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Review Article

Expression and prognostic value of miR-129-5p and miR-144 in acute ischemic stroke

Kuiyi Di[1], Min Pang[2], Chunlei Li[1]

[1]Department of Neurology, The Second People's Hospital of Dongying, Dongying, Shandong Province, China; [2]Department of Neurology, Dezhou Hospital of Traditional Chinese Medicine, Dezhou, Shandong Province, China

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Abstract: Objective: This study aimed to explore the diagnosis and prognostic value of miR-129-5p and miR-144 in patients with acute ischemic stroke (AIS). Methods: Altogether 128 AIS patients admitted to our hospital were selected as an AIS group, and 100 healthy people investigated during the same period were selected as a normal group. miR-129-5p and miR-144 level in both groups were detected. The diagnostic and prognostic values of the two microRNAs were analyzed by receiver operating characteristic curve, the correlation between the two was analyzed by Pearson correlation coefficient, and the risk factors affecting the poor prognosis of AIS patients were analyzed by multivariate Logistic regression. Results: miR-129-5p showed low expression in the AIS group, while miR-144 showed high expression in the AIS group. The AUC of patients diagnosed with AIS by miR-129-5p and miR-144 were 0.846 and 0.823, respectively. miR-129-5p was negatively correlated with miR-144 ($r=-0.666$, $P<0.001$). A low level of miR-129-5p was associated with poor prognosis,a high level of miR-144 was associated with poor prognosis, and the AUC of miR-129-5p and miR-144 for predicting poor prognosis of AIS patients were 0.829 and 0.811, respectively. Diabetes, smoking, miR-129-5p and miR-144 were independent risk factors for poor prognosis of AIS patients. Conclusion: miR-129-5p was decreased in AIS patients and miR-144 was increased in AIS patients, the two are potential biomarkers for diagnosis and prognosis of AIS patients.

Keywords: Acute ischemic stroke, miR-129-5p, miR-144, expression, prognostic value

Introduction

Acute ischemic stroke (AIS), as an acute disease caused by non-traumatic cerebrovascular diseases, clinically its characteristics have sudden onset, recurrent neurological deficits, and the onset of duration can exceed one day or even cause death within one day [1]. According to statistical data, about 70% of AIS appears in low-and middle-income countries. With the passage of time, the incidence rate of AIS in high-income countries has decreased by 12%, while that in low-and middle-income countries has increased by 12%. The main cause of AIS is atherosclerotic plaque on the vascular wall formed in the carotid artery, which leads to vascular stenosis and ischemia, thus causing a series of adverse reactions such as nerve cell injury, dysfunction and apoptosis [3]. Early intervention is helpful to relieve the disease. However, the early symptoms of AIS are relatively hidden and it is difficult to distinguish AIS from other neurological diseases or non-neurological diseases, which brings great challenges to the diagnosis [4]. Although neurological examination and imaging methods have emerged as diagnostic tools, their high cost may deter many AIS patients and the patients may miss the best opportunity for intervention [5]. Therefore, it is of great significance for early diagnosis and prognosis prediction of AIS patients to find a biomarker with low cost, rapid diagnosis, and high specificity and sensitivity.

The potential pathological mechanism of AIS involves apoptosis, oxidative stress, and neuroinflammation, etc. microRNA (miRNA), as a short-chain non-coding RNA is widely involved in the process of human diseases, and is also a biological regulator of pathological process of AIS patients. Its stability and differential expression in the blood are expected to be potential markers for early diagnosis and prognosis of AIS [6, 7]. In addition, miRNA is easy to detect,

which has the advantage of having low cost [8]. As a tumor inhibitor of the miRNA family, miR-129-5p can influence the protein expression of apoptosis markers bcl-2 and caspase-3 during cerebral ischemia-reperfusion (IR) and mediate the process of neuronal apoptosis, and IR is related to AIS pathophysiological process. This indicates that miR-129-5p may participate in the pathogenesis of AIS [9-11], and high-levels of miR-129-5p can inhibit the inflammatory cascade pathway, thus improving IR-induced neuroinflammation. We speculate that miR-129-5p also mediates the regulation of AIS neuroinflammation [12]. As a member of the miRNA family, miR-144 has been reported to have abnormally high expression in the plasma of the limb ischemia rat model, suggesting that miR-144 may participate in the operation process of IR [13]. In addition, the research of Chu et al. [14] on miR-144 of IR showed that the activity of miR-144 inhibited by ginsenoside Rg1 can activate Nrf2/ARW pathway at the post translation level and alleviate IR induced oxidative stress. We speculate that miR-144 may participate in the regulation of AIS oxidative stress.

At present, there is little research on the diagnostic and prognostic value of miR-129-5p and miR-144 in AIS patients. We will conduct diagnostic and prognostic analysis by testing miR-129-5p and miR-144 in AIS patients' serum, in order to to provide clinical reference for the diagnosis and prognosis of AIS patients.

Data and methods

General information

Altogether 128 AIS patients admitted to our hospital from March 2018 to May 2019 were chosen as an AIS group, including 75 men and 53 women, aged 38-79 years, with an average age of 76.23±6.89 years. Inclusion criteria: patients with AIS [15] confirmed by guidelines of the American Heart Association/American Stroke Association; patients met the scoring criteria of modified RANKIN scale (MRS) [16] (a score of less than 2 was regarded as the good prognosis group; a score of greater than 2 points or death was considered as the poor prognosis group, in which 0 points were considered to be completely asymptomatic; one point was considered to be symptomatic without apparent dysfunction; two points was considered as mild disability, patients unable to complete all activities before the disease, but able to live independently; three points was considered as moderate disability, patients can only walk independently; four points was considered as severe disability, patients unable to walk on their own, need assistance with everything; five points was considered as most-severe disability, patients are bedridden and incontinent and need continuous nursing); patients with no surgical history in the past three months; patients have not taken hormone drugs. Exclusion criteria: patients with acute respiratory and circulatory failure; patients complicated with malignant tumors, autoimmune diseases, infectious diseases and serious organ dysfunction; patients that have taken drugs that affect the indicators of this study within a half a year; patients with acute recurrent cerebral infarction or cerebral hemorrhage; patients with infection or fever. Another 100 healthy people undergoing physical examination during the same period were chosen as the normal group, containing 58 men and 42 women, aged 35-79 years, with an average age of 75.58±6.47 years. Our study was approved by the Ethics Committee of our hospital. The subjects and their families were fully informed and signed an informed consent form.

Detection method

A total of 5 mL of venous blood from the elbow from all subjects was collected at 8 o'clock in the morning, placed in a vacuum blood collection tube containing EDTA-K2, and centrifuged at 850 g for 15 min. Altogether 2 mL of upper serum was removed and transferred to EP tube, centrifuged at 16,000 g for 10 min to precipitate cell debris, and then the supernatant was stored in a new EP tube at -75°C for later use. The total RNA was extracted according to the instructions of mirVanaTM miRNA Isolation serum extraction kit (Zhen Shanghai and Shanghai Industrial Co., Ltd., hz90203-501). The concentration of RNA was determined by ultraviolet-visible spectrophotometer (ANATECH (Beijing) Co., Ltd, China, 1210000). Referring to TaqMan MicroRNA Reverse Transcription Kit (Hangzhou Woosen Biotechnology Co., Ltd, China, 4366596), RNA was reverse transcribed into cDNA, and PCR amplification experiment was conducted with cDNA as template, U6 was used as internal reference gene, and primer sequence was designed by Shanghai Qantu

Table 1. General information of two groups of patients [n (%), mean ± SD]

Factor	n	Normal group (n=100)	AIS group (n=128)	X^2/t	P
Gender				0.001	0.928
Male	133	58 (58.00)	75 (58.59)		
Female	95	42 (42.00)	53 (41.41)		
Age (years)				0.174	0.677
<75	90	41 (41.00)	49 (38.28)		
≥75	138	59 (59.00)	79 (61.72)		
Average age (years)	228	75.58±6.47	76.23±6.89	0.485	0.469
History of hypertension				1.219	0.270
No	139	65 (65.00)	74 (57.81)		
Yes	89	35 (35.00)	54 (42.19)		
History of diabetes				0.083	0.773
No	82	37 (37.00)	45 (35.16)		
Yes	146	63 (63.00)	83 (64.84)		
Drinking history				3.461	0.063
No	104	53 (53.00)	52 (40.63)		
Yes	124	47 (47.00)	76 (59.37)		
Smoking history				0.174	0.677
No	138	59 (59.00)	79 (61.72)		
Yes	90	41 (41.00)	49 (38.28)		
Prognosis					
MRS<2	50	-	50 (39.06)		
MRS≥2 or death	78	-	78 (60.94)		
HDL-C (mmol/L)	228	1.06±0.12	1.03±0.11	1.963	0.051
LDL-C (mmol/L)	228	3.00±0.31	3.08±0.35	1.800	0.073

Biotechnology Co., Ltd., China. miR-129-5p and miR-144 were quantitatively detected on a real-time fluorescence quantitative PCR system (Nanjing ZhongkeBio Medical Technology Co., Ltd, China, ZKBIO-003) with reference to miRNA RT-qPCR research kit (Genetimes Technology Inc., China, 110001S). PCR amplification conditions: 90°C for 5 min, 90°C for 5 s, 60°C for 30 s, 72°C for 5 s, with a total of 40 cycles. All samples were repeatedly detected 3 times, and the results were expressed by $2^{-\Delta CT}$.

Follow-up

The patients were followed up for 3 months after discharge, and the prognosis was evaluated by telephone, outpatient consultation, hospital visit and so on.

Statistical analysis

Statistical analysis was conducted by SPSS20.0 (EasyBio, Beijing, China), and Graphpad Prism6 (Graphpad Software, San Diego, USA) was used to visualize the data. The measurement data were expressed by mean ± SD. The comparison of measurement data between groups was conducted by independent sample t test, and the comparison before and after experiment was conducted by paired t test. The counting data was expressed by the number of cases/percentage [n (%)], for comparison of counting data between groups we adopted the chi-square test, and when the theoretical frequency in chi-square test was less than 5, the continuity correction chi-square test was used. Receiver operating characteristic curve (ROC) and Logistic regression models were applied to evaluate the diagnostic value of serum miR-129-5p and miR-144 in AIS patients. Pearson correlation coefficient was applied to analyze the correlation between miR-129-5p and miR-144. Multivariate Logistic regression analysis was used for risk factors affecting poor prognosis of AIS patients. A value of $P<0.05$ was considered as a statistically significant difference between the two groups.

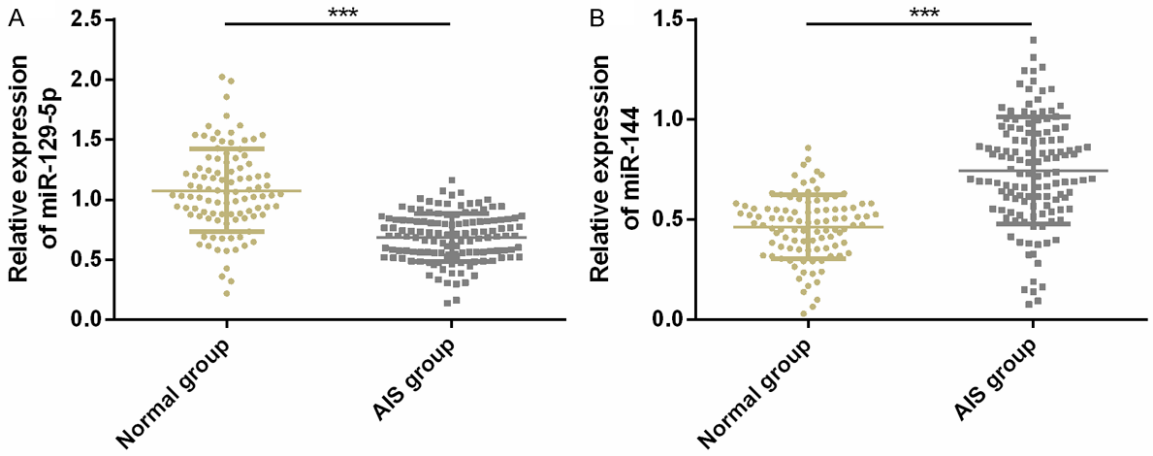

Figure 1. Relative expression of serum miR-129-5p and miR-144. A. The relative expression of serum miR-129-5p in the AIS group was lower than that in the normal group. B. The relative expression of serum miR-144 in the AIS group was higher than that in the normal group. Note: ***indicates that P<0.001.

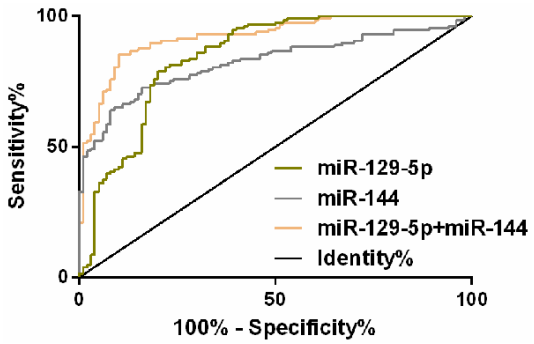

Figure 2. ROC curves of serum miR-129-5p and miR-144 diagnosis in the normal group and AIS group.

Result

General information of the two groups of patients

There was no clear difference in sex, age, average age, hypertension, diabetes, drinking, smoking, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) between the two groups (P>0.05). See **Table 1**.

Expression of serum miR-129-5p and miR-144 in AIS patients

miR-129-5p in the AIS group was significantly lower than that in the normal group, while miR-144 in the AIS group was significantly higher than that in the normal group (P<0.05). See **Figure 1**.

Diagnostic value of serum miR-129-5p and miR-144 in AIS patients

Analysis of the ROC curve showed that the AUC value of serum miR-129-5p in the normal group and the AIS group was 0.846, and the best cut-off value was 0.85. AUC value of serum miR-144 in the normal group and AIS group was 0.823, and the best cut-off value was 0.61. AUC value of serum miR-129-5p and miR-144 combined diagnosis in the normal group and AIS group was 0.922, and the best cut-off value was 0.59. Further binary Logistic regression analysis was carried out, miR-129-5p and miR-144 were used as independent variables, and Logistic regression model was obtained. Logit (P)=1.770+0.874miR-129-5p+4.097miR-144. AUC value of the normal group and AIS group diagnosed by miR-129-5p and miR-144 was 0.922. See **Figure 2** and **Table 2**.

Correlation analysis of serum miR-129-5p and miR-144

The relationship of serum miR-129-5p with miR-144 in AIS patients was analyzed by Pearson correlation coefficient. The results indicated that serum miR-129-5p and miR-144 were negatively correlated (r=-0.609, P<0.001). See **Figure 3**.

Predictive value of serum miR-129-5p and miR-144 for poor prognosis of AIS patients

All the patients were followed up successfully for three months, and the survival rate was

Table 2. ROC parameters of serum miR-129-5p and miR-144 in diagnosing AIS patients

Indicators	AUC	95% CI	S.E	Cut-off	Sensitivity (%)	Specificity (%)
miR-129-5p	0.846	0.792-0.900	0.028	0.85	78.91	80.00
miR-144	0.823	0.769-0.877	0.028	0.61	72.66	84.00
miR-129-5p+miR-144	0.922	0.887-0.957	0.018	0.59	85.16	90.00

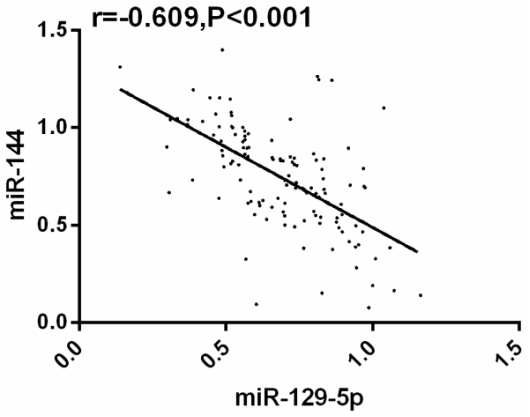

Figure 3. A negative correlation between miR-129-5p and miR-144 in serum of AIS patients.

90.63% (116/128). We took 50 AIS patients with MRS<2 as the good prognosis group and 78 AIS patients with MRS≥2 or dead patients as the poor prognosis group. After detecting miR-129-5p and miR-144 in the two groups of patients, we found that miR-129-5p in the serum of patients with good prognosis was higher than that of patients with poor prognosis, while miR-144 in the serum of patients with good prognosis was lower than that of patients with poor prognosis (P<0.05). After visualizing ROC curve, it was found that the AUC of miR-129-5p for predicting poor prognosis of AIS patients was 0.817, and the AUC of miR-144 for predicting poor prognosis of AIS patients was 0.826, all of which had good predictive value. See **Figure 4** and **Table 3**.

Univariate analysis of poor prognosis in AIS patients

Single factor analysis of patients with good prognosis and patients with poor prognosis showed that there were no clear differences in gender, age, average age, hypertension history, drinking history, HDL-C, LDL-C and other aspects between the two groups (P>0.05), but there were differences in diabetes history, smoking history, miR-129-5p, and miR-144 (P<0.05). See **Table 4**.

Multivariate analysis of poor prognosis in AIS patients

miR-129-5p and miR-144 were included in the analysis, and listed as dependent variables for assignment. Logistic regression model was used for multivariate analysis, and the results showed that diabetes history, smoking history, miR-129-5p and miR-144 were independent risk factors for poor prognosis of AIS patients. See **Tables 5**, **6**.

Discussion

AIS is a nervous system disease. It has high mortality and poor prognosis, it is also one of the main causes of human death and disability globally, which brings great burden and economic cost to the patient's family and to the society [17, 18]. At present, miRNA molecular markers from serum used in clinical diagnosis of AIS have not yet achieved the reliability by taking into account the sensitivity and specificity [19]. Therefore, exploring the potential of miRNA as an AIS diagnostic tool is of great significance for the diagnosis and prognosis of AIS patients.

Abnormal expression of miRNA is related to the occurrence and progress of various neurological diseases. Many studies have shown that miRNA in serum can be used as an index for diagnosis and prognosis evaluation of neurological diseases [20-22]. Previous studies have reported the imbalance of serum miRNA in AIS patients. For example, in Zeng et al. [23] studies, serum miR-210 was down-regulated in AIS patients, and low level of miR-210 was not conducive to the prognosis of AIS patients. Wang et al. [24] showed that over-expressed serum miR-146b was positively correlated with infarct volume and neurological deficit score. In this study, miR-129-5p in the AIS group was lower than that in normal group, while miR-144 in the AIS group was higher than that in normal group, suggesting that miR-129-5p and miR-144 may participate in the occurrence of AIS disease,

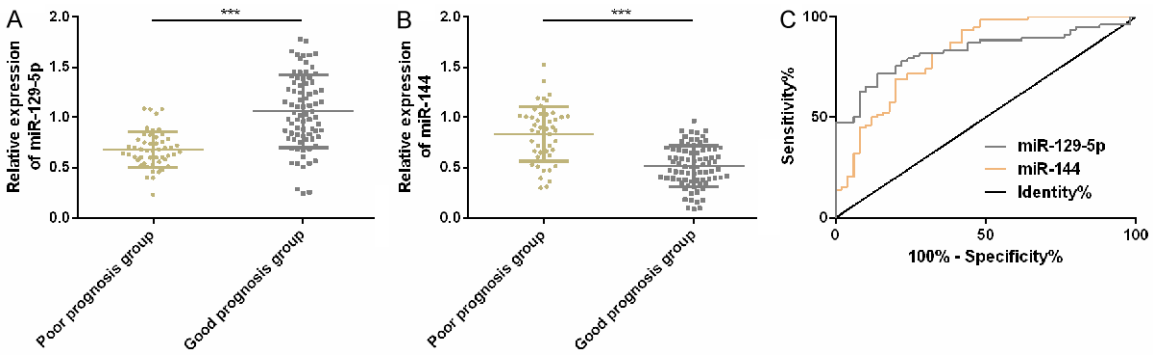

Figure 4. Predictive value of serum miR-129-5p and miR-144 for poor prognosis of AIS patients. A. The expression of serum miR-129-5p in AIS patients with poor prognosis was lower than that with good prognosis. B. The expression of serum miR-144 in AIS patients with poor prognosis was higher than that with good prognosis. C. ROC curve of serum miR-129-5p and miR-144 predicting poor prognosis of AIS patients.

Table 3. ROC parameters of serum miR-129-5p and miR-144 for predicting poor prognosis of AIS patients

Indicators	AUC	95% CI	S.E	Cut-off	Sensitivity (%)	Specificity (%)
miR-129-5p	0.829	0.758-0.900	0.036	0.83	71.79	86.00
miR-144	0.821	0.744-0.898	0.039	0.82	93.59	58.00

and the differential expression of the two in AIS patients and healthy people may be expected to be a specific sensitive marker for AIS patients. Therefore, we have drawn the ROC curves of serum miR-129-5p and miR-144 for AIS patients. The above results indicated that the AUC of miR-129-5p and miR-144 for AIS patients were 0.846 and 0.823 respectively, while the AUC of AIS patients with combined diagnosis was 0.922, indicating that miR-129-5p and miR-144 may be molecular markers for the diagnosis of AIS. We further analyzed the relationship of miR-129-5p with miR-144 through Pearson correlation coefficient. The results showed that serum miR-129-5p was negatively correlated with miR-144, suggesting that miR-129-5p and miR-144 may have antagonistic effects on the occurrence and progression of AIS patients, but the specific regulatory mechanisms of miR-129-5p and miR-144 on AIS patients have not been elaborated yet. By establishing a rat model of autoimmune encephalomyelitis (AE)-related epilepsy, the study has confirmed that down-regulating miR-129-5p can target and up-regulate HMGB1 and activate the inflammatory cascade sign, resulting in increased neuronal damage. This indicated that miR-129-5p may have a negative relationship with brain injury level [25]. Li et al. [26] studied the role of miR-144 in neuronal injury induced by IR. The results showed that overexpression of miR-144 was beneficial for apoptosis and oxidative stress, and significantly inhibited the activity of neurons. We were inspired by the fact that the increase of miR-129-5p and decrease of miR-144 may be beneficial to stimulate neuroprotection, thus recovering brain function in AIS patients. Both may become therapeutic targets for AIS patients.

MRS scoring system is a prognostic assessment tool for AIS patients, which can be used to assess the degree of disability of patients [27]. In this way, the patients with AIS were grouped by their prognosis. The results showed that low level of miR-129-5p was associated with poor prognosis, while high level of miR-144 was associated with poor prognosis, which indicated that low level miR-129-5p and high level miR-144 might not be beneficial to the prognosis of AIS patients. We also analyzed the value of predicting the poor prognosis of AIS patients. The results showed that the AUC of miR-129-5p and miR-144 for predicting the poor prognosis of AIS patients were 0.829 and 0.811, indicating that miR-129-5p and miR-144 may be able to predict the poor prognosis of AIS patients. Finally, we analyzed the risk factors that affected the prognosis of AIS patients. The results showed that AIS patients with diabetes history,

Table 4. Univariate analysis of poor prognosis in AIS patients [n (%), mean ± SD]

Factor	n	Good prognosis group (n=50)	Poor prognosis group (n=78)	X^2/t	P
Gender				0.228	0.633
Male	75	28 (56.00)	47 (60.26)		
Female	53	22 (44.00)	31 (39.74)		
Age (years)				0.480	0.488
<75	49	21 (42.00)	28 (35.90)		
≥75	79	29 (58.00)	50 (64.10)		
Average age (years)	128	73.79±6.21	75.08±6.46	1.119	0.265
History of hypertension				0.131	0.717
No	74	24 (48.00)	40 (51.28)		
Yes	54	26 (52.00)	38 (48.72)		
History of diabetes				8.268	0.004
No	45	10 (20.00)	35 (44.87)		
Yes	83	40 (80.00)	43 (55.13)		
Drinking history				0.234	0.628
No	52	19 (38.00)	33 (42.31)		
Yes	76	31 (62.00)	45 (57.69)		
Smoking history				7.083	0.008
No	79	38 (76.00)	41 (52.56)		
Yes	49	12 (24.00)	37 (47.44)		
HDL-C (mmol/L)	128	1.05±0.09	1.02±0.10	1.721	0.088
LDL-C (mmol/L)	128	3.05±0.28	3.10±0.32	0.905	0.367
miR-129-5p				6.043	0.014
<0.83	62	31 (62.00)	31 (39.74)		
≥0.83	66	19 (38.00)	47 (60.26)		
miR-144				4.478	0.034
<0.82	95	32 (64.00)	63 (80.77)		
≥0.82	33	18 (36.00)	15 (19.23)		

Table 5. Logistic multivariate regression analysis assignment

Factor	Variable	Assignment
History of diabetes	X1	No=0, Yes=1
Smoking history	X2	No=0, Yes=1
miR-129-5p	X3	≥0.83=0, <0.83=1
miR-144	X4	<0.82=0, ≥0.82=1

Table 6. Multivariate analysis of poor prognosis in patients with AIS

factor	β	S.E	Wald	P	OR	95% CI
History of diabetes	0.089	0.028	12.384	<0.001	1.102	1.039-1.148
History of smoking	0.120	0.027	13.194	<0.001	1.127	1.057-1.205
miR-129-5p	0.502	0.183	7.695	0.006	1.645	1.162-2.340
miR-144	0.334	0.012	9.068	0.003	1.387	1.121-1.732

smoking history, low level miR-129-5p and high level miR-144 had an increased risk of poor prognosis. In the study of Yang et al. [28], high expression of miR-144 in plasma of type 2 diabetes patients was associated with an increased risk of AIS, which was similar to the results of our study. Therefore, the expression of miR-129-5p and miR-144 in serum is a reliable index for early diagnosis and prognosis of AIS patients.

To sum up, serum miR-129-5p and miR-144 may be molecular markers for early diagnosis and prognosis prediction of AIS patients. Increase of miR-129-5p and decrease of miR-144 may be therapeutic targets for AIS patients. However, there is still room for improvement in this study. For example, we can increase the correlation study of serum miR-129-5p and miR-144 with the sever-

ity of AIS patients, further verifying whether the two can also predict AIS patients' conditions, and can supplement miR-129-5p, miR-144 for the study of cellular biological functions of AIS patients, and further explore the specific regulatory mechanisms of the two on AIS.

Disclosure of conflict of interest

None.

Address correspondence to: Chunlei Li, Department of Neurology, The Second People's Hospital of Dongying, Guangrao County, Dongying 257300, Shandong Province, China. E-mail: lichunlei116@163.com

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