Am J Transl Res 2022;14(6):4372-4379
www.ajtr.org /ISSN:1943-8141/AJTR0143082

Original Article

Expression of serum miR-126 in patients with intracranial aneurysm and its relationship with postoperative cerebral vasospasm

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Received March 24, 2022; Accepted May 16, 2022; Epub June 15, 2022; Published June 30, 2022

Abstract: Objective: To examine the expression of serum miR-126 in patients with intracranial aneurysm and to explore its relationship with postoperative cerebral vasospasm. Methods: In this retrospective study, 85 patients with intracranial aneurysms diagnosed and treated in our hospital were enrolled into the research group (RG), and 83 healthy volunteers who came to our hospital for physical examination were selected as the control group (CG). The serum miR-126 expression in both groups was examined by RT-PCR, and the relationship between the diagnostic value of miR-126 for intracranial aneurysm and postoperative cerebral vasospasm was analyzed. The serum inflammatory related factors in the patients were tested, and their correlation with miR-126 was assessed. The risk factors of postoperative cerebral vasospasm were evaluated by multiple factors. Results: The serum miR-126 expression in patients with intracranial aneurysm was obviously lower than that of participants in CG ($P<0.05$), and the AUC of miR-126 in diagnosing intracranial aneurysm was 0.945, which was of high diagnostic value. Serum inflammatory factors TNF-α and IL-6 were highly expressed in the serum of patients with intracranial aneurysm, which were positively correlated with the miR-126 level ($P<0.05$). After operation, the serum miR-126 level in patients with cerebral vasospasm was obviously higher than that of those without cerebral vasospasm, and the AUC of miR-126 for predicting cerebral vasospasm after operation was 0.859. Logistic regression analysis revealed that preoperative bleeding frequency, history of hypertension, Hunt-Hess grade and high expression of miR-126 were independent risk factors for cerebral vasospasm after operation in patients with intracranial aneurysm. Conclusion: miR-126 is highly expressed in the serum of patients with intracranial aneurysm, so it may be used as a potential biomarker for the diagnosis of patients with intracranial aneurysm and the prediction of cerebral vasospasm after operation.

Keywords: miR-126, intracranial aneurysm, expression, cerebral vasospasm

Introduction

Intracranial aneurysm, as a severe clinical disease, is mainly caused by abnormal changes in local blood vessels of the brain, resulting in hemangioma-like protrusion of the brain [1, 2]. If the intracranial aneurysm breaks, it may lead to massive intracranial and subarachnoid hemorrhage [3]. Clinical findings have revealed that the paroxysm of intracranial aneurysms is relatively insidious, and once the aneurysm ruptured, the mortality of patients is extremely high [4]. Therefore, patients with high-risk aneurysm rupture should be diagnosed early and prevented or treated actively to reduce serious complications.

Research has shown that miRNA plays a vital role in the formation and development of aneurysms [5]. There have been reports about the relationship between miRNA and arterial hemangioma. For example, a recent study [6] has revealed that miR-125b is abnormally downregulated in abdominal aortic aneurysm, and it regulates the vascular wall inflammation and anti-inflammatory balance. Another study [7] revealed that miRNA is closely bound up with the regulation of vascular inflammation, which

can affect the development and progression of intracranial aneurysms by regulating the expression of components (proteoglycan, elastin, etc.) responsible for the degradation of the endovascular elastic layer. Other studies [8] have clearly pointed out that inhibiting the expression of miR-29b in mice can slow down the progression of abdominal aorta. Some researches [9] have revealed that miR-126 is mainly expressed in vascular endothelial cells, which has the biological function of regulating vascular endothelial cells, and is closely associated with the process of angiogenesis and repair. However, there are few studies on the relevant expression of miR-126 in cases with intracranial aneurysm and its relationship with the prognosis of patients.

In this research, we examined the expression and clinical significance of serum miR-126 in patients with intracranial aneurysm, and analyzed the relationship between miR-126 and cerebral vasospasm after operation, so as to identify more effective molecular indicators for the diagnosis and treatment of intracranial aneurysm.

Methods and data

Clinical data

In this a retrospective study, 85 patients with intracranial aneurysms diagnosed and treated in our hospital were enrolled into the research group (RG) including 45 males and 40 females, with an average age of (58.5±4.1) years old. Eighty-three healthy volunteers who came to our hospital for physical examination in the same period were selected as the control group (CG).

Inclusion criteria: Patients who met the diagnostic criteria for intracranial aneurysms after CT and MRI examination and diagnosis [10].

Exclusion criteria: Patients with malignancy diseases; Patients with severe cardiovascular and cerebrovascular diseases; Patients with severe immune or infectious diseases.

This study was ratified by the hospital ethics committee (ethical approval number: LLSC20-200116). All subjects agreed to participate in the study and affixed a written informed consent form. This research conformed to Declaration of Helsinki. All patients received microsurgical treatment for intracranial aneurysm.

miR-126 test in serum

The serum miR-126 was tested via qRT-PCR. The fasting venous blood (5 mL) was drawn from subjects (in RG on the first day after admission and one week after surgery, and in CG during physical examination in the morning), and then centrifuged 10 min at 1500×g and 4°C. The supernatant was obtained in the refrigerator at -80°C for preservation. The total RNA in serum (200 μL) was extracted via TRIzol, and the concentration and purity of RNA solution were tested by Narodrop. The OD_{260}/OD_{280} was between 1.8 and 2.1. The total RNA was applied as template, and cDNA was synthesized by reverse transcription. The total reaction system of qRT-PCR was 20 μL, including template c DNA (1 μL), Taq polymerase (0.2 μL), forward primer and reverse primer (each 1 μL), 2×SYBR Green mix (1 μL), 20 mmol/L dNTPs (1 μL). Finally, the RNase-free water was supplemented to 20 μL. The reaction conditions were 95°C for 2 min, 95°C for 15 s, 60°C for 30 s and 70°C for 10 s, with a total of 40 cycles. The forward primer of miR-126 was 5'-ACACTCCAGCT-GGGTCGTACCGTGAGTAAT-3', and the reverse primer was 5'-CT-CAACTGGTGT CGTGGAGTCGGCAATTCAGTTGAGC-GCATTAT-3'. The forward primer of internal reference gene U6 was 5'-CTCGCT-TCGGCAGCACA-3', and the reverse primer was 3'-AACGCT-TCACGAATTTG-CGT-5'. The results were represented by relative quantitative method and calculated by $2^{-\Delta\Delta Ct}$.

Detection of serum inflammatory factors

The fasting venous blood (4 mL) was drawn from patients in both groups before and one month after operation, and the levels of serum inflammatory factors [tumor necrosis factor-α (TNF-α; ml077385), interleukin-6 (IL-6; ml058-097)] were measured by enzyme-linked immunosorbent assay. The kit was from Shanghai Enzyme-linked Biotechnology Co., Ltd.

Statistical methods

The experimental data were statistically analyzed by SPSS19.0. The enumeration data were assessed via Chi-square test. The measure-

Table 1. Comparison of baseline data

Factors	RG n=85	CG n=83	X^2	P
Gender			0.092	0.762
Male	45 (52.94)	42 (50.60)		
Female	40 (47.06)	41 (49.40)		
Age (years old)			0.001	0.990
≥58	46 (54.12)	45 (54.22)		
<58	39 (45.88)	38 (45.78)		
BMI (kg/m^2)			0.025	0.874
≥23	42 (49.41)	40 (48.19)		
<23	43 (50.59)	43 (51.81)		
Drinking or not			0.001	0.971
Yes	33 (38.82)	32 (38.55)		
No	52 (61.18)	51 (49.51)		
Surgical methods			-	-
Aneurysm clipping	26 (30.59)	-		
Aneurysm isolation	31 (36.47)	-		
Clipping combined with wrapping	28 (32.94)		-	-
Number of aneurysms		-		
1	52 (61.18)	-		
≥2	33 (38.82)	-		
Aneurysmal morphology			-	-
Scrotiform	47 (55.29)	-		
Prismatic	38 (44.71)	-		

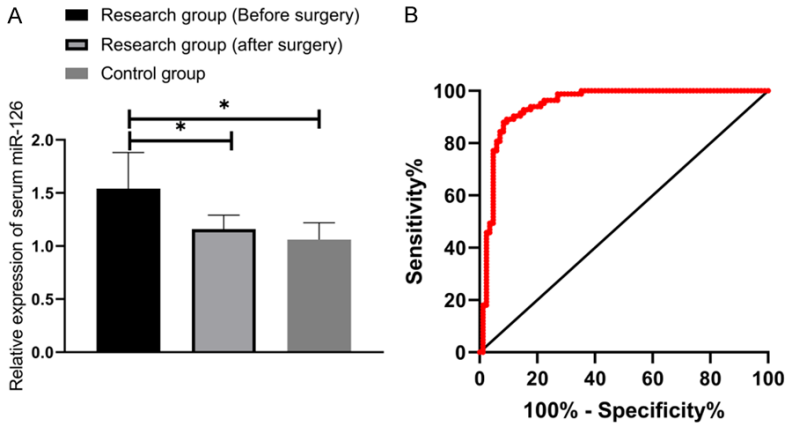

Figure 1. Analysis of miR-126 expression and its diagnostic value for intracranial aneurysm. A: Expression of serum miR-126 in patients with intracranial aneurysm; B: ROC of miR-126 in diagnosing intracranial aneurysm. * indicates P<0.05.

ment data were represented by mean number ± standard deviation, and independent t test was applied for comparison between the two groups. GraphPad Prism 6 was used to draw the experimental pictures. The diagnostic value of miR-126 for intracranial aneurysm was analyzed through ROC curve. Logistic regression model was applied to analyze the risk factors of postoperative cerebral vasospasm of patients. There was a statistical difference when P<0.05.

Results

Comparison of baseline data

There was no obvious difference in sex, age and BMI of subjects between both groups (P>0.05), showing comparability (**Table 1**).

Analysis on miR-126 expression and its diagnostic value for intracranial aneurysm

The serum miR-126 expression in patients in RG was obviously higher than that of subjects in CG. After treatment, the serum miR-126 of patients in RG declined, and the difference was statistically marked (P<0.05). The AUC of miR-126 in diagnosing intracranial aneurysm was 0.945, showing high diagnostic value (**Figure 1**).

Analysis on expression of serum inflammatory factors and its correlation with miR-126

The levels of serum TNF-α and IL-6 in RG were obviously higher than those of subjects in CG before operation (P<0.05). After operation, the levels of serum TNF-α and IL-6 in RG were obviously lower than those before operation (P>0.05). Correlation analysis revealed that the serum TNF-α and IL-6 levels in patients with intracranial aneurysm were positively correlated with miR-126 (P<0.05) (**Figure 2**).

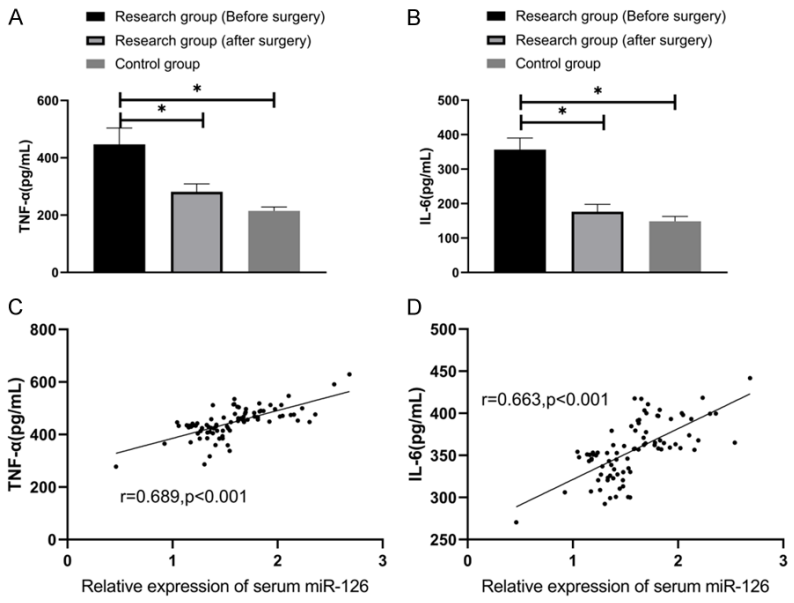

Figure 2. Analysis on expression of serum inflammatory factors and its correlation with miR-126 A: Expression of serum TNF-α; B: Expression of serum IL-6; C: Correlation analysis between serum miR-126 and TNF-α; D: Correlation analysis between serum miR-126 and IL-6. * indicates P<0.05.

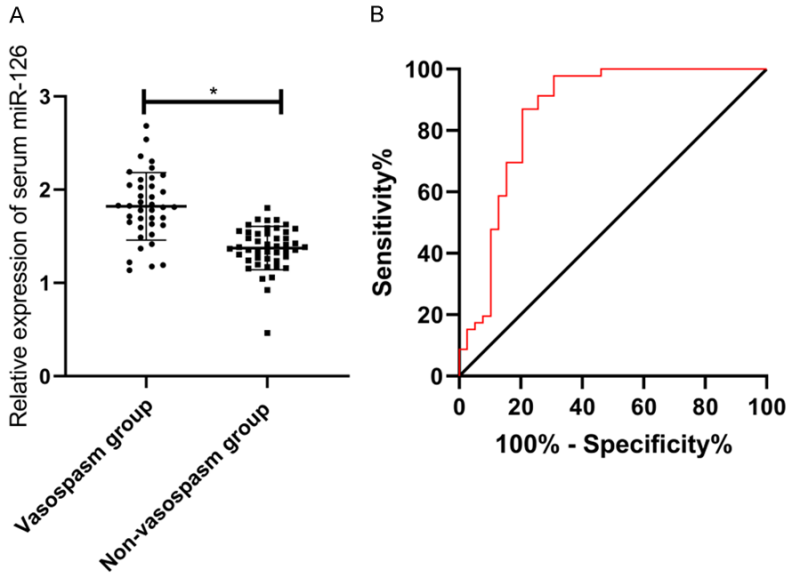

Figure 3. Expression and predictive value of serum miR-126 of patients with cerebral vasospasm after operation; A: Expression of serum miR-126 in patients with cerebral vasospasm after operation; B: ROC of miR-126 in predicting cerebral vasospasm after operation. * indicates P<0.05.

Expression and predictive value of serum miR-126 in patients with cerebral vasospasm after operation

According to whether cerebral vasospasm developed after operation, patients with intracranial aneurysm were divided into vasospasm group (39 cases) and non-vasospasm group (46 cases). Comparing the serum miR-126 of patients in both groups after operation, we found that the serum miR-126 of patients without vasospasm was obviously lower than that of patients with vasospasm. ROC analysis revealed that the ROC of miR-126 for predicting cerebral vasospasm after operation was 0.859, which had high predictive value (**Figure 3**).

Analysis on single factor influencing cerebral vasospasm in patients with intracranial aneurysm after operation

According to whether cerebral vasospasm developed after operation, patients were divided into vasospasm group (39 cases) and non-vasospasm group (46 cases). Univariate analysis manifested that preoperative bleeding frequency, history of hypertension, Hunt-Hess grade and miR-126 expression were related to postoperative cerebral vasospasm (P<0.05), while patients' age, BMI and sex had no significant correlation with postoperative cerebral vasospasm (P>0.05) (**Table 2**).

Analysis on multiple factors influencing cerebral vasospasm in patients with intracranial aneurysm after operation

We set preoperative bleeding frequency, history of hypertension, Hunt-Hess grade and miR-126 expression as independent variables and assigned values, and the postoperative cerebral vasospasm was applied as dependent variable for multivariate analysis by Logistic

Table 2. Analysis on single factor influencing cerebral vasospasm in patients with intracranial aneurysm after operation

Factors	Vasospasm group n=39	Non-vasospasm group n=46	X^2	P
Gender			0.024	0.878
Male (n=45)	21 (53.85)	24 (52.17)		
Female (n=40)	18 (46.15)	22 (47.83)		
Age (years old)			0.233	0.629
≥58 (n=46)	20 (51.28)	26 (56.52)		
<58 (n=39)	19 (48.72)	20 (43.78)		
BMI (kg/m^2)			0.014	0.906
≥23 (n=42)	19 (48.72)	23 (50.00)		
<23 (n=43)	20 (51.28)	23 (50.00)		
Preoperative bleeding frequency			21.73	<0.001
<2 (n=55)	15 (38.46)	40 (86.96)		
≥2 (n=30)	24 (61.54)	6 (13.04)		
Hunt-Hess grade			9.446	0.002
Grade 1-2 (n=56)	19 (48.72)	37 (80.43)		
Grade 3-4 (n=29)	20 (51.28)	9 (19.57)		
History of hypertension			4.438	<0.001
Yes (n=44)	10 (25.64)	34 (73.91)		
No (n=41)	29 (74.36)	12 (26.09)		

Note: BMI, body mass index.

Table 3. Multivariate analysis

Factor	β	S.E	Wald	HR	95% CI	P
Preoperative bleeding frequency	0.342	0.168	5.479	1.476	1.013-1.883	<0.05
History of hypertension	0.577	0.123	9.486	2.693	1.771-4.333	<0.05
Hunt-Hess grade	0.535	0.127	8.664	2.415	1.534-3.791	<0.05
miR-126	0.463	0.137	7.811	1.982	1.262-2.913	<0.05

regression analysis. It was shown that preoperative bleeding frequency, history of hypertension, Hunt-Hess grade and high expression of miR-126 were independent risk factors for cerebral vasospasm after operation in patients with intracranial aneurysm (**Table 3**).

Discussion

Intracranial aneurysm is mainly caused by the loss of elastic layer and muscular layer of artery, and the rupture of intracranial aneurysm is one of the most common causes of subarachnoid hemorrhage. Once the aneurysm ruptures, the life of patients will be threatened [11, 12]. For patients with intracranial aneurysm, timely diagnosis is most important, and surgical treatment is the best way to treat intracranial aneurysms. Although it has better efficacy, cerebral vasospasm is also a common serious complication after operation, which is not conducive to the prognosis of patients [13, 14]. Therefore, it is of great clinical significance to diagnose and treat patients with intracranial aneurysm in time and analyze the factors affecting the development of cerebral vasospasm.

Recently, more and more evidence has indicated that miRNA participates in atherosclerosis and vascular remodeling by regulating the functions of vascular endothelial cells, vascular smooth muscle cells and vessel extracellular matrix, and plays a vital role in the development and progression of aneurysms [15]. miR-126 gene is located on human chromosome 9 and mainly expressed in vascular endothelial cells. Mature miR-126 regulates the expres-

sion of vascular cell adhesion molecule 1 and affects the proliferation of vascular endothelial cells [16]. In this research, the expression of serum miR-126 in RG was obviously higher than that in CG, indicating that miR-126 was involved in the development and progression of intracranial aneurysm. Previous research [17] has pointed out that when intracranial aneurysms develop, the reactivation of the vasculature promotes the expression of miR-126 in the process of angiogenesis, and miR-126 can promote the expression of vascular endothelial growth factor and the angiogenesis by inhibiting the expression of budding-related proteins. In addition, we analyzed the expression of serum inflammatory factors and its correlation with miR-126 in patients with intracranial aneurysm. The results revealed that the expressions of serum TNF-α and IL-6 in patients were obviously higher than those in normal people, and the correlation analysis showed that the expression of miR-126 was positively correlated with TNF-α and IL-6, which suggested that the increased expression of miR-126 was also bound up with the inflammation in patients with intracranial aneurysm. Some studies [18] have pointed out that immune inflammation plays a vital role in the formation and rupture of intracranial aneurysms, in which inflammatory factors such as TNF-α and interleukin are highly expressed. Previous studies [19] have found that miR-126 can regulate inflammation by regulating AKT/Rac1 signaling pathway.

We also analyzed the relationship between miR-126 and postoperative cerebral vasospasm. Firstly, we compared the level of postoperative miR-126 between patients with cerebral vasospasm and those without cerebral vasospasm. The results revealed that the expression of serum miR-126 in patients with cerebral vasospasm was obviously higher than that in those without it, which suggested that the expression of miR-126 was related to the development of cerebral vasospasm after operation. Then, ROC curve analysis revealed that miR-126 had certain predictive value for the development of cerebral vasospasm after operation. Finally, we conducted a multi-factor analysis to further analyze the factors affecting the development of postoperative cerebral vasospasm. First of all, univariate analysis revealed that preoperative bleeding frequency, history of hypertension, Hunt-Hess grade and miR-126 expression were obviously correlated with postoperative cerebral vasospasm in patients with intracranial aneurysm. Multivariate analysis revealed that the preoperative bleeding frequency, history of hypertension, Hunt-Hess grade and high expression of miR-126 were independent risk factors for cerebral vasospasm after operation. Previous studies [20] have pointed out that vascular rupture can cause systemic stress response and vascular endothelial loss, and the more frequent the hemorrhage, the more severe the vascular endothelial loss, which is more likely to lead to the development of vasospasm. For patients with intracranial aneurysm who have a history of hypertension, long-term hypertension can cause varying degrees of atherosclerosis, which will also easily lead to vasospasm [21]. Hunt-Hess grade can predict patients' conditions and symptoms, and the severer the patients' condition, the more likely they are prone to suffer cerebral vasospasm [22]. However, high expression of miR-126 has rarely been counted as an independent risk factor for the development of postoperative cerebral vasospasm, the underlying specific mechanism needs to be further explored.

Although we analyzed the expression of miR-126 in patients with intracranial aneurysms, there are still some limitations in this research. First of all, we have not conducted a long-term follow up in patients, and the value of miR-126 in the prognosis of patients is still vague. Hence, we hope to improve our conclusions through long-term clinical follow up and further exploration of the potential mechanism of miR-126 in intracranial aneurysms through bioinformatics analysis.

To sum up, serum miR-126 is highly expressed in patients with intracranial aneurysm, showing certain predictive value for the diagnosis of intracranial aneurysm and the development of postoperative cerebral vasospasm. Hence, it may be used as a reference index for diagnosing and treating intracranial aneurysms.

Acknowledgements

Basic and Clinical Cooperative Research Promotion Program of Anhui Medical University, Project No. 2019xkjT021.

Disclosure of conflict of interest

None.

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