

EXPRESSION AND CLINICAL SIGNIFICANCE OF SERUM
miR-1247-3p IN ISCHEMIC STROKE

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Abstract

To investigate the expression and clinical significance of serum miR-1247-3p in patients with ischemic stroke, twenty-five patients with ischemic stroke were collected, and twenty-five healthy individuals were used as controls. RT-qPCR measured the expression of serum miR-1247-3p. Logistic regression analysis was applied to evaluate the effect of influencing factors, including miR-1247-3p, on ischemic stroke. Spearman analysis was applied to study the relationship between miR-1247-3p and clinicopathological characteristics. The sensitivity and specificity of serum miR-1247-3p in the diagnosis of ischemic stroke were evaluated by ROC curves. Results show that serum miR-1247-3p levels were significantly lower in patients with ischemic stroke than in controls ($P < 0.001$). miR-1247-3p expression levels negatively correlated with triglycerides ($r = -0.425$, $P < 0.05$). miR-1247-3p is a protective factor that can significantly influence the occurrence of ischemic stroke (OR, 0.401. 95% CI, 0.222–0.732; $P = 0.002$). miR-1247-3p had a sensitivity of 92% and a specificity of 88% for the diagnosis of ischemic stroke. We conclude that serum miR-1247-3p expression levels are significantly reduced in patients with ischemic stroke and could serve as a potential biological marker for diagnosing ischemic stroke.

Key words: ischemic stroke, miR-1247-3p, biomarkers, serum

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Introduction. Stroke is the leading cause of premature death in China [1] and is characterized by high mortality and disability [2], making it a costly public health and social issue. Ischemic stroke is the most common stroke type [3]. Currently, the only drug approved by the US FDA for treating ischemic stroke is the tissue-type fibrinogen activator [4], but due to the narrow therapeutic time window, only a few patients can be saved. It is crucial to find novel biomarkers to detect and control stroke risk factors as early as possible to limit their incidence.

MicroRNAs (miRNAs) are non-coding RNAs of 20–24 nucleotides in length that mediate post-transcriptional silencing of genes by binding to the 3'UTR region of messenger RNA (mRNA) to regulate body function [5]. Our previous study found that miR-1247-3p was aberrantly expressed in mice after cerebral ischemic injury and could play a neuroprotective role in focal cerebral ischemic injury by inhibiting post-stroke neuronal apoptosis and reducing the size of cerebral infarcts in mice with stroke [6]. Further investigation of the clinical significance of miR-1247-3p in the diagnosis of ischemic stroke by comparing miR-1247-3p expression levels in patients with ischemic stroke and healthy subjects could provide a theoretical basis for the clinical search for serum biomarkers in the acute stroke phase. Therefore, this study aimed to investigate the expression of miR-1247-3p in patients with ischemic stroke and its correlation with the disease, as well as to determine whether it has the potential to serve as a diagnostic biomarker for ischemic stroke.

Objects and methods. Objects. From April to July 2022, twenty-five patients with first-onset ischemic stroke were admitted to the Department of Neurology at CRW General Hospital, and 25 healthy controls admitted for physical examination were selected. The ischemic stroke patient group ranged in age from 52 to 88 years, with a mean age of (70.3 ± 10.7) years, while the control group ranged in age from 50 to 63 years, with a mean age of (54 ± 3.8) years. The ethics committee of CRW General Hospital approved the study protocol, and all participants signed an informed consent form. Inclusion criteria: Patients presented to the hospital within 24 h of the onset of symptoms, and patients with ischemic stroke should conform to the Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke 2018 [7]; clinicians diagnosed enrolled patients as having ischemic stroke based on their medical history, neurological deficits, combined with CT as well as MRI examinations, and all patients with ischemic stroke were first-time patients. Exclusion criteria: patients with recent surgery or trauma, hemorrhagic stroke, transient ischemic attack, malignant tumour, systemic inflammatory disorder, end-stage renal disease, end-stage liver disease, myocardial infarction, and major thromboembolic events within the last month.

Serum sample collection. On the day of physical examinations, 5 ml of fasting venous blood was collected from healthy individuals. The same sample was collected within 24 h of hospitalization in patients with first ischemic stroke using a vacuum blood tube without additives. The blood tube was kept vertical

for approximately 30 min to form a clot, followed by centrifugation at 3000 r/min at room temperature for 12 min and then at 12 000 r/min at 4 °C for 10 min. The serum was finally collected and stored at –80 °C until further analysis.

Extraction of total RNA and RT-qPCR assay. Total RNA was extracted according to the instructions of the Trizol LS kit, lysed in RNA-free enzyme water, and the RNA concentration and purity were checked using an ultra-micro spectrophotometer. The cDNAs of miR-1247-3p and U6 were obtained by reversing the first-strand cDNA reverse transcription kit from Thermo Fisher, respectively. miR-1247-3p and U6 were detected by real-time fluorescence quantitative PCR according to the kit instructions of Hieff® qPCR SYBR® Green Master Mix (11201ES03) (Shanghai Yisheng Biotechnology Co., Ltd.). The expression of miR-1247-3p was detected by real-time fluorescence PCR. Reaction conditions: pre-denaturation at 95° for 5 min, denaturation at 95° for 10 s, annealing at 55° for 20 s, and extension at 72° for 20 s. A total of 40 cycles were performed. Finally, the specificity of the amplified products was analyzed by melting curve. The relative expression of miR-1247-3p was calculated using the 2- $\Delta\Delta C_t$ method with U6 as the internal reference.

Statistical analysis. SPSS version 22.0 was used to perform all statistical analyses. The results of the categorical variables are expressed as a percentage (%), which is calculated by dividing the number of events by the total number of patients, excluding lost or unknown cases. For non-normal or normal distributions, results for continuous variables are expressed as median (interquartile range) (M(Qn)) or mean $\pm$ standard deviation ($x \pm s$), respectively. The independent sample *t*-test was used to detect the differences in normally distributed continuous variables between the two groups, while the Mann–Whitney U test was used for non-normally distributed variables. The χ^2 test was used to compare categorical variables between the two groups. Spearman correlation analysis was used to calculate the Correlation coefficient (*r*) between miR-1247-3p levels and clinical features in patients with ischemic stroke. Positive correlation was assumed when $r > 0$, negative correlation when $r < 0$, and nonlinear correlation (zero correlation) when $r = 0$. Binary logistic regression was used to ascertain the impact of the factors associated with stroke occurrence. The diagnostic value of serum miR-1247-3p for ischemic stroke was assessed using the receiver operating characteristic curve (ROC) curve and subline area (AUC). $P < 0.05$ indicates statistical significance.

Results. Comparison of baseline clinical features of ischemic stroke patients and control groups. The two groups' general characteristics, such as their sex, heart rate, fasting blood glucose, creatinine, total cholesterol, and triglycerides, revealed no statistically significant differences ($P > 0.05$). Patients with ischemic stroke were older (54.0 vs. 70.3; $P < 0.001$), with higher systolic (120.5 vs. 143.8; $P < 0.001$) and diastolic blood pressure (73.6 vs. 84.1; $P < 0.001$), increased urea nitrogen (4.16 vs. 5.29; $P = 0.008$), increased low-density

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Comparison of baseline clinical characteristics in patients with ischemic stroke and normal controls

Clinical features	Control	IS	<i>P</i> -value
<i>n</i>	25	25	
Age, $\bar{x} \pm s$	54.0±3.8	70.3±10.7	< 0.001
Male, <i>n</i> (%)	21 (84)	19 (76)	0.335
Systolic pressure (SBP), mmHg, $\bar{x} \pm s$	120.5±11.7	143.8±18.5	< 0.001
Diastolic pressure (DBP), mmHg, $\bar{x} \pm s$	73.6±7.0	84.1±10.7	< 0.001
HR, $\bar{x} \pm s$	75±6	74±7	0.334
Fasting glucose (FPG), mmol/L, <i>M(Qn)</i>	5.42 (5.17–5.97)	5.58 (5.05–13.86)	0.143
Creatinine (Cre), $\mu\text{mol/L}$, <i>M(Qn)</i>	67.55 (58.33–76.80)	75.20 (55.60–91.30)	0.234
Blood urea nitrogen (BUN), mmol/L, <i>M(Qn)</i>	4.16 (3.54–4.88)	5.29 (4.50–6.81)	0.008
Total cholesterol (TCHO) mmol/L, $\bar{x} \pm s$	4.82±0.87	4.65±0.90	0.526
Triglyceride (TG), mmol/L, <i>M(Qn)</i>	1.25 (0.80–1.53)	1.25 (1.01–1.37)	0.782
High-density lipoprotein (HDL), mmol/L, $\bar{x} \pm s$	1.96±0.99	1.18±0.21	0.001
Low-density lipoprotein (LDL), mmol/L, $\bar{x} \pm s$	2.17±0.78	2.67±0.68	0.024
miR-1247-3p, <i>M(Qn)</i>	11.63 (6.19–18.68)	2.32 (0.99–4.31)	< 0.001

lipoprotein (2.17 vs. 2.67; $P = 0.024$), and decreased high-density lipoprotein (1.96 vs. 1.18, $P = 0.001$) (Table 1).

Expression of serum miR-1247-3p in patients with ischemic stroke.

When compared to the control group, patients with ischemic stroke had significantly lower serum miR-1247-3p levels (11.63 vs. 2.32; $P^{***} < 0.001$, Fig. 1a, Table 1).

Relationship between miR-1247-3p levels and clinical features in patients with ischemic stroke. Spearman's correlation analysis (Table 2) found a negative association between miR-1247-3p expression levels and triglycerides ($r = -0.425$, $P < 0.05$, Table 2). There was no correlation between miR-1247-3p expression and other parameters such as HDL, LDL, total cholesterol, age, gender, systolic blood pressure, diastolic blood pressure, heart rate, fasting blood glucose, creatinine, and urea nitrogen ($P > 0.05$).

Univariate binary logistic regression analysis of influencing factors of ischemic stroke. In a binary logistic regression analysis of risk factors for

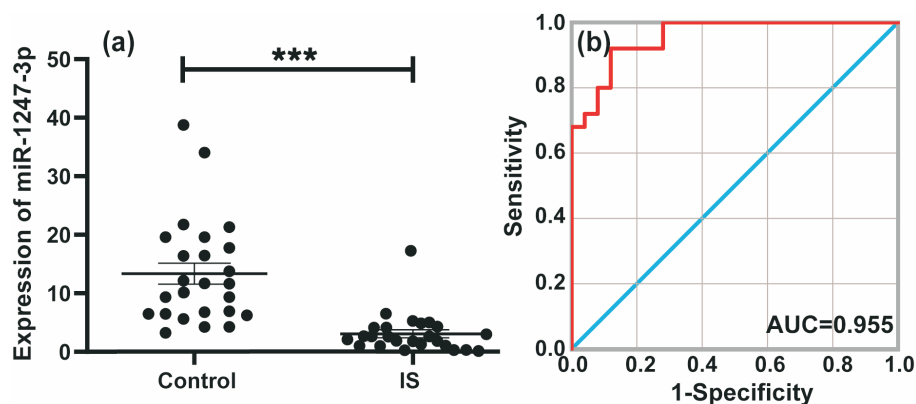


Fig. 1. (a) Comparison of serum miR-1247-3p levels in patients with ischemic stroke and normal controls; (b) ROC curve of miR-1247-3p diagnostic efficacy

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Correlation between the relative expression of miR-1247-3p in serum and clinical characteristics of patients with ischemic stroke

Feature	miR-1247-3p	HDL	LDL	TCHO	TG	Sex	Age
Correlation coefficient	1.000	0.069	−0.089	−0.125	−.425*	0.013	−0.131
<i>P</i> -value		0.750	0.679	0.561	0.038	0.951	0.532
Feature	SBP	DBP	HR	FPG	Cre	BUN	
Correlation coefficient	0.078	0.072	−0.252	−0.305	0.188	0.150	
<i>P</i> -value	0.712	0.732	0.224	0.139	0.369	0.474	

stroke, the following were found to have a statistically significant impact: age (OR, 1.386, 95% CI, 1.161–1.656; $P < 0.001$), systolic blood pressure (OR, 1.102; 95% CI, 1.044–1.163; $P < 0.001$), diastolic blood pressure (OR, 1.163; 95% CI, 1.056–1.281; $P = 0.002$), urea nitrogen (OR, 1.790; 95% CI, 1.115–2.874; $P = 0.016$), HDL (OR, 0.020; 95% CI, 0.001–0.333; $P = 0.007$), LDL (OR, 2.565; 95% CI, 1.095–6.004; $P = 0.03$), the miR-1247-3p level (OR, 0.401; 95% CI, 0.222–0.732; $P = 0.002$) (Table 3).

Diagnostic efficacy of miR-1247-3p in ischemic stroke. The findings of the ROC curve analysis showed that miR-1247-3p had a significantly higher area under the curve (AUC) for stroke diagnosis than the control group (0.955 vs. 0.5, 95% CI 0.906–1.000, $P < 0.001$). This diagnostic threshold value of 5.35 showed 92% sensitivity and 88% specificity. In cases of ischemic stroke, miR-1247-3p is a good prognostic marker (Fig. 1b).

Discussion and conclusion. Numerous studies have investigated the relationship between ischemic stroke and serum biomarkers, with most papers con-

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Single-factor binary logistic regression analysis of factors influencing stroke

Risk factor	Regression coefficient	Standard error	Wald	<i>P</i> -value	OR-value	OR (95% CI)
Age	0.327	0.091	12.973	< 0.001	1.386	1.161–1.656
Sex	0.506	0.719	0.494	0.482	1.658	0.405–6.785
SBP	0.097	0.028	12.337	< 0.001	1.102	1.044–1.163
DBP	0.151	0.049	9.428	0.002	1.163	1.056–1.281
HR	−0.044	0.045	0.954	0.329	0.957	0.876–1.045
FPG	0.375	0.206	3.305	0.069	1.455	0.971–2.181
Cre	0.025	0.017	2.259	0.133	1.025	0.992–1.060
BUN	0.582	0.242	5.803	0.016	1.790	1.115–2.874
TCHO	−0.219	0.338	0.419	0.517	0.804	0.414–1.558
TG	0.337	0.462	0.535	0.465	1.401	0.567–3.463
HDL	−3.929	1.444	7.405	0.007	0.020	0.001–0.333
LDL	0.942	0.434	4.708	0.030	2.565	1.095–6.004
miR-1247-3p	−0.914	0.301	9.225	0.002	0.401	0.222–0.723

centrating on serum proteins as potential diagnostic and prognostic indicators [8–10]. Inflammatory molecules such as C-reactive protein (CRP), interleukin-6 (IL-6), tumour necrosis factor- α (TNF- α), serum amyloid A (SAA), and intercellular adhesion molecule, ICAM-1) is associated with an increased risk of stroke [11–13]. In addition, increased levels of thrombo-markers D-dimer and fibrinogen [14,15] may indicate increased stroke risk. miRNAs are involved in almost all biological processes, including cell proliferation, development, differentiation, and metabolism [5,16]. Currently, polymerase chain reaction (PCR) is the main technique for detecting miRNAs, and the specific detection method of miRNA magnifies the initial differences between samples, allowing for earlier and easier detection than traditional markers, making miRNAs more sensitive biomarkers [17]. Some studies have revealed the feasibility of employing miRNA expression levels as biomarkers for stroke [18,19], such as serum levels of miR-15a, miR-16, miR-17-5p, and miR-145, which have been reported as biomarkers of acute ischemic stroke.

There was a significant reduction in miR-1247-3p expression following cerebral ischemic injury, and our previous research showed that overexpression of miR-1247-3p had anti-apoptotic effects on nerve cells, reduced cerebral infarction area in ischemia/reperfusion mice and improved mouse behavioural function [6]. Therefore, we hypothesize that the abnormal expression of miR-1247-3p during the acute phase of ischemic stroke may serve as a potential molecular marker for early diagnosis of ischemic stroke. In this study, serum expression of miR-1247-3p was compared between patients with ischemic stroke and healthy subjects.

Consistent with findings at the animal and cellular levels, results showed that miR-1247-3p expression was significantly lower in patients with ischemic stroke compared to healthy subjects. In addition, miR-1247-3p expression levels were inversely correlated with triglyceride levels. Excessive triglycerides frequently induce atherosclerosis [20], which leads to stroke, so serum miR-1247-3p may also indicate the degree of dyslipidemia.

A 3-miRNA combinatorial model (miR-1268b, miR-4433b-3p, and miR-6803-5p) was constructed for predicting stroke risk with a sensitivity of 84%, specificity of 98%, and an AUC of 0.95 (95% CI, 0.92–0.98). In this study, miR-1247-3p has the highest sensitivity (up to 92%), specificity (up to 88%), and AUC of 0.955. Compared to the previously reported 3-miRNA combination model of stroke patients, miR-1247-3p had a higher sensitivity and lower specificity than that of the reported 3-miRNA combination model of stroke patients. According to the univariate binary logistic regression analysis, miR-1247-3p was a protective factor against ischemic stroke (OR, 0.401; 95% CI, 0.222–0.732; $P = 0.002$). Therefore, we propose that miR-1247-3p is a promising serum marker for diagnosing ischemic stroke in clinical practice, allowing for more accurate differentiation between healthy individuals and patients with ischemic stroke.

This study had several limitations. Firstly, the sample size was inadequate to conduct effective internal stratified analyses of patients with ischemic stroke. Secondly, there is a difference in age between the groups, as the control group was recruited from the population that typically undergoes physical examinations, predominantly young and middle-aged. Furthermore, brain imaging was not performed on those who had undergone physical examinations and had no stroke history; a small proportion of these control participants may have asymptomatic cerebrovascular disease. Finally, the diagnostic performance of miR-1247-3p has not been further verified. These questions suggest that future research could involve a larger sample of patients and control groups with matched characteristics, verify the marker role of miR-1247-3p and stratify the patients to investigate the impact of miR-1247-3p on stroke development and prognosis.

In conclusion, serum miR-1247-3p expression is reduced in patients with ischemic stroke, which can be used as a protective factor to affect the incidence of ischemic stroke and as a potential biological marker for the diagnosis of ischemic stroke and can also reflect the degree of dyslipidemia. This work is limited by its small sample size and experimental design, and additional research is necessary to improve the miR-1247-3p evaluation system for ischemic stroke.

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