

Expression and Clinical Value of miR-185 and miR-424 in Patients with Acute Ischemic Stroke

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Background: To investigate the expression of serum miR-185 and miR-424 in patients with acute ischemic stroke (AIS) and their predictive value. A total of 142 patients with AIS and 50 healthy controls were enrolled.

Methods: According to the modified Rankin scale (mRS) score, AIS patients were divided into the good prognosis group and the poor prognosis group. Based on the National Institutes of Health Stroke Scale (NIHSS) score, AIS patients were divided into the mild group, the moderate group, and the severe group. RT-qPCR was used to determine the expression. ROC curve and Pearson correlation analysis were adopted to predict poor prognosis and analyze the correlation between the expression and NIHSS, mRS score.

Results: Compared with the control group, the expression of miR-185 and miR-424 in the AIS group was significantly higher ($P < 0.01$). Similarly, significantly higher expressions could be found in the poor prognosis group and the severe group ($P < 0.01$). The ROC curve revealed that the optimal cut-off values were 2.14 and 4.08, respectively. The area under the ROC curve (0.928, 95% CI: 0.870–0.993) was the largest, with sensitivity and specificity of 92.0% and 85.7%. Pearson correlation analysis showed that their expression was positively correlated with NIHSS score and mRS score in AIS patients ($r = 0.735, 0.802, 0.796, 0.873, P < 0.01$).

Conclusion: There are two factors related to the up-regulated expression of serum miR-185 and miR-424, one is the severity degree of neurological impairment of patients with AIS and the other is their prognosis. These two combined indicators can contribute to predicting the prognosis of AIS patients.

Keywords: acute ischemic stroke, miR-185, miR-424, prognostic evaluation

Introduction

Acute ischemic stroke (AIS) is a dangerous cerebrovascular disease that brings non-reversible damage to our brain.¹ It brings great mental stress and economic burden on patients and their families, especially the middle-aged and elder people.^{2–4} AIS is always recurrent and a very complex disease.⁵ Therefore, finding a helpful biomarker for AIS prognosis is of great importance. microRNA (miRNA), a new type of gene regulation molecule, participates in the occurrence and development of stroke by regulating gene expression, and is expected to serve as a potential biomarker of AIS.⁶ Recent studies have found that miR-185 and miR-424 are involved in initiating a series of complex pathophysiological processes of stroke.^{7–10} They play a vital role in the damage of atherosclerotic plaque, neuronal destruction and repair, and local inflammation in the lesion.^{11,12} However, further exploration is still needed, since the mechanisms and potential therapeutic effects of miR-185 and miR-424 in AIS are not

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clear. Therefore, in this study, the expression of serum miR-185 and miR-424 in patients with AIS was detected, and the predictive value of miR-185 and miR-424 in patients with AIS was analyzed, to provide a reference for the early treatment of patients with AIS.

Materials and Methods

Study Population

One hundred and forty-two patients with AIS admitted to People's Hospital of Danzhou from January 1, 2019 to July 31, 2021 were enrolled, including 97 males and 45 females, aged 35 to 87 (53.60 ± 13.25) years. The inclusion criteria: (a) diagnosis based on "Chinese Stroke Association guidelines for clinical management of cerebrovascular disorders";¹³ and (b) the patients were hospitalized within 24 hours of onset, and the ischemic lesions were revealed by CT or MRI. The exclusion criteria of patients are as follows: (a) patients with hemorrhagic stroke, traumatic brain injury, hypertensive encephalopathy, encephalitis, and other types of cerebrovascular diseases; (b) patients with cardiopulmonary disease, multiple sclerosis, malignant tumors, and blood system diseases; and (c) patients with consciousness disorder or mental symptoms. Fifty cases of healthy controls were selected as the control group, including 32 males and 18 females, aged from 40 to 75 (57.20 ± 9.28) years. This study was approved by the hospital ethics committee of People's Hospital of Danzhou. All patients signed informed consent before the experiment was conducted. According to the modified Rankin scale (mRS) score, AIS patients were divided into the good prognosis group and the poor prognosis group. Based on the National Institutes of Health Stroke Scale (NIHSS) score, AIS patients were classified into the mild group, the moderate group, and the severe group. The NIHSS score was applied to evaluating the degree of neurological impairment of patients. Among them, 42 cases were patients with mild neurological impairment (the mild group, score ≤ 5), 64 cases were patients with moderate neurological impairment (the moderate group, score 5–20), and 36 cases were patients with severe neurological impairment (the severe group, score > 20). The general information of all patients at the time of admission was recorded, including age, gender, body mass index, basic diseases, hyperlipidemia, history of smoking and drinking.

Primer Design and Synthesis

miR-185 Primer Upstream sequence: 5'-ACGTATGCTACTGTGAGCTG-3', Downstream sequence: 5'-GACTGCGAGCTAGCATCG-3'; miR-424 Primer Upstream sequence: 5'-GAGTACTCTATGACTGTCAG-3', Downstream sequence: 5'-ATCGTACTAGATACTACG-3'.

Detection of miR-185 and miR-424

A sample of 5 mL of venous blood was taken from every patient on admission to the hospital, and placed in a centrifuge tube without anticoagulant. Then, centrifuge at 2500 r/min for 15 min at room temperature, pipet 400 μ L of the upper serum and add 1 mL of extractant (Trizol) into it, and store that at -70°C . Real-time fluorescent quantitative polymerase chain reaction (RT-PCR) was performed on the ABI 7500 fluorescent quantitative PCR instrument. The reaction system was 20.00 μ L, including 1.00 μ L TaqMan MicroRNA Assay, 1.33 μ L cDNA, 10.00 μ L TaqMan 2 \times Universal PCR Master Mix, 7.67 μ L ddH₂O. The mixture was centrifuged and put into a quantitative PCR instrument. The condition of PCR amplified reaction was 95°C pre-degeneration for 10 minutes, 95°C degeneration for 15 seconds, 60°C annealing for 60 seconds, and 95°C extension for 2 seconds which cycle for 45 times. Take U6 as the internal reference; the relative expression of serum miR-185 and miR-424 were solved by the method of $2^{-\Delta\Delta\text{Ct}}$, $\Delta\text{Ct} = \text{Ct}_{\text{target gene}} - \text{Ct}_{\text{U6}}$.

Statistical Analysis

In the analysis, SPSS 12.0 was applied, and the measurement data were represented by $\bar{X} \pm S$, and the comparison of the means of two independent samples was conducted by *t*-test. The counting data were expressed as the percentage (%) and were performed by χ^2 test. ROC curve and Pearson correlation analysis was also applied. $P < 0.05$ indicated the difference was statistically significant.

Results

The Expression of Serum miR-185 and miR-424

There was no significant difference in gender, body mass index (BMI), basic diseases, atrial fibrillation, smoking history, drinking history, heart rate, systolic blood pressure, and diastolic blood pressure between the good prognosis group and the poor prognosis group ($P > 0.05$) (Table 1). Compared with the control group, the expression of miR-185 and miR-424 in the AIS group was

Table I Clinical Characteristics of the Study Population

Variable	AIS Group (n=142)	Cerebral Infarction (n=44)	Control (n=50)	P
Age (mean±SD)	52.40±2.35	52.80±2.14	52.15±2.83	0.758
BMI (kg/m ²)	23.78±2.34	23.18±2.14	23.60±2.78	0.316
Gender				
Man	82 (57.7)	24 (54.5)	30 (60.0)	0.824
Woman	60 (42.3)	20 (45.5)	20 (30.0)	
History of Diabetes, n (%)				
Yes	61 (43.0)	21 (47.7)	23 (46.0)	0.760
No	81 (57.0)	23 (52.3)	27 (54.0)	
History of Hypertension, n (%)				
Yes	85 (59.9)	27 (61.4)	31 (62.0)	0.624
No	57 (40.1)	17 (38.6)	19 (38.0)	
Hyperlipidemia, n (%)				
Yes	82 (57.7)	30 (68.2)	32 (64.0)	0.514
No	60 (42.3)	14 (31.8)	18 (36.0)	
History of Drinking, n (%)				
Yes	55 (38.7)	16 (36.4)	17 (34.0)	0.698
No	87 (61.3)	28 (63.6)	33 (66.0)	
History of Smoking, n (%)				
Yes	89 (62.7)	35 (79.5)	38 (76.0)	0.114
No	53 (37.3)	9 (20.5)	12 (24.0)	

Abbreviation: AIS, acute ischemic stroke.

significantly higher ($P<0.01$) (Figure 1). Similarly, significantly higher expressions could be found in the poor prognosis group and the severe group ($P<0.01$) (Figures 2 and 3). Furthermore, the expression levels of serum miR-185 and miR-424 in the large area cerebral infarction group were significantly higher than those in the medium area cerebral infarction group and small area cerebral infarction group ($P<0.01$) (Figure 4).

ROC Curve

The optimal cut-off values of the expression of serum miR-185 and miR-424 for predicting poor prognosis of AIS patients were 2.14 and 4.08, respectively. The AUC of the two combined predictors for poor prognosis of AIS patients was 0.928 (0.870~0.993), which was significantly higher than that of single miR-185 [0.795 (0.737~0.856)] and single miR-424 [0.841 (0.785~0.897)], the differences

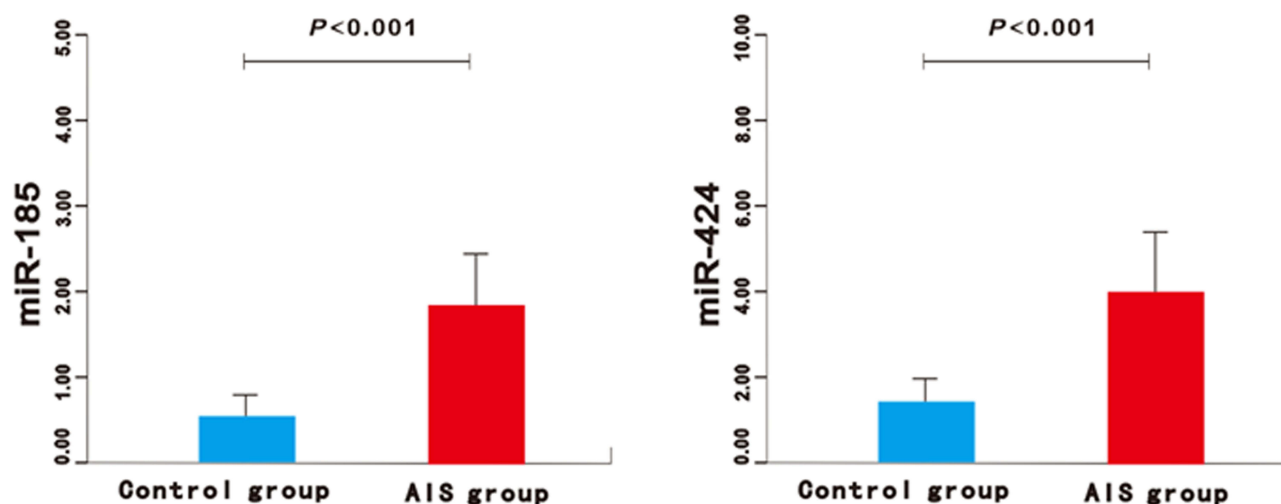


Figure 1 The expression of miR-185 and miR-424 in the AIS group and control group.

Abbreviation: AIS, acute ischemic stroke.

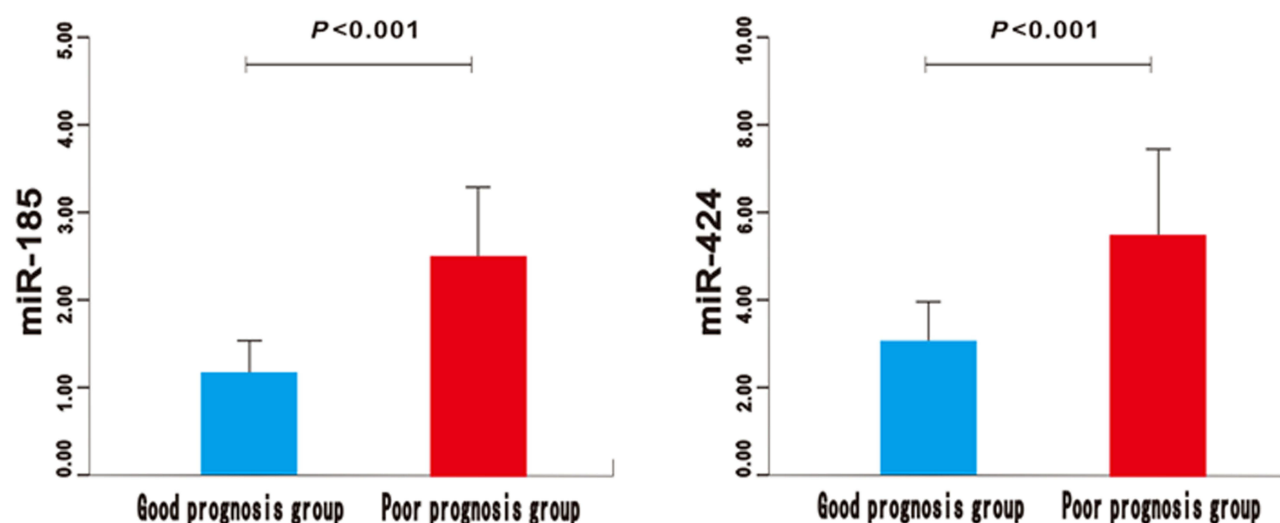


Figure 2 The expression of miR-185 and miR-424 in poor prognosis group and good prognosis group of AIS patients.

Abbreviation: AIS, acute ischemic stroke.

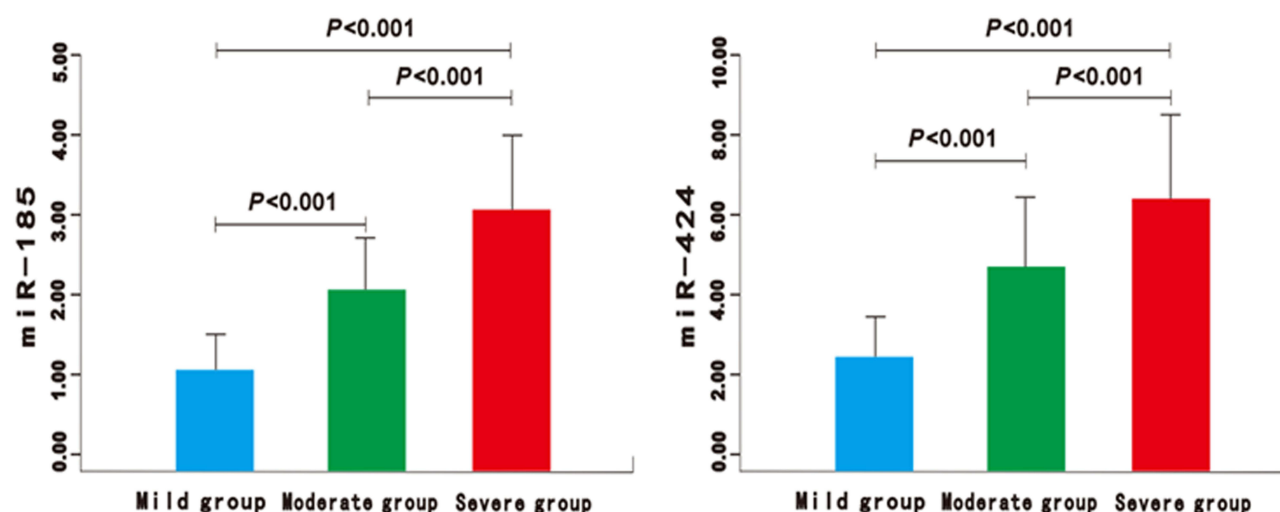


Figure 3 The expression of miR-185 and miR-424 in the mild, moderate, and severe group of AIS patients.

Abbreviation: AIS, acute ischemic stroke.

were statistically significant ($Z=7.118, 5.214, P<0.05$), and the sensitivity and specificity were 92.7% and 86.5%, respectively (Table 2 and Figure 5).

Pearson Correlation Analysis

Pearson correlation analysis showed that the expression of serum miR-185 was positively related to NIHSS score and mRS score ($r=0.735, 0.802, P<0.01$) (Figure 6). Similarly, the expression of serum miR-424 was positively related to NIHSS score and mRS score ($r=0.796, 0.873, P<0.01$) (Figure 7).

Discussion

AIS is a common vascular disease of the central nervous system, which can trigger a series of ischemic cascade pathological events, and finally lead to the apoptosis and necrosis of cerebrovascular endothelial cells and nerve cells. Inflammatory reaction, endothelial dysfunction, and atherosclerosis play a vital role in the pathophysiological process of AIS.¹⁴ miRNA, composed of 18–25 nucleotides in length, is an endogenous single-stranded non-coding RNA molecule. By affecting the expression of target genes, it participates in the initiation of a series of complex

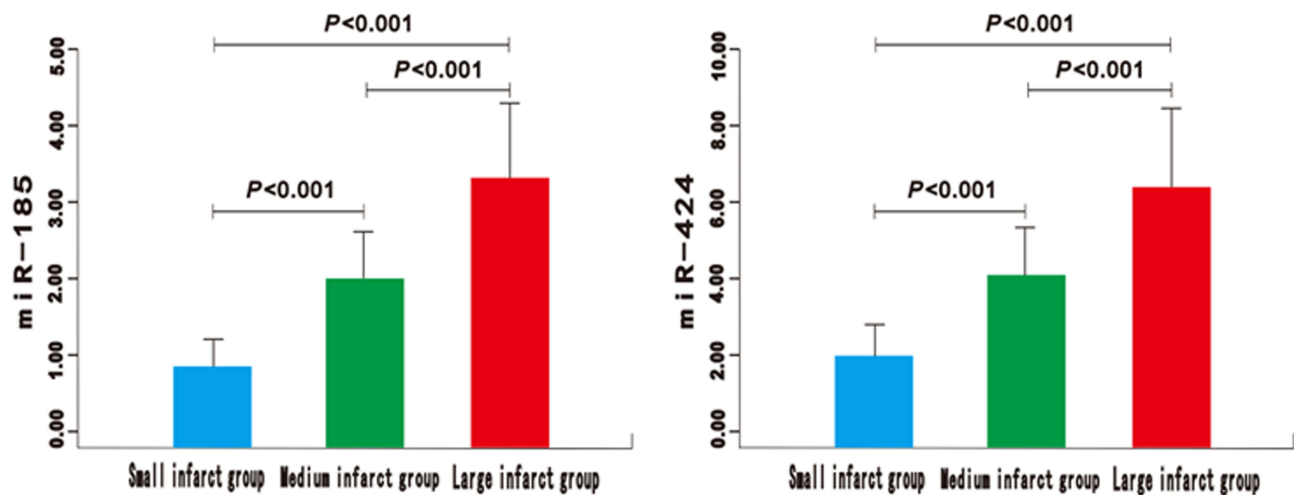


Figure 4 The expression of miR-185 and miR-424 in small, medium, and large area cerebral infarction group of AIS patients.

Abbreviation: AIS, acute ischemic stroke.

pathophysiological processes of AIS.¹⁵ Numerous studies have reported that miRNAs participate in degrading extracellular matrix, regulating cell apoptosis in plaques, regulating vascular endothelial function and vascular remodeling. miRNAs also can affect the formation and

rupture of atherosclerotic plaques, and are involved in neuron destruction, repair, and local inflammatory reaction. miRNA may provide a new target for the treatment of AIS.^{16,17} Xu et al¹⁸ found that the differential expression of miRNAs in AIS may be involved in the release of

Table 2 Clinical Characteristics of the Good Prognosis Group and Poor Prognosis Group

Variable	Good Prognosis (n=89)	Poor Prognosis (n=53)	P
Age (mean±SD)	51.48±9.35	57.15±8.63	0.006
BMI (kg/m ²)	23.28±2.24	23.45±2.36	0.584
Heart rate (beats/min)	95.20±7.15	96.36±7.40	0.473
Systolic blood pressure (mmHg)	143.26±10.28	145.40±10.37	0.751
Diastolic blood pressure (mmHg)	85.73±7.26	86.48±7.42	0.557
NIHSS (score)	10.50±3.25	17.62±3.58	<0.001
mRS (score)	1.42±0.28	4.17±0.56	<0.001
Gender			
Man	58	39	0.297
Woman	31	14	
History of Diabetes, n (%)			
Yes	20 (22.5)	14 (26.4)	0.594
No	69 (77.5)	39 (73.6)	
History of Hypertension, n (%)			
Yes	27 (30.3)	19 (35.8)	0.561
No	62 (69.7)	34 (64.2)	
History of Drinking, n (%)			
Yes	37 (41.6)	25 (47.2)	0.515
No	52 (58.4)	28 (52.8)	
History of Smoking, n (%)			
Yes	29 (32.3)	15 (28.3)	0.594
No	60 (67.7)	38 (71.7)	
Atrial fibrillation, n (%)			
Yes	7 (7.9)	5 (9.4)	0.745
No	82 (92.1)	58 (90.6)	

Abbreviations: NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin scale.

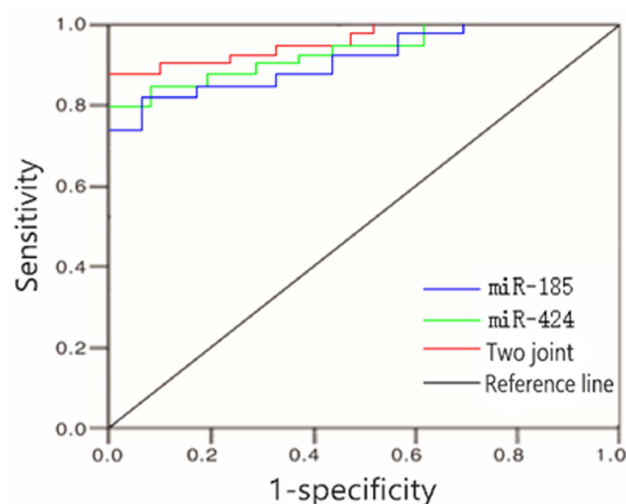


Figure 5 ROC curve for predicting poor prognosis of AIS patients by the expression of serum miR-185 and miR-424.

Abbreviation: AIS, acute ischemic stroke.

inflammatory mediators and endothelial cell dysfunction. This may also promote the formation and rupture of atherosclerotic plaque and affect the occurrence of stroke. Other studies pointed out that miRNA can identify the 3' non-coding region of the target gene by complementary base pairing, degrade the target messenger RNA, or inhibit the translation of the target messenger RNA. It can participate in the regulation of the pathophysiological process of AIS as well, such as atherosclerosis, cerebral edema, and cerebral ischemia–reperfusion damage.¹⁹

The present study suggests that the up-regulated expression of serum miR-185 and miR-424 may be involved in the pathogenesis of AIS. Moreover, there are differences in the expression of serum miR-185 and miR-424 in patients with different degrees of neurological impairment. The more severe the neurological impairment, the more obvious the increased expression of serum miR-185 and miR-424. This conclusion suggests that the increased expression of serum miR-185 and miR-424 is strongly associated with poor prognosis, and can indicate the severity of neurological impairment and may become biomarkers of AIS patients. The research by Gacoñ et al²⁰ showed that miRNA was closely linked with the pathogenesis of AIS. Wu et al²¹ found that the expression of miRNA was closely linked with the degree of neurological impairment in stroke patients. Thus, the expression of miRNA can be used as a biological indicator for evaluating the degree of neurological impairment of AIS. The ROC curve indicated that the optimal cut-off values of the expression of serum miR-185 and miR-424 for predicting poor prognosis of AIS patients were 2.14 and 4.08, respectively. The area under the curve of the two combined indicators for predicting was the largest, with relatively higher sensitivity and specificity. Jin and Xing's²² research concluded that the expression of serum miR-185 in patients with AIS was significantly higher and was positively linked with NIHSS score. miR-185 can be used as an independent biomarker of AIS risk and has a good

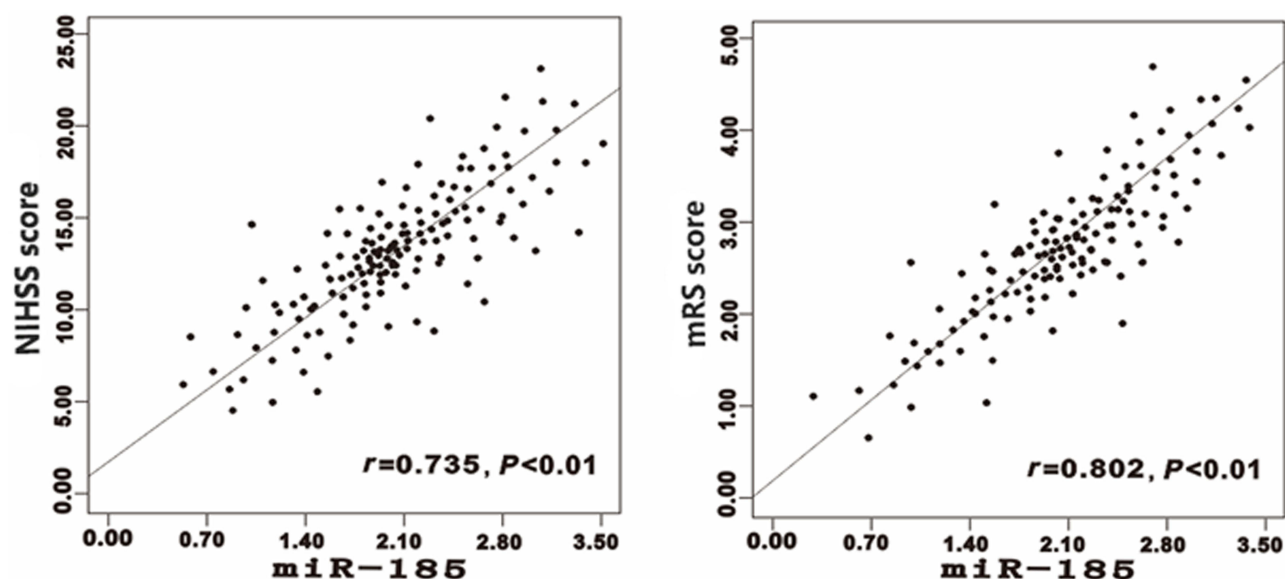


Figure 6 Correlations between the expression of serum miR-185 and NIHSS, mRS score in patients with AIS.

Abbreviations: AIS, acute ischemic stroke; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin scale.

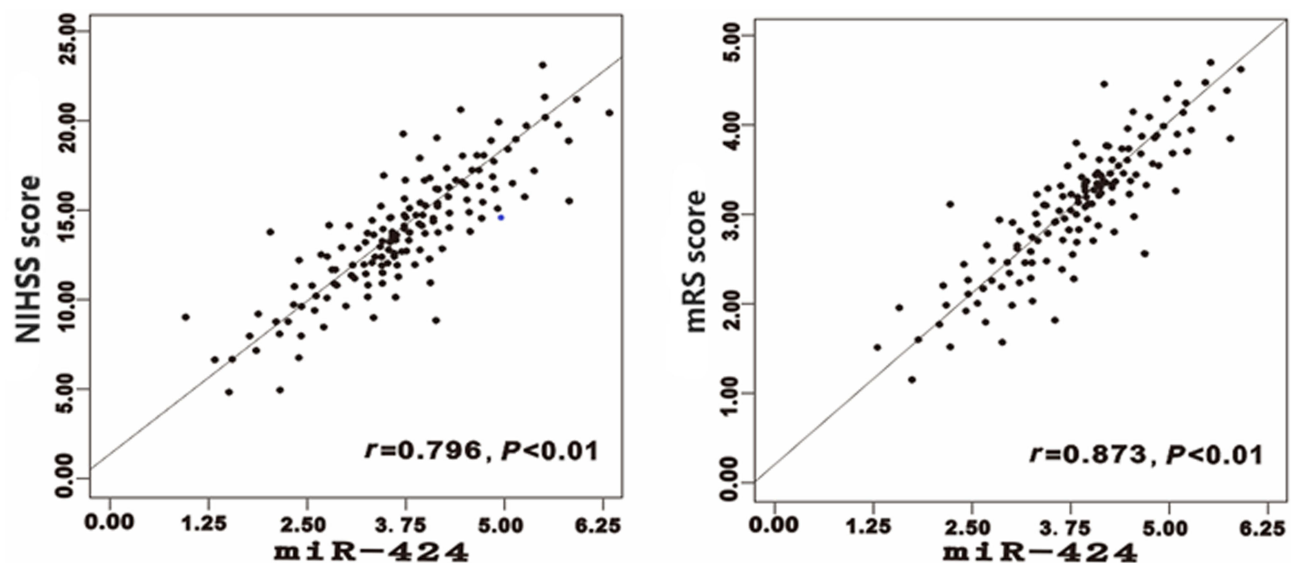


Figure 7 Correlations between the expression of serum miR-424 and NIHSS, mRS score in patients with AIS.

Abbreviations: AIS, acute ischemic stroke; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin scale.

diagnostic value for AIS. Li et al's research¹⁰ showed higher expression of serum miR-424 in AIS patients, and its expression was linked with the severity of the disease; suggests that miR-424 can be a biomarker for the diagnosis of AIS and can provide clues for the individualized treatment of AIS. At present, the most commonly used stroke scale globally is the NIHSS score, which can be used not only to evaluate neurological impairment of stroke patients, but also to regularly evaluate the treatment effect on stroke patients. The degree of disability or independent ability in daily activities of post-stroke patients were evaluated by mRS. In this study, the expression of serum miR-185 and miR-424 is positively related to the NIHSS score and mRS score. The increased expressions of serum miR-185 and miR-424 are closely linked with the severity of neurological impairment and poor prognosis in AIS patients. They can become potential markers for predicting poor prognosis in AIS patients as well.

Conclusion

There are two factors related to the up-regulated expression of serum miR-185 and miR-424, one is the severity degree of neurological impairment of patients with AIS and the other is their prognosis. These two combined indicators can contribute to predicting the prognosis of AIS patients. However, the specific mechanism of serum miR-185 and miR-424 involved in AIS remains unclear. We will investigate further in the future experiment. Furthermore, more tests on other classical miRNAs as

the contrast for highlighting the specificity of miR-182 and miR-424 are also necessary and beneficial in future study.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article.

Ethical Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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Author Contributions

HC conceived study design; CG and YY conceived the content concept; QL and YG performed the data collection, extraction and analyzed the data. CG, YY and HC interpreted and reviewed the data and drafts. CG and HC reviewed the final draft. All authors were involved in literature search, writing the paper and had final approval of the

submitted and published versions. All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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