

Diagnostic and Prognostic Significance of miR-675-3p in Patients With Atherosclerosis

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Abstract

In recent years, a rising number of studies have confirmed that microRNA (miRNA) plays a prominent role in the early diagnosis and prognostic value assessment of cardiovascular diseases. The current study was conducted to examine the expression of miR-675-3p in atherosclerosis (AS) patients and to evaluate its clinical diagnosis and prognostic value. 110 AS patients and 70 healthy controls were included in the study. Serum miR-675-3p levels were detected by quantitative real-time PCR (qRT-PCR). The clinical diagnostic significance of serum miR-675-3p in AS patients were investigated by the receiver operating characteristic (ROC) curve. The correlation between miRNA and carotid intima-media thickness (CIMT) was analyzed by the Spearman correlation coefficient. The prognostic significance of serum miR-675-3p was evaluated by the Kaplan-Meier method and Cox regression analysis. The patient's serum miR-675-3p was significantly increased than the healthy individuals ($P < 0.05$). An increase of carotid intima-media thickness (CIMT) was positively correlated with the promotion of serum miR-675-3p levels. The area under the ROC curve (AUC) was 0.918, with high sensitivity and specificity. miR-675-3p is a key independent predictor of cardiovascular adverse events in AS patients (HR = 5.375, 95%CI = 1.590-18.170, $P = 0.007$), and patients with elevated miR-675-3p were more likely to have cardiovascular adverse events (log-rank $P = 0.030$). Increased miR-675-3p can be used as a potential marker for the diagnosis of AS, and was associated with the poor prognosis of AS.

Keywords

miR-675-3p, asymptomatic AS, diagnosis, prognosis

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Introduction

Atherosclerosis (AS) is a cardiovascular disease caused by the thickening and hardening of arterial walls caused by the accumulation of cells, cholesterol, and extracellular matrix.¹ Epidemiological studies have shown that smoking, hypercholesterolemia, hypertension, and diabetes mellitus are major risk factors for AS and related disease progression.² Although significant prognosis has been made in lifestyle improvement and drug treatment, AS is still the main cause of morbidity and mortality in developed countries.³ At the same time, the occurrence of AS is the main pathological basis of other cardiovascular and cerebrovascular diseases. Therefore, there is an urgent need to develop new and reliable diagnostic or prognostic biomarkers and treatment options to reduce the burden of AS treatment.

miRNAs are short non-coding RNAs that are involved in the post-transcriptional regulation of gene expression and play key roles in many biological processes.⁴ Previous research has evidence that miRNAs are stably expressed in peripheral blood

circulation and exhibit tolerance to pH, temperatures, and store time, showing good physiological characteristics.⁵ Besides, other studies have shown that the changes of miRNA can be detected in different cardiovascular diseases, and its expression is conducive to the early diagnosis and prognosis assessment of cardiovascular diseases such as AS.⁵ For example, miR-574-5p is a new candidate target for severe coronary artery disease.⁶ Plasma miR-29c is a feasible marker for the early identification of AS.⁷ As one of the miRNAs, miR-675-3p can regulate colorectal cancer, esophageal squamous cell carcinoma, and skeletal muscle differentiation and regeneration.⁸⁻¹⁰ Recently, Yi et al

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confirmed 19 miRNAs that are abnormally expressed during lipid droplet formation, including miR-675-3p.¹¹ Besides, Li et al in 2018 studied atherosclerotic diabetic rat tissues and found 9 differentially expressed miRNAs, including miR-675-3p.¹² What's more, elevated miR-675-3p is associated with hallmarks of heart failure.¹³ As the first exon located in H19, miR-675 is derived from the conserved transcript of lncRNA H19 and can produce 2 kinds of function miRNA, namely miR-675-3p and miR-675-5p.¹⁴ In addition, miR-675 regulated by H19 was initially proved to be involved in placental growth before birth. Currently, more and more studies have confirmed that miR-675-3p has different tissue sources and multiple functions.¹⁵ Although miR-675-3p has been reported in adipogenesis, atherosclerotic diabetic rat, and complication heart failure, the expression pattern, clinical diagnosis, and predictive value of miR-675-3p in AS patients remain unclear.

In the present work, the serum miR-675-3p expression level and clinical diagnosis, and prognostic value of asymptomatic AS patients were evaluated, in order to provide a theoretical basis for early screening and intervention of AS.

Materials and Methods

Subject' Recruitment

The present survey was approved by the Medical Ethics Committee of Binzhou Medical University Hospital, and all subjects signed informed consent. 110 asymptomatic AS patients admitted to Binzhou Medical University Hospital from June 2010 to June 2014 were selected to be included in this study. The asymptomatic AS were identified based on the carotid intima-media thickness (CIMT) >0.7 mm in the previous studies.^{16,17} Patients with heart disease, stroke, heart failure, angina, transient ischemic time, hypertension, renal insufficiency, and other cardiovascular diseases were excluded from the subjects. 70 healthy people of similar age were selected from the health examination center as the control group. Physical examination was performed on all subjects and basic information was recorded. Measurement of carotid intima-media thickness (CIMT) of subjects was also performed using the ATL HDI 3000 ultrasound system. The upper limb venous blood of the subjects was collected after 12 h of fasting. After centrifugation, the upper serum was separated and stored at -80°C .

Fluorescence Quantitative Polymerase Chain Reaction (qRT-PCR)

Serum samples of the subject's total RNA were extracted by TRIzol reagent. According to the manufacturer's instructions of the miRNA cDNA Synthesis Kit (CWBiotech), the RNA extracted was synthesized the first strand of complementary DNA. The qRT-PCR reaction was performed in 7500 FAST RT-PCR system (Thermo Fisher Scientific, Inc) as required by the miRcute Plus miRNA qPCR Kit (SYBR Green). $2^{-\Delta\Delta\text{Ct}18}$

method to calculate the relative expression level of miR-675-3p in standardized to the endogenous controls U6 small nuclear RNA.¹⁹ The primer sequence was used in the qRT-PCR were as follows: miR-675-3p forward 5'-GCCGAGCATCTTACCG-GACGT-3' and reverse 5'-CTCAACTGGTGTCTGGGA-3'; U6 forward 5'-AACGCTTACGAATTGCGT-3' and reverse 5'-CTCAACTGGTGTCTGGGA-3'. The thermocycling condition used were as follows: 95°C for 10 min followed by 40 cycles of 94°C for 15 sec, 55°C for 30 sec and 70°C for 30 sec.²⁰

Follow-Up Plan and Content

According to the definition of cardiovascular end events in the 2014 American College of Cardiology/American Heart Association clinical Trial as the endpoints of this study.²¹ Hospitalization or death due to events such as myocardial infarction, stroke, transient, ischemic attack, coronary intervention (including stent thrombosis), peripheral vascular intervention, and unstable angina was defined as cardiovascular endpoints.²¹ The follow-up time of this study was 5 years. Participants' cardiovascular endpoints events were recorded during telephone follow-up.

Statistical Analysis

Statistical calculations of experimental data were performed using GraphPad Prism 7.0 and SPSS 23.0 software. The expression level of miRNAs in control and AS patients were compared by unpaired two-tailed student tests. The ROC curve to investigate the diagnostic significance of serum miR-675-3p in AS patients. The correlation between miRNA and CIMT was analyzed by the Spearman correlation coefficient. Kaplan-Meier and log-rank methods detect the ability of miR-675-3p to predict adverse cardiovascular events in patients. The prognostic significance of miR-675-3p in AS was measured by multivariate Cox regression analysis. The level of statistical significance was set at $P < 0.05$.

Results

Clinical Indicators of the Patients Involved

The clinicopathological characteristics of the subjects were counted in Table 1. Studies have found that C-reactive protein, a marker of inflammation, was generally elevated in AS patients ($P < 0.05$). There was no statistical significance in age, gender, BMI, total cholesterol, and other indicators between the 2 groups ($P > 0.05$).

Serum miR-675-3p Is Upregulation in AS Patients

To clarify the expression pattern of miR-675-3p in AS, we first detected the level of serum miR-675-3p in subjects. qRT-PCR reactions confirmed that serum miR-675-3p was markedly increased in AS patients compared with the

Table 1. Demographic and Clinical Characteristics Between Groups.

Features	Healthy controls (n = 70)	AS patients (n = 110)	P value
Age (years)	54.61 ± 8.60	53.33 ± 5.70	0.208
Gender (male/female)	35/35	58/52	0.761
BMI (kg/m ²)	25.73 ± 1.71	26.01 ± 1.52	0.252
Total cholesterol (mg/dl)	198.91 ± 13.26	200.15 ± 14.10	0.557
HDL-C (mg/dl)	45.92 ± 3.52	46.82 ± 2.62	0.069
LDL-C (mg/dl)	122.00 ± 12.36	125.09 ± 12.76	0.110
Triglyceride (mg/dl)	174.64 ± 16.61	179.42 ± 20.54	0.088
SBP (mm Hg)	138.77 ± 8.34	141.19 ± 8.92	0.071
DBP (mm Hg)	82.48 ± 6.95	83.55 ± 6.30	0.062
CRP (mg/l)	0.81 ± 0.20	2.72 ± 0.60	0.000

Abbreviations: AS, atherosclerosis; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein.

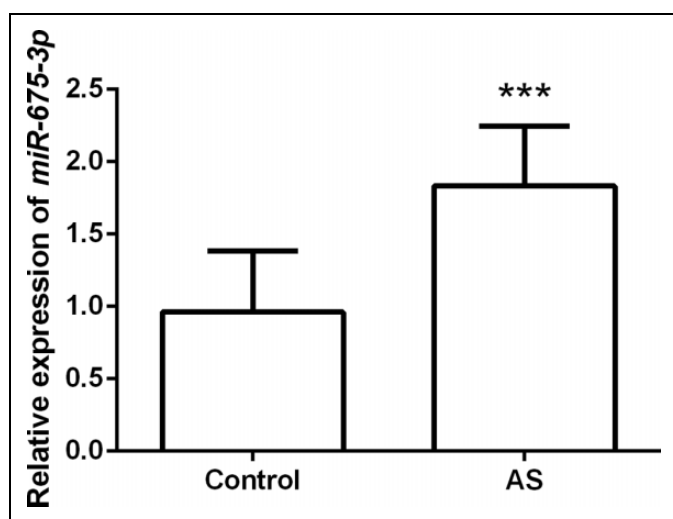


Figure 1. Serum miR-675-3p expression levels in AS patients and controls. Compared with control, serum miR-675-3p expression in AS patients was significantly increased, *** $P < 0.001$.

control group ($P < 0.001$, Figure 1). The results prompt that the dysregulation of miR-675-3p may be involved in the progression of AS.

Serum miR-675-3p Is Positively Correlated With the CIMT Value

A noninvasive approach before the onset of symptoms of AS can reduce morbidity and mortality. Elevated CIMT is considered to be a subclinical index of AS independent of age, gender, and cardiovascular risk factors, and is widely used in the early detection of AS.^{22,23} In this study, the mean CIMT of AS patients was 0.90 ± 0.09 mm. Spearman correlation coefficient analysis detected that the increase of serum levels of miR-675-3p was markedly correlated with the CIMT ($r = 0.701$, $P < 0.001$, Figure 2).

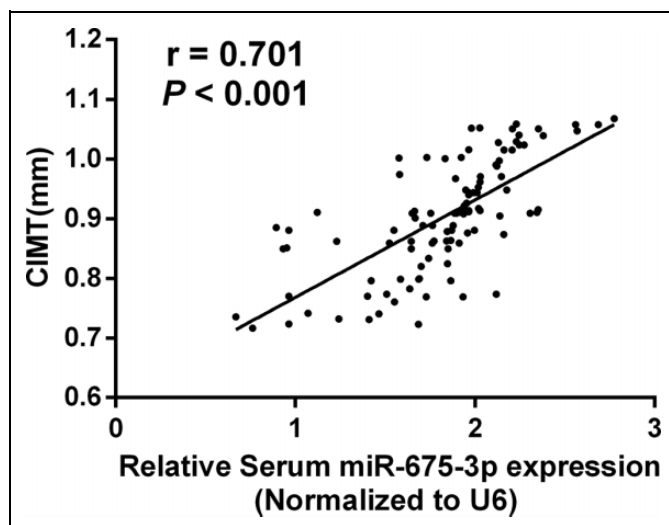


Figure 2. Correlation between serum miR-675-3p levels and CIMT value. Serum miR-675-3p were significantly positively correlated with CIMT in AS patients, $r = 0.701$, $P < 0.001$.

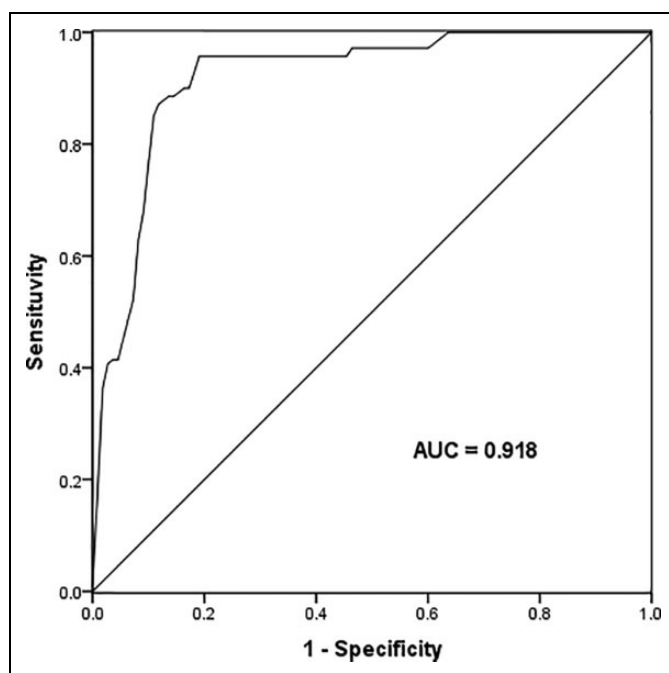


Figure 3. The potential diagnostic significance of miR-675-3p was evaluated by Receiver Operating Characteristic (ROC) curves. When the cut-off value is 1.368, the AUC of serum miR-675-3p was 0.918, sensitivity was 88.60%, and specificity was 89.09%.

Diagnostic Potential of Serum miR-675-3p in AS Patients

Because of the positive correlation between serum miR-675-3p and CIMT, we further studied the diagnostic potential of miR-675-3p in AS patients by receiver operating characteristic curve (ROC) analysis. As shown in Figure 3, when the cut-off value is 1.368, the AUC of serum miR-675-3p was 0.918, sensitivity was 88.60%, and specificity was 89.09%. Studies

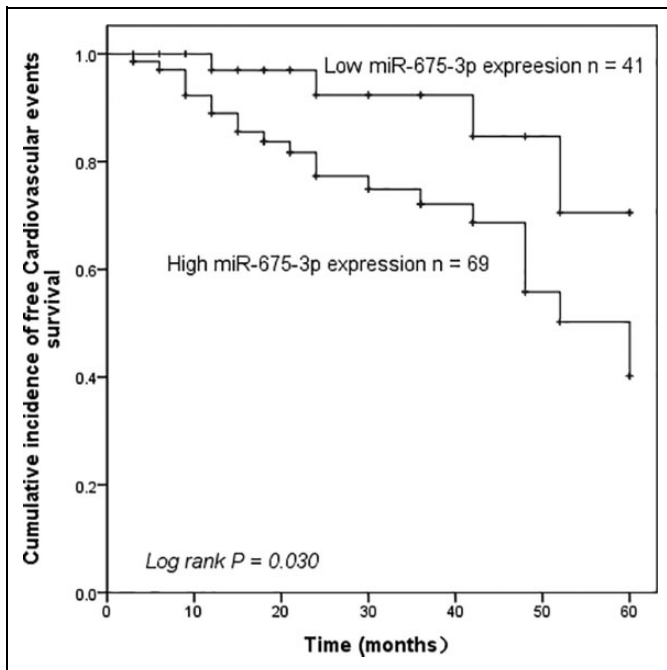


Figure 4. Kaplan-Meier analysis was used to calculate the predictive potential of miR-675-3p for cardiovascular adverse events in asymptomatic AS patients. Patients with high miR-675-3p expression are more likely to have cardiovascular adverse events, log-rank $P = 0.030$.

have shown that miR-675-3p has a high diagnostic value and can clearly distinguish AS patients from healthy people.

Patients With High Serum miR-675-3p Have a Poor Prognosis

Then we analyzed the prognostic effect of serum miR-675-3p levels on the survival rate of cardiovascular adverse events. Based on the mean levels of miR-675-3p (1.832 ± 0.413), the patients were divided into the miR-675-3p high expression group and the low expression group. Survival curves were drawn based on cardiovascular endpoints during the 5-year follow-up. The results showed that compared with the low expression of miR-675-3p, patients with high levels of miR-675-3p had a markedly higher probability of cardiovascular adverse events (log-rank $P = 0.030$, Figure 4). Besides, to further study the prognostic significance of serum miR-675-3p in AS, multivariate Cox regression analysis verified the independent predictive value of miR-675-3p for the occurrence of cardiovascular adverse events in AS patients ($P < 0.05$, Table 2).

Discussion

AS is a chronic inflammatory disease related to the systemic arterial wall, which can lead to myocardial infarction, ischemic stroke, peripheral artery disease, and other cardiovascular diseases, with high morbidity and mortality.²⁴ Several studies have reported that inflammation is involved in all stages of

Table 2. Multivariate Cox Regression Analysis for the Overall Survival of AS Patients.

Parameters	Multivariate analysis		
	Hazard ratio	95% CI	P value
miR-675-3p	5.375	1.590-18.170	0.007
Age (years)	0.615	0.240-1.579	0.312
Gender (male/female)	0.894	0.374-2.136	0.801
BMI (kg/m^2)	0.543	0.218-1.354	0.190
Total cholesterol (mg/dl)	0.711	0.281-1.800	0.472
HDL-C (mg/dl)	0.910	0.376-2.202	0.835
LDL-C (mg/dl)	0.773	0.339-1.763	0.541
Triglyceride (mg/dl)	0.676	0.269-1.704	0.407
SBP (mm Hg)	0.752	0.312-1.813	0.526
DBP (mm Hg)	0.451	0.182-1.117	0.085
CRP (mg/l)	2.728	1.054-7.064	0.039

Abbreviations: AS, atherosclerosis; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein.

AS formation, including early AS, plaque instability, thrombosis, and cardiovascular events.²⁵ CRP, a nonspecific marker of inflammation, is a strong predictor of future cardiovascular events.²⁶ In the present study, we found that the patient's CRP concentration was significantly increased, which is consistent with the results of existing studies.

Previous studies have revealed key signaling and molecular pathways involved in the initiation and progression of AS.^{27,28} As an important physiological process regulator of diseases, miRNAs, such as cell adhesion, proliferation, lipid uptake and efflux, and inflammatory response, has identified new therapeutic targets.^{29,30} Besides, miRNAs can be detected outside the cell, including in circulating blood, urine, and other body fluids, and their expression is relatively stable, which improves their potential as biomarkers for diagnosis, prognosis, or response to cardiovascular treatment.³¹ For example, aberrant expression of plasma miR-33a in AS patients can be used as a candidate biomarker for AS.³² miR-374 is significantly elevated in patients and is a potential diagnostic marker for AS.³³

Our current research confirmed that miR-675-3p was generally upregulated in AS patients. In 2018, Li et al conducted a miRNA microarray analysis on diabetic atherosclerotic rats and identified 9 differentially expressed genes including miR-675-3p.¹² Liu et al confirmed that miR-675-3p was markedly up-regulated in human heart failure patients, and it also increased in the myocardial hypertrophy mice model induced by aortic constriction.¹³ Our results are consistent with those of common complications of AS. So far as we know, this is the first study to detect the expression level of miR-675-3p in AS patients, and the study suggests that the dysregulation of miR-675-3p may play a major part in the progression of AS.

The incidence of cardiovascular events was generally associated with CIMT. Moreover, the increase in CIMT is recognized as a subclinical indicator of AS.³⁴ In the present survey, it was confirmed that the serum miR-675-3p level was markedly positively correlated with the CIMT. Furthermore, the present

investigation also evaluated the diagnostic potential of miR-675-3p in AS through the ROC curve. The results indicate that miR-675-3p elevation is a new potential diagnostic biomarker for AS, which can significantly distinguish AS patients from healthy personnel.

Next, to further study the clinical prognostic value of serum miR-675-3p levels in AS. We analyzed the adverse cardiovascular events of patients based on the 5-year follow-up records. The results showed that patients with high expression of miR-675-3p had a poor prognosis, and we proved that miR-675-3p was an independent prognostic factor for cardiovascular adverse events in AS patients by multivariate Cox regression analysis. Present investigation indicates that miR-675-3p elevation is a new prognostic marker for AS.

This study verified that miR-675-3p is highly expressed in AS patients. Elevated miR-675-3p may be a potential diagnostic biomarker for asymptomatic AS, and indicates a poor prognosis, providing a theoretical basis for early screening and intervention of AS.

Authors' Note

The present survey was approved by the Medical Ethics Committee of Binzhou Medical University Hospital, and all subjects signed informed consent. Written informed consent was obtained from the patient(s) for their anonymized information to be published in this article.


Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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