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Correlation Between Serum microRNA-18a Level and Endothelial Function and Prognosis in Female Coronary Heart Disease Patients

ABSTRACT

Background: The aim was to analyze the correlation between serum microRNA (miR)-18a level, endothelial function, and prognosis in female coronary heart disease (CHD) patients.

Methods: One hundred sixtyfemale patients admitted to our hospital for the first occurrences of chest pain and tightness were divided into CHD and non-CHD groups based on the coronary angiography results. Clinical data, laboratory indexes, serum miR-18a level, and endothelial function [flow-mediated dilation (FMD) function, endothelin 1 (ET-1), and nitric oxide (NO)] were compared.

Results: There were no significant differences in clinical data (except CHD family history) between 2 groups. Coronary heart disease group had significantly lower levels of NO and FMD, while significantly higher levels of miR-18a and ET-1 than non-CHD group ($P < .05$). Pearson correlation showed that serum miR-18a level was positively correlated with ET-1 ($r = 0.492$, $P < .001$), and negatively correlated with NO and FMD ($r = -0.504$, -0.307, $P < .001$). The receiver operating characteristic) curve showed that the area under the curve of serum miR-18a level in predicting the occurrence of CHD in women was 0.878 (95% CI: 0.828-0.928). Compared with good prognosis group, poor prognosis group had significantly lower NO, and FMD levels, while higher proportions of acute coronary syndrome, multi-vessel disease, miR-18a, and ET-1 levels ($P < .05$).

Conclusion: The expression of serum miR-18a in female CHD patients was high, which was related to endothelial function. The increase in serum miR-18a level was a risk factor for the occurrence of MACE in female CHD patients during follow-up, and the serum miR-18a level could effectively predict the occurrence of CHD in female patients.

Keywords: Female, coronary heart disease, miR-18a, endothelial function, prognosis

ORIGINAL INVESTIGATION

INTRODUCTION

Coronary heart disease (CHD), also known as coronary atherosclerotic heart disease, is primarily a heart condition caused by lumen stenosis or blockage due to coronary atherosclerotic lesions, leading to myocardial necrosis, hypoxia, or ischemia.[1,2] After women enter menopause, the levels of serum estrogen, such as estradiol decreases, the cardiovascular protective effect of estrogen is lacking, and ventricular remodeling worsens and cardiac function decreases, resulting in a nearly 4-fold increase in the incidence of CHD compared with premenopausal women.[3] Epidemiological investigations have found that the incidence of CHD in premenopausal women was only 1/10-3/10 of that in men, but the incidence and mortality of CHD in perimenopausal and postmenopausal women were significantly increased.[4] Due to the strong compensation of myocardium, some patients have no obvious symptoms in the early stage, and typical symptoms are usually accompanied by severe coronary stenosis, which not only delays treatment but also induces major adverse cardiovascular events (MACE), which is not conducive to the prognosis. Therefore, uncovering the pathogenesis of female CHD and exploring molecular markers for early diagnosis may provide a new theoretical foundation for improving the prognosis of female CHD patients. Studies have found that during the early formation of CHD plaques, the cellular components

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within the plaques released microRNAs (miRNAs) into the blood, which may serve as biomarkers for early prediction of CHD occurence.[5,6] An *in vitro* cell experiment in China has confirmed that overexpression of miR-18a can inhibit the expression of estrogen receptors (ERs). Downregulation of miR-18a expression can participate in the proliferation and apoptosis of human umbilical vein endothelial cells by targeting ER levels and the PI3K/Akt/mTOR signaling pathway, which may provide a promising method for molecular targeted therapy of female CHD.[7] However, there are few clinical studies on the correlation and mechanism of action between miR-18a levels and female CHD. Based on this, this study observed the expression of serum miR-18a in female CHD patients, analyzed the correlation between serum miR-18a level and endothelial function and prognosis of patients, and aimed to explore a serum biomarker for predicting the occurrence of female CHD and improving the prognosis of patients.

METHODS

General Information

A total of 160 female patients, aged 45-68 years, with an average age of 57.49 ± 5.71 years, sought their initial medical consultation at our hospital between June 2020 and June 2021, presenting with their first occurrences of chest pain and chest tightness symptoms. The patients had a body mass index (BMI) ranging from 19.5 to 28.6 kg/m^2, with an average BMI of 24.79 ± 2.28 kg/m^2. Among them, 10 patients had a history of smoking, and the medical history included 39 cases of hypertension, 26 cases of diabetes, 19 cases with a family history of CHD, 7 cases of hypothyroidism, and 3 cases of autoimmune diseases. Based on the coronary angiographic results, 160 female patients were divided into a CHD group (75 cases) and a non-CHD group (85 cases).

Inclusion and Exclusion Criteria

Inclusion criteria: (1) postmenopausal women; (2) patients with first-time occurrence of chest pain and chest tightness symptoms; (3) patients whose age is ≥45 years old; (4) in the CHD group, coronary angiography showed at least 1 coronary artery with stenosis of ≥50%; (5) patients who voluntarily signed informed consent forms; and (6) patients who cooperated with treatment and follow-up. Exclusion criteria: (1) patients with a history of aortic valve disease, acute myocardial infarction, or old myocardial infarction; (2) pregnant or lactating women; (3) patients with cerebral infarction, new cerebral hemorrhage, or surgical treatment within 6 months before enrollment; (4) patients with a history of major operations such as coronary artery bypass grafting and coronary stent implantation; (5) patients with valvular heart disease and cardiomyopathy; (6) patients combined with conditions like disseminated intravascular coagulation, shock, or other critical conditions; (7) patients with malignant tumors; (8) patients with severe liver or kidney dysfunction; (9) patients complicated with heart failure or other serious primary diseases or mental disorders; (10) patients accompanied by bipolar disorder, schizophrenia, or other mental illnesses; (11) patients with acute or chronic inflammatory disorders; (12) patients lost to follow-up; (13) patients with coagulation disorders; and (14) patients who received treatment with vasoactive drugs before enrollment.

HIGHLIGHTS

- microRNA-18a levels were higher in the coronary heart disease (CHD) group than in the non-CHD group.
- microRNA-18a levels were negatively correlated with endothelial function-related factors nitric oxide (NO) and flow-mediated dilation (FMD), and positively correlated with endothelin 1 (ET-1).
- Serum miR-18a level has a high predictive efficacy for the occurrence of CHD in women.

Serum miR-18a Level

On the day of the medical appointment, 3 mL of fasting cubital venous blood was collected from the patient, centrifuged at 3000 rpm for 10 minutes at 4℃, and the upper layer of serum was collected in a 1.5 mL EP tube. Total RNA in serum was extracted according to the instructions of the Trizol Kit (Zhejiang Yuxiang Biotechnology Co., Ltd.) and the Total RNA Extraction Kit (Guangzhou BayBio Technology Co., Ltd.), and it was reverse-transcribed into cDNA by the Reverse Transcription Kit (Shanghai Umibio Science and Technology Co., Ltd.), following the manufacturer's instructions. The expression of miR-18a in serum was detected by real-time PCR (PRISM 7000 quantitative PCR instrument) using reverse-transcribed cDNA as a template. The reverse transcription system was 10 μL, and the reaction conditions were: 95°C for 90 seconds (1 cycle), 95°C for 30 seconds, 63°C for 30 seconds, 72°C for 15 seconds (40 cycles), fluorescence was collected, and U6 was used as an internal control. U6: upstream: 5′-CTCGCTTCGGCAGCACA-3′, downstream: 5′-AACGCTTCACGAATTTGCGT-3′; miR-18a: upstream: 5′-TGTAGGGTAGGTTATGACA-3′, downstream: 5′-TTCGGACTGGCCCATAGACTA-3′. The relative expression of miR-18a was calculated using the $2^{-\Delta\Delta Ct}$ method.

Endothelial Function and Other Laboratory Indexes

A total of 3 mL of fasting elbow venous blood was collected on the day of the patient's visit, centrifuged at 3000 rpm for 10 minutes at 4℃, and the upper serum was taken for determination of endothelin 1 (ET-1) level by radioimmunoassay (kit purchased from Shanghai X-Y Biotechnology Co., Ltd.). The level of nitric oxide (NO) was measured by indirect calorimetry (kit purchased from AmyJet Scientific Co., Ltd.). Fasting blood glucose (FBG) was measured by the hexokinase method (kit purchased from Shanghai Xinfan Biotechnology Co., Ltd.). Glycated hemoglobin (HbA1c) was measured by high-performance liquid chromatography (kit purchased from BiotechPack ANALYTICAL (Beijing) Co., Ltd.). Low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured by direct assay method (kit purchased from Shanghai Yuduo Biotechnology Co., Ltd.). Total cholesterol (TC) and triglyceride (TG) were determined by the enzyme reagent method (kit purchased from Beijing APPLYGEN Genetic Technology Co., Ltd.). A Hitachi 7600 automatic biochemical analyzer was used as the instrument. A color Doppler ultrasonic

diagnostic instrument (Siemens, ACUSON X300) was used to detect the internal diameter of the basic brachial artery and the internal diameter of the brachial artery after reactive congestion. The flow-mediated dilatation (FMD) function = (internal diameter of the brachial artery after reactive congestion–internal diameter of the basic brachial artery)/internal diameter of the basic brachial artery × 100%.[8]

Clinical Data

The patients' age, BMI, smoking history (>20 packs/year), past medical history [hypertension, diabetes, hypothyroidism, autoimmune disease, family history of CHD (parents, siblings, or other first-degree relatives with CHD)], and blood pressure level were recorded.

Follow-up

Patients with CHD were followed up for 24 months by telephone and outpatient follow-up after discharge from the hospital after standardized treatment. During this period, the occurrences of MACE were recorded, including heart failure (Killip grade II and above), malignant arrhythmia (which appeared on ECG or ECG monitor, including third-degree atrioventricular block, sick sinus syndrome, persistent ventricular tachycardia, atrial fibrillation, etc.), shock [systolic blood pressure (SBP) dropping below 80 mm Hg (1 mm Hg = 0.133 kPa), accompanied by clinical manifestations such as pallor, decreased urine output, rapid pulse, cold limbs, etc.], and cardiac arrest (the beating of the aorta and the disappearance of heart sounds, and the sudden stop of cardiac ejection function). Patients who experienced MACE were categorized into the poor prognosis group, while those without MACE were classified into the good prognosis group.

Statistical Analysis

Statistical analysis was performed using software IBM SPSS® v.27 (National Opinion Research Center, Chicago, Ill, USA), and the measurement data were expressed as "mean ± standard deviation," using independent sample *t*-test. The enumeration data were presented as percentages and analyzed using the χ^2 test. Pearson's correlation analysis was employed to assess correlations. Variables with $P < .05$ in the univariate analysis were entered into the binary logistic regression analysis model as covariables, with the dichotomous variables of the prognostic status of female CHD patients as dependent variables, and $P < .05$ was taken as the criteria for stepwise regression screening variables. Receiver operating characteristic (ROC) curves were plotted to obtain area under curve (AUC), and the value of serum miR-18a level in predicting the occurrence of CHD in women was analyzed. A *P*-value of <.05 was considered statistically significant.

Artificial intelligence-Assisted Technologies Statement

We did not use artificial intelligence (AI)-assisted technologies (such as large language models (LLMs), chatbots, or image creators) in the production of submitted work.

RESULTS

Comparison of Clinical Data

There were no significant differences in age, BMI, SBP, diastolic blood pressure (DBP), FBG, HbA1c, TG, TC, LDL-C, HDL-C, smoking history, previous hypertension history, previous diabetes history, previous hypothyroidism history, and previous autoimmune disease history between CHD group and non-CHD group (all $P > .05$). However, the proportion of CHD family history in the CHD group was higher than that in the non-CHD group ($P < .05$). See Table 1.

Comparison of Serum miR-18a Level and Endothelial Function

Coronary heart disease group had significantly lower levels of NO and FMD, while a significantly higher levels of miR-18a and ET-1 compared to the non-CHD group (all $P < .05$). See Table 2.

Table 1. Comparison of Clinical Data

Index		CHD group (n = 75)	Non-CHD group (n = 85)	t/χ^2	*P*
Age (years)		57.93 ± 5.68	57.39 ± 5.79	0.594	.553
BMI (kg/m^2)		25.10 ± 2.14	24.69 ± 2.28	1.168	.245
SBP (mm Hg)		131.25 ± 10.25	130.35 ± 9.86	0.566	.573
DBP (mm Hg)		81.68 ± 6.54	79.58 ± 7.76	1.837	.068
FBG (mmol/L)		6.63 ± 2.74	6.14 ± 2.69	1.140	.256
HbA1c (%)		10.36 ± 2.89	10.68 ± 2.38	0.768	.444
TG (mmol/L)		2.36 ± 0.53	2.28 ± 0.49	0.992	.323
TC (mmol/L)		4.53 ± 0.49	4.48 ± 0.47	0.658	.511
LDL-C (mmol/L)		2.96 ± 0.37	2.89 ± 0.35	1.229	.221
HDL-C (mmol/L)		1.62 ± 0.41	1.65 ± 0.41	0.462	.645
History of smoking		4	6	0.015	.902
Previous history	Hypertension	19	20	0.070	.791
	Diabetes	11	15	0.260	.610
	Family history of CHD	15	4	8.906	.003
	Hypothyroidism	3	4	0.028	.866
	Autoimmune diseases	2	1	0.012	.913

BMI, body mass index; CHD, coronary heart disease; DBP, diastolic blood pressure; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triglyceride; TC, total cholesterol.

Table 2. Comparison of Serum miR-18a Level and Endothelial Function (±SD)

Group	miR-18a	NO (ng/L)	ET-1 (ng/L)	FMD (%)
Non-CHD group (n = 85)	0.92 ± 0.12	87.98 ± 7.40	76.67 ± 4.31	3.08 ± 0.57
CHD group (n = 75)	1.14 ± 0.14	76.02 ± 6.43	83.28 ± 6.75	2.79 ± 0.60
t	10.869	10.840	7.465	3.132
P	<.001	<.001	<.001	.002

CHD, coronary heart disease; ET-1, endothelin 1; FMD, flow-mediated dilatation; miR-18a: microRNA (miR)-18a; NO, nitric oxide.

Correlation between Serum miR-18a Level and Endothelial Function

Pearson correlation showed that serum miR-18a level was positively correlated with ET-1 (r = 0.492, P < .001) and negatively correlated with NO and FMD (r = −0.504, −0.307, P < .001). See Table 3 and Figures 1, 2, and 3 for further details.

Value Analysis of Serum miR-18a Level in Predicting the Occurrence of CHD in Women

Taking the occurrence of female CHD (1 = Yes, 0 = No) as the state variable and the serum level of miR-18a as the test variable, the ROC curve was plotted. It was found that the AUC of predicting female CHD by the serum level of miR-18a was 0.878 (95% CI: 0.828-0.928). The optimal cutoff value was 1.025, the specificity was 0.874, the sensitivity was 0.885, and the Youden index was 0.759. See Figure 4.

Table 3. Correlation Between Serum miR-18a Level and Endothelial Function

Coefficient	NO	ET-1	FMD
r	−0.504	0.492	−0.307
P	<.001	<.001	<.001

ET-1, endothelin 1; FMD, flow-mediated dilatation; miR-18a, microRNA 18a; NO, nitric oxide.

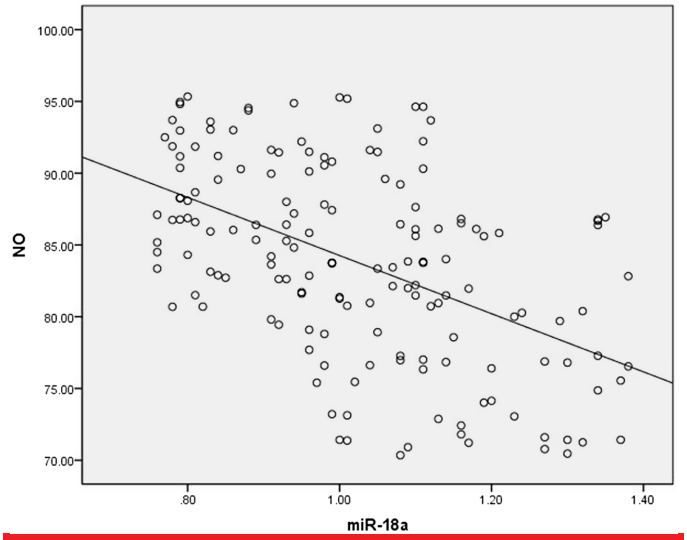

Figure 1. Scatter plot of the correlation between miR-18a and NO. NO, nitric oxide.

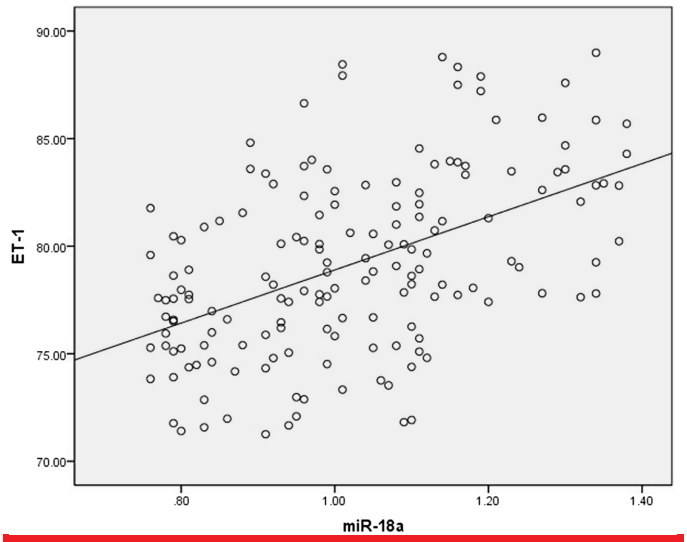

Figure 2. Scatter plot of the correlation between miR-18a and ET-1. ET-1, endothelin 1.

Comparison of Clinical Data of Patients with Different Prognoses in CHD Group

During 24 months of follow-up, MACE occurred in 18 of 75 female CHD patients (24.0%). Age, BMI, SBP, DBP, FBG, HbA1c, TG, TC, LDL-C, HDL-C, smoking history, past hypertension history, past diabetes history, past CHD family history, past hypothyroidism history, and past autoimmune disease history were compared between the poor prognosis group and the good prognosis group, and the differences were not statistically significant (all P > .05). The poor prognosis group had significantly lower levels of NO and FMD, while significantly higher proportions of acute coronary syndrome, multi-vessel disease, miR-18a, and ET-1 levels compared with the good prognosis group (all P < .05). See Table 4.

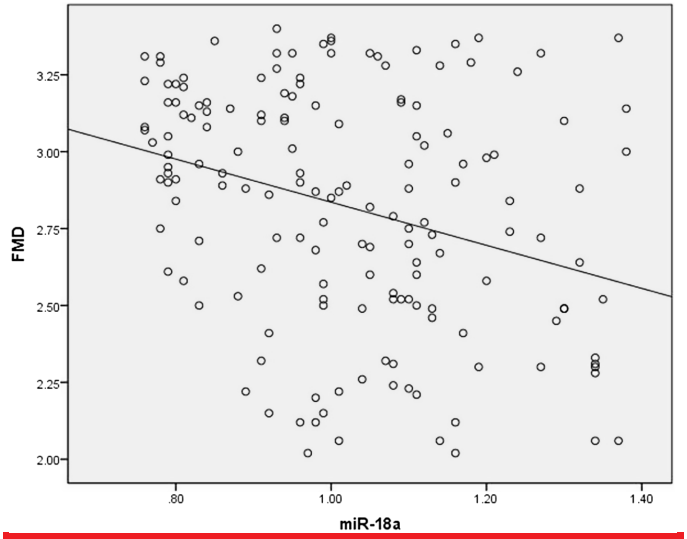

Figure 3. Scatter plot of the correlation between miR-18a and FMD. FMD, flow-mediated dilatation.

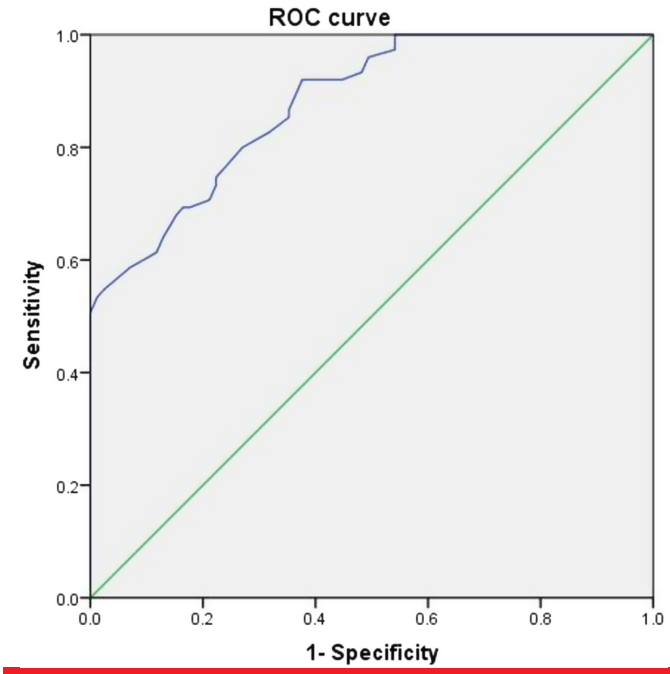

Figure 4. ROC curve of serum miR-18a level for predicting the occurrence of CHD in women. CHD, coronary heart disease; miR-18a, microRNA 18a; ROC, receiver operator characteristic.

Binary Logistic Regression Analysis Affecting Prognosis of Female Patients with CHD

Variables with $P < .05$ (disease type, number of lesions, miR-18a, NO, ET-1, FMD) in the univariate analysis were entered into the binary logistic regression analysis model as covariates and assigned values (Table 5). Dichotomous variables of prognostic status of female CHD patients (1 = poor prognosis, 0 = good prognosis) were taken as dependent variables. By binary logistic regression analysis, the results revealed that the type of disease (acute coronary syndrome), the number of diseased vessels (multi-vessel disease), miR-18a, and elevated ET-1 levels were independent risk factors affecting poor prognosis in female CHD patients (OR > 1, $P < .05$). Conversely, increased levels of NO and FMD were protective factors (OR < 1, $P < .05$). See Table 6.

DISCUSSION

At present, the specific pathogenesis of postmenopausal female CHD in clinical practice has not been fully elucidated, and it is often believed to be related to estrogen deficiency, thrombosis theory, lipid infiltration theory, and smooth muscle cloning theory.[9] This disease is a complex and dynamic development process involving multiple factors. Therefore, in-depth study of the pathogenesis is of great significance for exploring biomarkers for early diagnosis.

Table 4. Comparison of Clinical Data of Patients with Different Prognoses in CHD group

Index		Poor Prognosis Group (n = 18)	Good Prognosis Group (n = 57)	t/χ^2	*P*
Age (years)		58.02 ± 6.32	57.85 ± 5.76	0.107	.915
BMI (kg/m^2)		25.36 ± 2.25	24.85 ± 2.36	0.808	.422
SBP (mm Hg)		132.15 ± 9.68	130.74 ± 8.59	0.589	.558
DBP (mm Hg)		81.26 ± 7.15	81.87 ± 6.98	0.321	.749
FBG (mmol/L)		6.68 ± 2.58	6.14 ± 2.69	0.750	.456
HbA1c (%)		10.42 ± 2.68	10.21 ± 2.74	0.285	.777
TG (mmol/L)		2.39 ± 0.47	2.33 ± 0.52	0.436	.664
TC (mmol/L)		4.58 ± 0.48	4.42 ± 0.52	1.158	.251
LDL-C (mmol/L)		2.93 ± 0.41	2.99 ± 0.38	0.573	.568
HDL-C (mmol/L)		1.65 ± 0.39	1.60 ± 0.38	0.484	.630
miR-18a		1.26 ± 0.20	0.85 ± 0.15	12.056	<.001
NO (ng/L)		68.76 ± 4.22	78.31 ± 5.19	7.099	<.001
ET-1 (ng/L)		89.54 ± 5.06	81.30 ± 5.99	5.263	<.001
FMD (%)		2.14 ± 0.60	3.00 ± 0.43	6.706	<.001
History of smoking (n = 4)		1	3	0.306	.685
Previous history	Hypertension (n = 19)	5	14	0.001	.970
	Diabetes (n = 11)	3	8	0.011	.915
	Family history of CHD (n = 15)	5	10	0.370	.543
	Hypothyroidism (n = 3)	1	2	0.092	.902
	Autoimmune diseases (n = 2)	0	2	0.001	.970
Types of disease	Stable angina pectoris (n = 36)	4	32	6.626	.010
	Acute coronary syndrome (n=39)	14	25		
Number of diseased vessels	Single vessel disease (n = 25)	3	22	12.733	.002
	Double vessel disease (n = 38)	7	31		
	Multi-vessel disease (n = 12)	8	4		

BMI, body mass index; CHD, coronary heart disease; DBP, diastolic blood pressure; ET-1, endothelin 1; FBG, fasting blood glucose; FMD, flow-mediated dilatation; HDL-C, high-density lipoprotein cholesterol; HbA1c, glycated hemoglobin; SBP: systolic blood pressure; LDL-C, low-density lipoprotein cholesterol; miR-18a, microRNA 18a; NO, nitric oxide; TG, triglyceride; TC, total cholesterol.

Table 5. Binary Logistic Regression Analysis of Each Variable Assignment

		Assignment (Point)		
Independent Variable	**Description of Variable**	**0**	**1**	**2**
Types of disease	Categorical variable	Stable angina pectoris	Acute coronary syndrome	–
Number of diseased vessels	Categorical variable	Single vessel disease	Double vessel disease	Multi-vessel disease
miR-18a	Continuous variable	–	–	–
NO	Continuous variable	–	–	–
ET-1	Continuous variable	–	–	–
FMD	Continuous variable	–	–	–

ET-1, endothelin-1; FMD, flow-mediated dilatation; miR-18a, microRNA (miR)-18a; NO, nitric oxide.

microRNAs are endogenous single-stranded non-coding RNA small molecules, which are composed of about 22 nucleotides. It can participate in the process of cardiac development and cardiovascular diseases (myocarditis, atherosclerosis, myocardial hypertrophy, myocardial fibrosis, etc.) by activating a variety of intracellular signaling pathways.[10,11] microRNA-18a is located on human chromosome 13q31.3 and is a member of the highly conserved miR-17–92 gene cluster. Previous studies have confirmed that the expression of miR-18a is significantly increased in colon cancer, breast cancer, and other tumors, but there are few studies on miR-18a in cardiovascular diseases.[12-14] In this study, we found that the level of miR-18a in the CHD group was higher than that in the non-CHD group. When the optimal cutoff value of miR-18a was 1.025, the specificity, sensitivity, and Youden index for predicting female CHD were 0.874, 0.885, and 0.759, respectively, indicating that miR-18a may be involved in the pathogenesis of female CHD patients. The reason may be that estrogen may reduce the expression of the Notch1 signaling pathway in mononuclear cells, inhibit the release of inflammatory factors and the activation of inflammatory cells, and reduce the inflammatory injury and apoptosis in the vascular wall. It can prevent the transformation of vascular wall cells into the osteogenic phenotype, thereby preventing the occurrence and development of coronary artery calcification. It has been reported that the target gene of miR-18a may be ERα (ER includes ERα and ERβ2 subtypes), and the 2 are negatively correlated.[15] Therefore, it is speculated that the upregulation of miR-18a expression can reduce the protective effect of estrogen on the cardiovascular by inhibiting ER expression, thus inducing the occurrence of coronary heart disease. Lin et al[16] found in a rat experiment that miR-18a might protect acute myocardial infarction by inhibiting Akt/mTOR axis activity and reducing the number of senescent cells. Kraus et al[17] also reported that miR-18a could regulate the transformation and differentiation of fibroblasts into myofibroblasts, providing a solution for the cardiac repair response after myocardial injury. Establishing a binary logistic regression analysis model revealed that the increase of serum miR-18a level was a risk factor for the prognosis of female CHD patients [OR: 1.841 (95% CI: 1.456-2.328)]. It was confirmed that the increase of serum miR-18a level may increase the risk of MACE in female CHD patients during follow-up. It is suggested that the change of serum miR-18a level should be closely and dynamically monitored in the clinic, and targeted treatment should be taken to reduce the expression of serum miR-18a, further reducing the incidence of MACE and improving the prognosis of patients.

The barrier between vascular endothelial cells and peripheral blood circulation and vascular smooth muscle plays a dual role. On the one hand, it can secrete many vasoactive factors and participate in the regulation of the fibrinolytic system and anticoagulant function; on the other hand, it can participate in the permeability barrier of blood vessels and regulate the selective permeability of biological macromolecular substances, gases, and liquids, which is closely related to the stability of the circulatory system and vascular function.[18] A study found that the proliferation and migration of vascular endothelial cells were regulated by a variety of endothelial cell proliferation inhibitory factors [such as angiostatin and thrombospondin-1 (TSP-1)], endothelial cell proliferation-promoting factors (angiopoietin

Table 6. Binary Logistic Regression Analysis Affecting Prognosis of Female Patients with CHD

Independent Variable	***β***	**SE**	**Wald**	***P***	**OR**	**95% CI**
Constant quantity	18.562	4.365	14.966	<.001	–	–
Type of disease (acute coronary syndrome)	1.500	0.627	5.727	.017	4.480	1.312-15.299
Number of diseased vessels (multi-vessel disease)	2.686	0.868	9.568	.002	14.667	2.675-80.418
miR-18a	0.610	0.120	25.950	<.001	1.841	1.456-2.328
NO	−0.582	0.172	11.437	.001	0.559	0.399-0.783
ET-1	0.315	0.087	13.084	<.001	1.370	1.155-1.624
FMD	−3.583	0.957	14.006	<.001	0.028	0.004-0.181

ET-1, endothelin 1; FMD, flow-mediated dilatation; miR-18a, microRNA 18a; NO, nitric oxide.

and vascular endothelial growth factor (VEGF)), and other cytokines. Some of the above regulators have been confirmed to be downstream target genes or upstream regulators of miRNA.[19] For example, Wang et al[20] reported that miR-18a could induce blood–brain barrier damage and increase endothelial permeability in ischemic stroke through the VEGF axis. Chiba et al[21] reported that miR-18a could participate in the process of angiogenesis by up-regulating the expression of TSP-1. From the aforementioned foreign experiments, it was evident that miR-18a was related to vascular endothelial cells, and vascular endothelial dysfunction was the main cause of atherosclerosis. Therefore, it was speculated that miR-18a might be involved in the occurrence and development of CHD by regulating vascular endothelial function. ET-1 could maintain basal vascular tone and cardiovascular system homeostasis, and stimulate vasoconstriction when its level was abnormally increased. Nitric oxide was released and synthesized by vascular endothelial cells, which inhibited coronary atherosclerosis and promoted endothelial repair by promoting the opening of potassium channels, vasodilation, and scavenging oxygen-free radicals. In this study, Pearson's correlation test showed that the serum miR-18a level was positively correlated with ET-1 and negatively correlated with NO and FMD, suggesting that the serum miR-18a level was closely related to endothelial function in female CHD patients, and the increase of serum miR-18a level could cause the formation of coronary atherosclerotic plaques, weaken the reserve function of vascular dilatation and endothelial relaxation function, thus increasing ET-1 secretion, reducing NO formation, promoting vascular endothelial dysfunction, damaging vascular endothelial function, and further aggravating the condition of female CHD patients.

In conclusion, serum miR-18a expression was high in female CHD patients, which was related to endothelial function. The increase of serum miR-18a level was a risk factor for the occurrence of MACE in female CHD patients during follow-up, and the serum miR-18a level could effectively predict the occurrence of CHD in female patients. However, the targets and signaling pathways of miR-18a in female CHD are still unclear. In the future, GO and KEGG enrichment analysis and gene network interaction analysis should be further performed to screen key genes and signaling pathways.

Data Availability: The simulation experiment data used to support the findings of this study are available from the corresponding author upon request.

Ethics Committee Approval: The study was reviewed and approved by the Ethics Committee of Second Affiliated Hospital of Fujian Medical University (approval number: 2020YB278).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

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