid metabolism,¹⁶ VSMC proliferation,^{17,18} inflammatory responses,¹⁹ lipid uptake and efflux,²⁰ plaque formation,²¹ and other processes is strongly associated with the initiation

and the development of CAD.^{22,23} In the present study, the expression levels of four miRNAs, including miR-22, miR-30c, miR-145, miR-519d, and inflammation-associated markers, IL-6 and TGF- β , were evaluated in PBMCs of patients with CAD, and the results were compared with the healthy control group.

Table 4. Expression of inflammation-associated biomarker

Inflammation biomarker	CAD	Control	P*
IL-6	4.086 ± 3.090 (median: 4.51, IQR: -4.37)	0.003 ± 3.139 (median: -0.74, IQR: -4.00)	< 0.0001
TGF- β	3.945 ± 2.855 (median: 4.13, IQR: -5.00)	0.001 ± 3.062 (median: 0.13, IQR: -2.98)	< 0.0001

Data are presented as mean ± standard deviation (SD)

*P-value less than 0.05 was statistically significant

The Mann-Whitney U test was used to compare the expression level of cellular miRNAs between the case and control groups
IL-6: Interleukin 6; TGF- β : Transforming growth factor beta; CAD: Coronary artery disease; IQR: Interquartile range

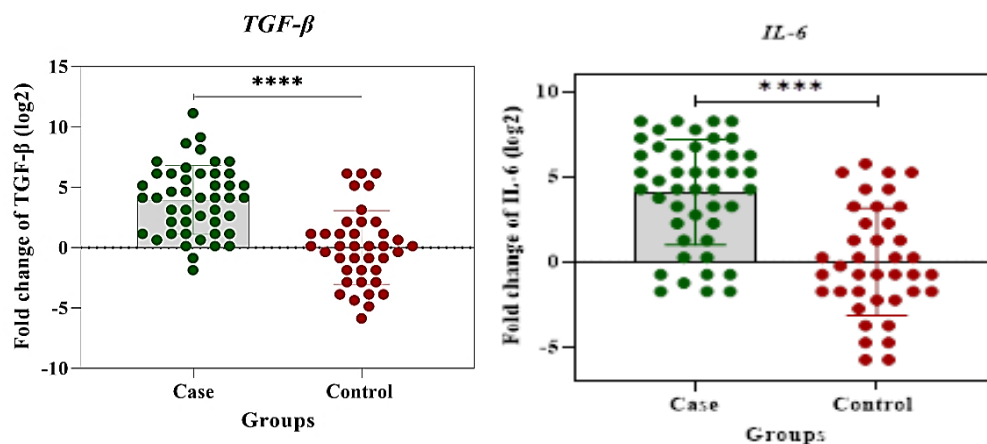


Figure 2. Comparison of the expression levels of inflammation-associated cytokines [interleukin 6 (IL-6) and transforming growth factor beta (TGF- β)] in the patients with coronary artery disease (CAD) ($n = 46$) and healthy control ($n = 39$) samples; the mean expression levels of IL-6 (4.08-fold), and TGF- β (3.8-fold) were upregulated in patients with CAD compared to healthy controls. Quantitative polymerase chain reaction (qPCR) was used to determine the expression levels of IL-6 and TGF- β . The unpaired t-test was used to compare the expression levels of cytokines between the CAD and healthy control groups (**** $P \leq 0.0001$).

Table 5. Spearman's correlation coefficient between the micro-ribonucleic acid (miRNA) and the inflammation markers

	MiR-22	MiR-30c	MiR-145	MiR-519d
IL-6	-0.2001	-0.2100	-0.5330**	0.4348**
TGF- β	-0.2696*	-0.2572*	-0.5780**	0.4518**

P-value less than 0.05 was statistically significant

* $P < 0.05$; ** $P < 0.001$

IL-6: Interleukin 6; TGF- β : Transforming growth factor beta

Moreover, the correlations between the expression levels of these miRNAs and IL-6 and TGF- β in patients with CAD and the control group were assessed. Our results showed that the expression levels of miR-22, miR-30c, and miR-145 were significantly down-regulated among patients with CAD compared to the healthy control. However, the expression level of miR-519d was significantly up-regulated among patients with CAD compared to the healthy controls. We also investigated the expression levels of IL-6 and TGF- β , and our results showed that the expression levels of IL-6 and TGF- β were significantly up-regulated among patients with CAD compared to the healthy controls.

CAD is a chronic inflammatory disease associated with a variety of inflammatory cytokines.²⁴ In this study, the expression levels of inflammatory cytokines were measured by real-time PCR assay. Our results showed that the expression levels of TGF- β and IL-6 were unregulated; the up-regulation of IL-6 and TGF- β was strongly positively associated with miR-519d and negatively associated with miR-145. MiR-22 also had a

significant association with TGF- β , while no correlation was found between the expression levels of miR-22 and miR-30c with IL-6. MiR-22 has been initially identified as a tumor suppressor miRNA in many cancers.²⁵ Besides, miR-22 overexpression can suppress the inflammation process in patients with rheumatoid arthritis (RA).²⁶ Huang et al. investigated the protective effect of miR-22 against EC injury. Their results showed that the miR-22 reduced the activity and apoptosis of coronary arterial ECs (CAECs) by suppressing the inflammasome signaling pathway in a rat model of coronary heart disease. Their study also indicated that miR-22 could down-regulate the levels of inflammatory cytokines by suppressing NLRP3 inflammasome activation, which exerts an active role in protecting CAECs.²⁷

Proinflammatory factors play important roles in the pathogenesis of atherosclerosis and CAD. Among them, IL-6 is an upstream inflammatory cytokine that has been proven to have a crucial role in the initiation and the development of the diseases.²⁸ The potential target site between miR-22 and IL-6 receptor (IL6R) was predicted by Targetscan.²⁶ Yang et al.'s study showed that the overexpression of miR-22 inhibited the inflammatory injury in RA by targeting IL6R.²⁶ Besides, Chen et al. investigated the role of miR-22 in the pathogenesis of CAD by targeting monocyte chemoattractant protein-1 (MCP-1). Their study showed that the expression of miR-22 was down-regulated in patients with CAD, which correlated with the severity of the disease.

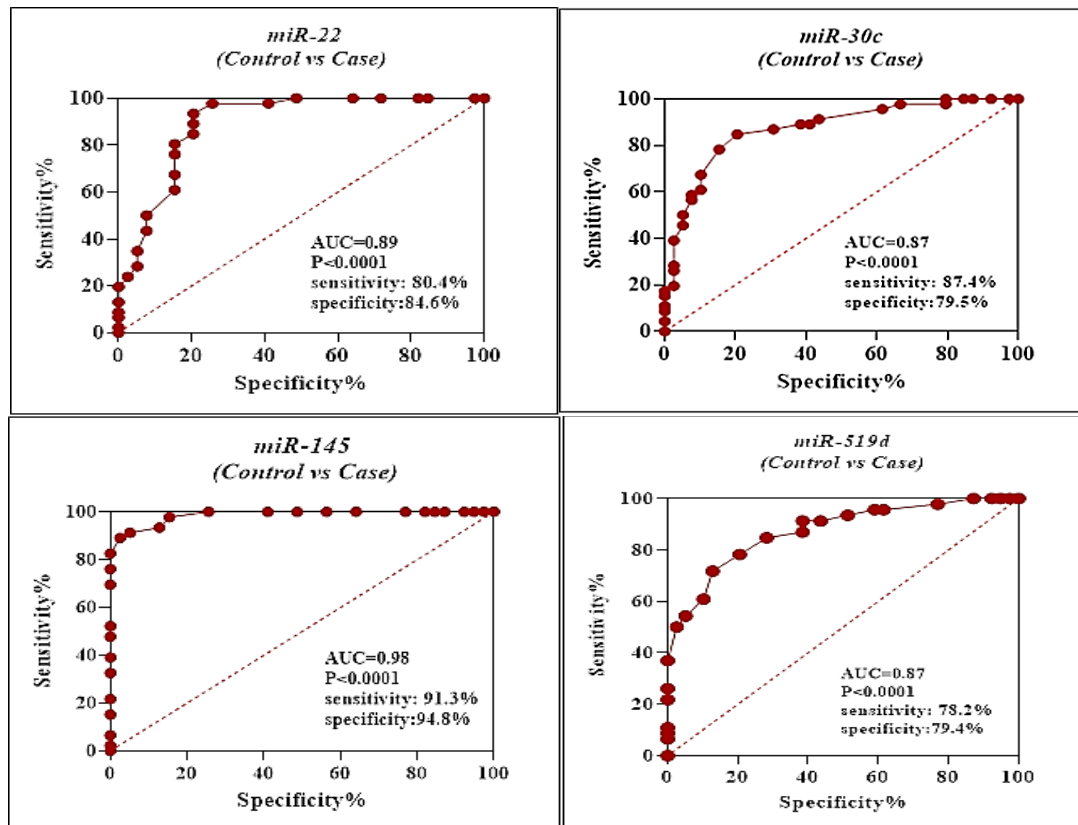


Figure 3. Receiver operating characteristic (ROC) curve analysis using peripheral blood mononuclear cells (PBMCs) miR-22, miR-30c, miR-145, and miR-519d for discriminating between patients with coronary artery disease (CAD) ($n = 46$) and healthy controls ($n = 39$); PBMC miR-145 [area under the curve (AUC) = 0.98] probably can be a good biomarker for discriminating between patients with CAD and healthy controls.

In patients with CAD, the expression of inflammatory cytokines is increased following a decrease in miR-22 expression. It may serve as a biomarker of CAD development and progression. It may be possible to reduce the expression of inflammatory cytokines by increasing the expression of miR-22.²⁹ The reduction of inflammatory cytokines may be helpful in preventing the progression of atherosclerotic plaques.

MiR-30c has been highly expressed in the heart tissues, but its role in heart development is unknown.³⁰ Modulating the expression of miR-30c may affect vascular calcification. The study conducted by Soh et al. showed that miR-30c interacted with the microsomal triglyceride transfer protein (MTP) mRNA and reduced its activity and media apolipoprotein B (apoB). It has also been documented that miR-30c decreases atherosclerosis and hyperlipidemia in the Western diet-fed mice by reducing lipid synthesis and secretion of triglyceride-rich apoB-containing lipoproteins.^{30,31} Previous studies have indicated a direct correlation between the increased levels of low-density

lipoprotein (LDL), cholesterol, and apoB with the risk of atherosclerotic cardiovascular diseases (ASCVD).^{32,33} Accordingly, miR-30c can coordinately lead to a reduction in the lipid biosynthesis and lipoprotein secretion to control plasma and hepatic lipids, and it may be useful to treat hyperlipidemias and related disorders.³⁰ The study conducted by Ceolotto et al. demonstrated that the reduction of miR30c was a promoter for early atherosclerosis by conveying proinflammatory-proapoptotic signals and impairing endothelial healing.³⁴ Therefore, stimulation of miR-30c can be a candidate for direct anti-atherosclerotic treatment.

Previous studies have reported that miR-145 is the most abundant miRNA expressed in the VSMCs of vascular walls. MiR-145 participates in VSMC function regulation, including the proliferation and migration, via its target genes.³⁵ It has been reported that TGF- β receptor II (TGFBR2) and cluster of differentiation 40 (CD40) are direct targets for miR-145 in the proliferation and phenotypic modulation of VSMCs.³⁶ Wang et al. showed that TGF- β 1 led to a decrease in miR-

145 expression and an increase in the proliferation and migration of VSMCs. Overexpression of miR-145 prevented cell autophagy, whereas the inhibition of miR-145 promoted autophagy in TGF- β 1-stimulated VSMCs.³⁷ He et al. showed that the overexpression of miR-145 had an anti-inflammatory function via targeting the osteoprotegerin (OPG).³⁸ Nevertheless, miR-145 may play a protective role in atherosclerosis models. Some studies reported that the expression of miR-145 in atherosclerosis and patients with CAD was reduced. Faccini et al. showed that the reduction of circulating miR-155, miR-145, and let-7c expression could be used as diagnostic biomarkers of CAD.³⁹ In addition, several clinical studies have demonstrated an association between the miR-145 dysregulation and many cardiovascular diseases (CVDs) like atherosclerosis, essential HTN, pulmonary arterial HTN, and CAD.³⁵ This suggests that miR-145 can be a potential and prognostic biomarker for progressing stages of CVDs.

Martinelli et al. showed that the peroxisome proliferator-activated receptor α (PPARA) was a target for miR-519d. Their results showed that miR-519d suppressed translation of the PPARA protein and increased lipid accumulation during preadipocyte differentiation.⁴⁰ Alteration of PPARA protein expression and the overexpression of miR-519d could be associated with obesity.⁴⁰ Previously, Satoh et al. investigated the expression levels of some miRNAs such as miR-519d among patients with CAD. They reported that the expression level of miR-519d had no association with CAD.¹³ In contrast, our results showed that the expression of miR-519d was increased in patients with CAD. A variety of internal and external factors, including age, sex, patients' conditions, medications received, and small sample size may lead to this difference. Moreover, our results suggested that miR-519d could be used as a potential biomarker in patients with CAD. ROC analysis showed that these four miRNAs had a great potential to provide sensitive and specific diagnostic values. However, the mechanism of miR-519d up-regulation is not clear yet.

Conclusion

The change in expression levels of miR-22, miR-30c, miR-145, and miR-519d in PBMCs of patients with CAD could be considered as a potential biomarker for CAD. MiRNAs profile needs to be quantified, analyzed, and manipulated in different stages of chronic diseases, such as CAD

for the determination of biomarker and can open a new gate for controlling these diseases. However, the data of the study suggest that it is required to warrant further investigation.

Acknowledgments

None.

Conflict of Interests

Authors have no conflict of interests.

Authors' Contribution

SG, SHM, SHS, and AT conceived and designed the study. SG and SHS collected samples. AG, AAP, FBS, LB, DJ, and HG performed laboratory tests. JSN, LH, and SG analyzed the data. SG, SJK, LB, AT, and SHM wrote and revised the paper. All authors read and approved the final manuscript.

References

1. Fazmin IT, Achercouk Z, Edling CE, Said A, Jeevaratnam K. Circulating microRNA as a biomarker for coronary artery disease. *Biomolecules* 2020; 10(10): 1354.
2. GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; 388(10053): 1545-602.
3. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; 388(10053): 1459-544.
4. Chen LJ, Lim SH, Yeh YT, Lien SC, Chiu JJ. Roles of microRNAs in atherosclerosis and restenosis. *J Biomed Sci* 2012; 19(1): 79.
5. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005; 352(16): 1685-95.
6. Gimbrone MA, Garcia-Cardena G. Endothelial cell dysfunction and the pathobiology of atherosclerosis. *Circ Res* 2016; 118(4): 620-36.
7. Thakore AH, Guo CY, Larson MG, Corey D, Wang TJ, Vasan RS, et al. Association of multiple inflammatory markers with carotid intimal medial thickness and stenosis (from the Framingham Heart Study). *Am J Cardiol* 2007; 99(11): 1598-602.
8. Campos H, Genest JJ, Blijlevens E, McNamara JR, Jenner JL, Ordovas JM, et al. Low density

- lipoprotein particle size and coronary artery disease. *Arterioscler Thromb* 1992; 12(2): 187-95.
9. Saadati S, Eskandari V, Rahmani F, Mahmoudi MJ, Rahnemoon Z, Rahmati Z, et al. Gene expression and levels of TGF- β in PBMC is associated with severity of symptoms in chronic heart failure. *Avicenna J Med Biotechnol* 2020; 12(2): 132-4.
 10. Bai Y, Zhang P, Zhang X, Huang J, Hu S, Wei Y. LTBP-2 acts as a novel marker in human heart failure - a preliminary study. *Biomarkers* 2012; 17(5): 407-15.
 11. Wang GK, Zhu JQ, Zhang JT, Li Q, Li Y, He J, et al. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *Eur Heart J* 2010; 31(6): 659-66.
 12. Keshavarz M, Dianat-Moghadam H, Sofiani VH, Karimzadeh M, Zargar M, Moghoofei M, et al. miRNA-based strategy for modulation of influenza A virus infection. *Epigenomics* 2018; 10(6): 829-44.
 13. Satoh M, Takahashi Y, Tabuchi T, Tamada M, Takahashi K, Itoh T, et al. Circulating Toll-like receptor 4-responsive microRNA panel in patients with coronary artery disease: Results from prospective and randomized study of treatment with renin-angiotensin system blockade. *Clin Sci (Lond)* 2015; 128(8): 483-91.
 14. Kadam P, Bhalerao S. Sample size calculation. *Int J Ayurveda Res* 2010; 1(1): 55-7.
 15. Chen KC, Juo SH. MicroRNAs in atherosclerosis. *Kaohsiung J Med Sci* 2012; 28(12): 631-40.
 16. Flowers E, Froelicher ES, Aouizerat BE. MicroRNA regulation of lipid metabolism. *Metabolism* 2013; 62(1): 12-20.
 17. Guo X, Li D, Chen M, Chen L, Zhang B, Wu T, et al. miRNA-145 inhibits VSMC proliferation by targeting CD40. *Sci Rep* 2016; 6: 35302.
 18. Wang D, Atanasov AG. The microRNAs regulating vascular smooth muscle cell proliferation: A minireview. *Int J Mol Sci* 2019; 20(2).
 19. Essandoh K, Li Y, Huo J, Fan GC. MiRNA-mediated macrophage polarization and its potential role in the regulation of inflammatory response. *Shock* 2016; 46(2): 122-31.
 20. Moore KJ, Rayner KJ, Suarez Y, Fernandez-Hernando C. The role of microRNAs in cholesterol efflux and hepatic lipid metabolism. *Annu Rev Nutr* 2011; 31: 49-63.
 21. Bidzhikov K, Gan L, Denecke B, Rostalsky A, Hristov M, Koepfel TA, et al. microRNA expression signatures and parallels between monocyte subsets and atherosclerotic plaque in humans. *Thromb Haemost* 2012; 107(4): 619-25.
 22. Zhang Y, Zhang L, Wang Y, Ding H, Xue S, Qi H, et al. MicroRNAs or long noncoding RNAs in diagnosis and prognosis of coronary artery disease. *Aging Dis* 2019; 10(2): 353-66.
 23. Melak T, Baynes HW. Circulating microRNAs as possible biomarkers for coronary artery disease: a narrative review. *EJIFCC* 2019; 30(2): 179-94.
 24. Roifman I, Beck PL, Anderson TJ, Eisenberg MJ, Genest J. Chronic inflammatory diseases and cardiovascular risk: A systematic review. *Can J Cardiol* 2011; 27(2): 174-82.
 25. Ling B, Wang GX, Long G, Qiu JH, Hu ZL. Tumor suppressor miR-22 suppresses lung cancer cell progression through post-transcriptional regulation of ErbB3. *J Cancer Res Clin Oncol* 2012; 138(8): 1355-61.
 26. Yang QY, Yang KP, Li ZZ. MiR-22 restrains proliferation of rheumatoid arthritis by targeting IL6R and may be concerned with the suppression of NF- κ B pathway. *Kaohsiung J Med Sci* 2020; 36(1): 20-6.
 27. Huang WQ, Wei P, Lin RQ, Huang F. Protective effects of microRNA-22 against endothelial cell injury by targeting NLRP3 through suppression of the inflammasome signaling pathway in a rat model of coronary heart disease. *Cell Physiol Biochem* 2017; 43(4): 1346-58.
 28. Anderson DR, Poterucha JT, Mikuls TR, Duryee MJ, Garvin RP, Klassen LW, et al. IL-6 and its receptors in coronary artery disease and acute myocardial infarction. *Cytokine* 2013; 62(3): 395-400.
 29. Chen B, Luo L, Zhu W, Wei X, Li S, Huang Y, et al. miR-22 contributes to the pathogenesis of patients with coronary artery disease by targeting MCP-1: An observational study. *Medicine (Baltimore)* 2016; 95(33): e4418.
 30. Soh J, Iqbal J, Queiroz J, Fernandez-Hernando C, Hussain MM. MicroRNA-30c reduces hyperlipidemia and atherosclerosis in mice by decreasing lipid synthesis and lipoprotein secretion. *Nat Med* 2013; 19(7): 892-900.
 31. Gatling AM. The Role of MicroRNA-30C in Regulating Plasma Lipids in MicroRNA-30C Double Knockout Mice [MSc Thesis]. Albany, NY: State University of New York; 2018.
 32. AIM-HIGH Investigators. The role of niacin in raising high-density lipoprotein cholesterol to reduce cardiovascular events in patients with atherosclerotic cardiovascular disease and optimally treated low-density lipoprotein cholesterol Rationale and study design. The Atherothrombosis Intervention in Metabolic syndrome with low HDL/high triglycerides: Impact on Global Health outcomes (AIM-HIGH). *Am Heart J* 2011; 161(3): 471-7.
 33. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J* 2017; 38(32): 2459-72.

34. Ceolotto G, Giannella A, Albiero M, Kuppusamy M, Radu C, Simioni P, et al. miR-30c-5p regulates macrophage-mediated inflammation and pro-atherosclerosis pathways. *Cardiovasc Res* 2017; 113(13): 1627-38.
35. Zhao W, Zhao SP, Zhao YH. MicroRNA-143/-145 in cardiovascular diseases. *Biomed Res Int* 2015; 2015: 531740.
36. Zhao N, Koenig SN, Trask AJ, Lin CH, Hans CP, Garg V, et al. MicroRNA miR145 regulates TGFBR2 expression and matrix synthesis in vascular smooth muscle cells. *Circ Res* 2015; 116(1): 23-34.
37. Wang W, Chen L, Shang C, Jin Z, Yao F, Bai L, et al. miR-145 inhibits the proliferation and migration of vascular smooth muscle cells by regulating autophagy. *J Cell Mol Med* 2020; 24(12): 6658-69.
38. He M, Wu N, Leong MC, Zhang W, Ye Z, Li R, et al. miR-145 improves metabolic inflammatory disease through multiple pathways. *J Mol Cell Biol* 2020; 12(2): 152-62.
39. Faccini J, Ruidavets JB, Cordelier P, Martins F, Maoret JJ, Bongard V, et al. Circulating miR-155, miR-145 and let-7c as diagnostic biomarkers of the coronary artery disease. *Sci Rep* 2017; 7: 42916.
40. Martinelli R, Nardelli C, Pilone V, Buonomo T, Liguori R, Castano I, et al. miR-519d overexpression is associated with human obesity. *Obesity (Silver Spring)* 2010; 18(11): 2170-6.
- Joseph AT, Bhardwaj SK, Srivastava LK. Role of prefrontal cortex anti- and pro-inflammatory cytokines in the development of abnormal behaviors induced by disconnection of the ventral hippocampus in neonate rats. *Front Behav Neurosci* 2018; 12: 244.
42. Chu C, Liu X, Bai X, Zhao T, Wang M, Xu R, et al. MiR-519d suppresses breast cancer tumorigenesis and metastasis via targeting MMP3. *Int J Biol Sci* 2018; 14(2): 228-36.
43. Gu W, Zhan H, Zhou XY, Yao L, Yan M, Chen A, et al. MicroRNA-22 regulates inflammation and angiogenesis via targeting VE-cadherin. *FEBS Lett* 2017; 591(3): 513-26.
44. Xia Y, Chen Q, Zhong Z, Xu C, Wu C, Liu B, et al. Down-regulation of miR-30c promotes the invasion of non-small cell lung cancer by targeting MTA1. *Cell Physiol Biochem* 2013; 32(2): 476-85.
45. Hua M, Qin Y, Sheng M, Cui X, Chen W, Zhong J, et al. miR145 suppresses ovarian cancer progression via modulation of cell growth and invasion by targeting CCND2 and E2F3. *Mol Med Rep* 2019; 19(5): 3575-83.
46. Chen R, Zhou S, Chen J, Lin S, Ye F, Jiang P. LncRNA BLACAT1/miR-519d-3p/CREB1 axis mediates proliferation, apoptosis, migration, invasion, and drug-resistance in colorectal cancer progression. *Cancer Manag Res* 2020; 12: 13137-48.
47. Lee JS, Ko EJ, Hwang HS, Lee YN, Kwon YM, Kim MC, et al. Antiviral activity of ginseng extract against respiratory syncytial virus infection. *Int J Mol Med* 2014; 34(1): 183-90.