



The Role of Serum miR-497 on the Predictive Index of Early Diagnosis and Poor Prognosis of Atherosclerosis Cerebral Infarction

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Abstract

Background: Serum miR-497 can be used as a predictive index of the early diagnosis and poor prognosis of atherosclerosis cerebral infarction (ATCI).

Methods: Overall, 135 ATCI patients, treated in The Second Affiliated Hospital of Nantong University, Nantong 226001, P.R.China from Apr 2012 to Jan 2015, were included in ATCI group. Whereas, 77 patients with non-atherosclerosis cerebral infarction were put in the control group. RT-qPCR was performed for detecting serum miR-497 expression, whose relationship with the patients' clinicopathological parameters was analyzed. Receiver operating characteristic (ROC) curves were plotted to evaluate values of serum miR-497 for diagnosing ATCI patients and their 3-year and 5-year overall survival rates (OSRs). Cox regression analysis was conducted on prognostic factors of ATCI patients.

Results: miR-497 remarkably rose in the serum of ATCI patients, and was correlated with histories of hypertension, smoking and diabetes mellitus (DM). Its areas under curves (AUCs) for diagnosing these pathological parameters were 0.803, 0.817 and 0.819, respectively. Its expression was higher in the serum of the patients with recurrence and poor prognoses. Its AUCs for predicting the two conditions were 0.924 and 0.937, respectively. The 3- and 5-year OSRs of patients with low expression were remarkably higher than those of patients with high expression.

Conclusion: miR-497 and histories of hypertension, smoking and DM were independent prognostic factors affecting the 3-year OSR of ATCI patients. miR-497 expression rises in ATCI patients, so this miR is expected to become a serum diagnostic marker for ATCI.

Keywords: Serum microRNA-497; Atherosclerosis cerebral infarction; Prognosis; Predictive index

Introduction

Atherosclerosis cerebral infarction (ATCI) is a disease with a relatively high disability rate and mortality rate in clinical practice (1). Atherogenesis and thrombosis in the brain lead to the luminal stenosis or occlusion of cerebral vessels,

thus resulting in acute insufficiency of cerebral blood supply and the ischemic necrosis of the local brain tissue (2, 3). ATCI usually causes aphasia, hemiplegia and other symptoms of focal cerebral damage (4), but clinically therapeutic



schemes for it are few, because it is easy to have a relapse and results in complex adverse reactions of prognoses (5). Therefore, exploring biomarkers for the early diagnosis and prognosis of ATCI patients is of great value for improving their poor prognoses.

As non-coding single-stranded RNAs that are composed of 19-24 nucleotides, microRNAs (miRNAs) are involved in most biological processes, and their loss and disorders have a great effect on some diseases, such as cancer (6,7). They have certain clinical values for the diagnosis, prognosis and recurrence prediction of the diseases (8,9). For instance, serum miR-320b is a specific serum marker for carotid atherosclerosis cerebral infarction and vulnerable plaques (10). miR-29b and miR-424 are prognostic markers for patients with acute cerebral infarction (ACI) (11). In this study, we focused on serum miR-497. This miR is abnormally expressed in the serum of with hepatocellular carcinoma, and associated with the patients' invasive clinicopathological features and poor prognoses (12). Serum miR-497 rises in ACI patients, and it can be used as a prognostic marker, diagnostic marker and therapeutic target for ACI (13). All these indicate the potential of miR-497 to diagnose diseases and evaluate their prognoses.

Therefore, in this study, miR-497 expression in the serum of ATCI patients was detected, and the correlation of this miR with the patients' pathological features was analyzed, so as to discuss the clinical value of miR-497 for ATCI.

Materials and Methods

General data

Overall, 135 ATCI patients, treated in The Second Affiliated Hospital of Nantong University, Nantong 226001, P.R.China from April 2012 to January 2015, were included in ATCI group. Whereas, 77 patients with non-atherosclerosis cerebral infarction were put in the control group. Inclusion criteria: Patients confirmed with ATCI by CT or MRI (14); patients aged >18 years and with the initial attack of acute ATCI; patients

with the time of onset ≤ 72 hours; patients with complete general clinical data.

The included patients were informed of this study and signed a written informed consent form. The experimental processes were approved by the Ethics Committee of our hospital and in line with the Declaration of Helsinki.

Exclusion criteria: Those accompanied by cerebral hemorrhage or with infection and fever during hospitalization; those accompanied by renal or cardiac diseases; those complicated with severe organ dysfunction, malignant tumors or mental illness; those withdrawing from the experiment midway; those who did not cooperate in follow-ups and were lost to visits.

Sample collection

The serum (5 mL each) was collected from the patients in both groups, and centrifuged at 1500Xg (4°C, 10 min), to obtain the supernatant for carrying out follow-up experiments.

Detection of miR expression

TRIzol reagents (Simgen, Hangzhou, China, 5301100) were applied to the extraction of total RNA from the collected serum and tissues, with an UV spectrophotometer (Noted Scientific Instrument Co., Ltd, Hangzhou, China, 760 CRT) and agarose gel electrophoresis (Biolab Science and Technology Co., Ltd., Beijing, China, GL1177-VYF) used for detecting its purity, concentration and integrity. Reverse transcription kits (Kemin Biotechnology Co., Ltd., Shanghai, China, 205311) were applied to the reverse transcription of the total RNA, with the steps strictly carried out based on the manufacturer's instructions. After that, cDNA was collected for its amplification with miRNA Realtime qRT-PCR kits (Yu Bo Biotech Co., Ltd., Shanghai, China, YB131042-25) and ABI 7500 (Ai Biological Research, Shanghai, China, 4365463). The amplification system was mirVana 5X PCR Buffer (5 μ L), 50X ROX™ (0.5 μ L), cDNA (1 μ L), upstream and downstream primers (0.5 μ L each), and Nuclease-free Water that was finally added to make up to 20 μ L. The conditions were pre-

denaturation (95 °C, 3 min), denaturation (95°C, 15 sec), and annealing and extension (60 °C, 30 sec), which were cycled for 40 times. There were 3 same wells for each sample. Three repeated experiments were performed. In this study, U6 was

used as the internal reference and $2^{-\Delta\Delta ct}$ was applied to data analysis. (Primer sequences are shown in Table 1).

Table 1: Primer sequences

	<i>Forward</i>	<i>Reverse</i>
miR-497	5'-AGTCCAGTTTTCCAGGAATCCCT-3'	5'-ACCAGCAGCACACTGTGGTTTGT-3'
U6	5'-TCCGATCGTGAAGCGTTC-3'	5'-GTGCAGGGTCCGAGGT-3'

Follow-ups

Through telephones, WeChat and outpatient medical records, the patients were followed up for 5 years, once every 4 months, to record their survival status. Overall survival (OS) was the time from starting treatment to the patients' deaths or the end time of the last follow-up.

Statistical analysis

SPSS20.0 (Easy Bio System Inc., Beijing, China) was applied to statistical analysis. GraphPad Prism 6 (GraphPad Software, San Diego, USA) was used for plotting figures. Count data were expressed as the number of cases/percentage [n (%)], and compared between groups by a chi-square test. Measurement data were expressed as mean \pm standard deviation (mean \pm SD), and compared between groups by an independent samples t test, with the comparison before and after treatment conducted by a paired t test. Receiver operating characteristic (ROC) curves were plotted to evaluate values of serum miR-497 for diagnosing ATCI patients and predicting their recurrence and prognoses. The Kaplan-Meier method was used to plot survival curves of the patients' 3-year and 5-year OS, which was compared by a Log-rank test. Univariate and multivariate Cox regression analyses were conducted

on relevant prognostic factors. $P < 0.05$ indicated a statistically significant difference.

Results

Comparison of general information

The differences were not significant between the ATCI and control groups in gender, age, body mass index (BMI), history of hypertension, history of drinking, history of smoking, history of diabetes mellitus (DM), hyperlipidemia, cholesterol (Chol), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (Table 2).

miR-497 remarkably upregulated in serum of ATCI patients

For knowing whether miR-497 was imbalanced in ATCI patients, its expression in the patients' serum was detected. The expression in the ATCI group was remarkably higher than that in the control group ($P < 0.05$). The ROC curve of miR-497 for identifying ATCI was also plotted. Its area under the curve (AUC), sensitivity, specificity and optimal cut-off value were 0.868 (95%CI: 0.821-0.915), 80.00%, 81.82% and 2.15, respectively (Fig. 1).

Table 2: Comparison of general information [n(%), mean±SD]

Categories	ATCI group (n=135)	Control group (n=77)	t/ χ^2 value	P value
Gender			0.974	0.324
Male	83 (61.48)	42 (54.55)		
Female	52 (38.52)	35 (45.45)		
Age (Years)			0.129	0.719
≥53	79 (58.52)	47 (61.04)		
<53	56 (41.48)	30 (38.96)		
BMI (kg/m ²)			0.438	0.507
≥23	62 (45.93)	39 (50.65)		
<23	73 (54.07)	38 (49.35)		
History of hypertension			0.484	0.486
Yes	74 (54.81)	46 (59.74)		
No	61 (45.19)	31 (40.26)		
History of drinking			0.536	0.463
Yes	79 (58.52)	49 (63.64)		
No	56 (41.48)	28 (36.36)		
History of smoking			0.316	0.573
Yes	86 (63.70)	52 (67.53)		
No	49 (36.30)	25 (32.47)		
History of DM			0.775	0.378
Yes	74 (54.81)	47 (61.04)		
No	61 (45.19)	30 (38.96)		
Hyperlipemia			0.020	0.886
Yes	82 (60.74)	46 (59.74)		
No	53 (39.26)	31 (40.26)		
Chol (mmol/L)			1.575	0.117
	4.78±1.03	4.55±1.01		
LDL (mmol/L)			1.183	0.238
	3.02±0.94	3.18±0.96		
HDL (mmol/L)			1.857	0.064
	1.18±0.35	1.09±0.32		

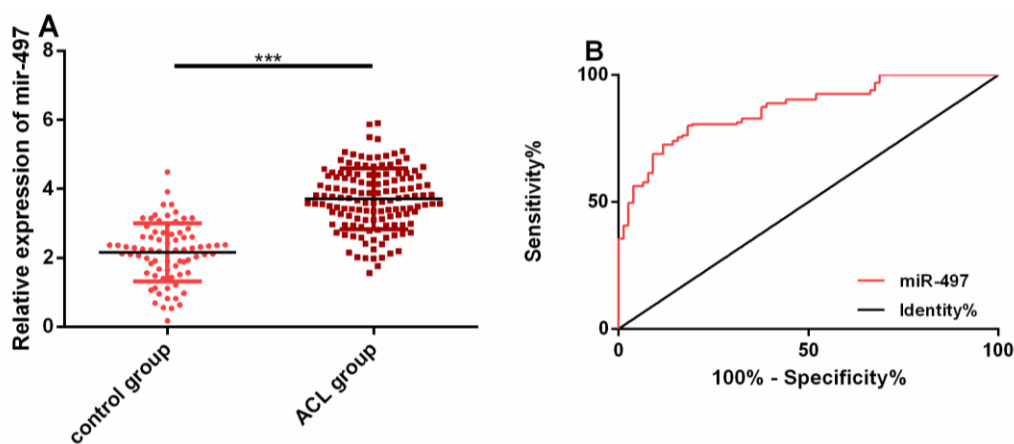


Fig. 1: miR-497 remarkably upregulated in serum of ATCI patients.

A: miR-497 expression in the serum of patients in both groups.

B: The ROC curve of miR-497 for identifying ATCI.

Note: *** indicates P<0.001 when there is a comparison between two groups

Correlation of miR-497 with pathological parameters

According to the observation of the correlation of miR-497 with ATCI patients' pathological parameters, this miR was correlated with histories

of hypertension, smoking and DM ($P < 0.001$). According to the plotted ROC curves, the AUCs of miR-497 for these pathological parameters were 0.803, 0.817 and 0.819, respectively. (Table 3, and 4 and Fig. 2).

Table 3: Correlation of miR-497 with pathological parameters (mean \pm SD)

Parameters	n	miR-497	t	P
Gender			1.448	0.149
Male	125	2.31 \pm 0.75		
Female	87	2.16 \pm 0.73		
Age (yr)			1.456	0.147
≥ 53	126	2.29 \pm 0.72		
< 53	86	2.14 \pm 0.76		
BMI (kg/m ²)			1.473	0.142
≥ 23	101	2.19 \pm 0.73		
< 23	111	2.34 \pm 0.75		
History of hypertension			5.351	< 0.001
Yes	120	2.76 \pm 0.79		
No	92	2.19 \pm 0.74		
History of drinking			1.866	0.063
Yes	128	2.36 \pm 0.79		
No	84	2.16 \pm 0.72		
History of smoking			4.289	< 0.001
Yes	138	2.73 \pm 0.81		
No	74	2.24 \pm 0.76		
History of DM			5.873	< 0.001
Yes	121	2.88 \pm 0.79		
No	91	2.25 \pm 0.75		
Hyperlipemia			1.515	0.131
Yes	128	2.34 \pm 0.74		
No	84	2.18 \pm 0.77		
Chol (mmol/L)			0.634	0.526
High expression	131	2.21 \pm 0.81		
Low expression	81	2.14 \pm 0.73		
LDL (mmol/L)			0.854	0.393
High expression	129	2.36 \pm 0.76		
Low expression	83	2.27 \pm 0.73		
HDL (mmol/L)			1.225	0.221
High expression	138	2.23 \pm 0.82		
Low expression	74	2.09 \pm 0.74		

Table 4: Diagnostic values of serum miR-497 for clinicopathological parameters

Pathological parameters	AUC	95%CI	S.E	Cut-off value	Sensitivity (%)	Specificity (%)
History of hypertension	0.804	0.744-0.863	0.030	> 2.476	75.83	79.35
History of smoking	0.816	0.756-0.878	0.031	> 2.224	71.74	78.38
History of DM	0.819	0.762-0.876	0.028	> 2.908	59.50	80.01

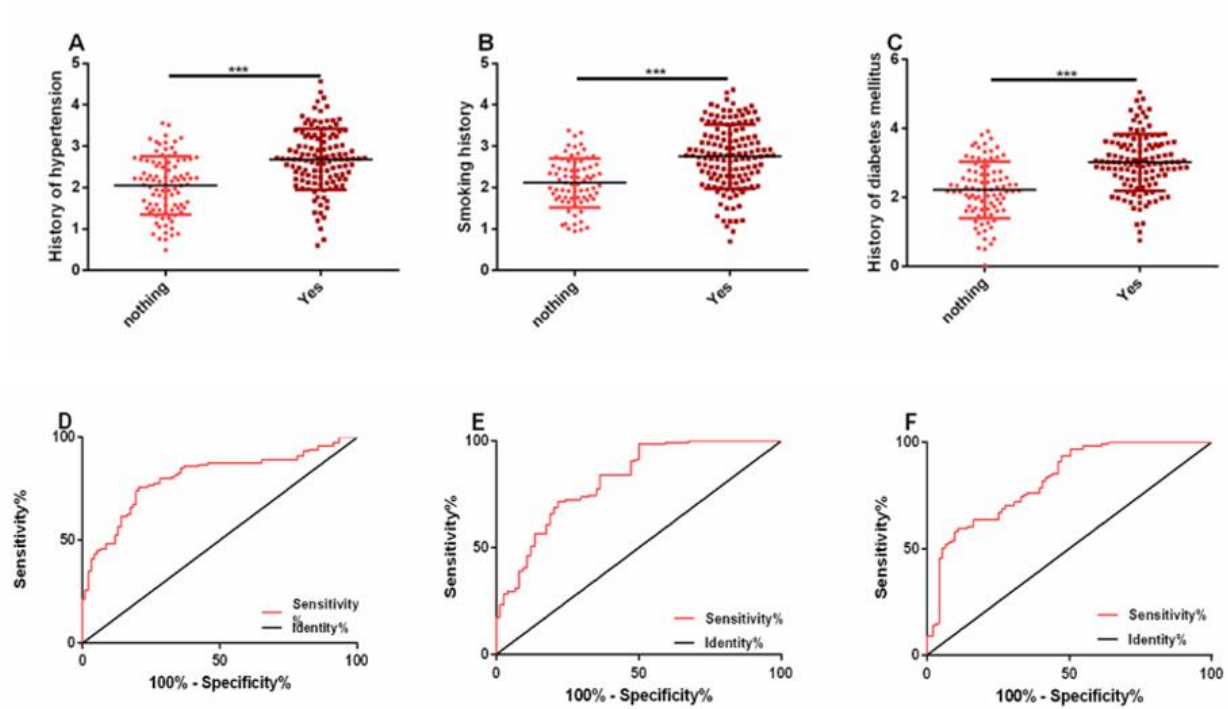


Fig. 2: Correlation of miR-497 with pathological parameters.

The correlation of miR-497 with histories of hypertension (A), smoking (B) and DM (C). The diagnostic values of miR-497 for histories of hypertension (D), smoking (E) and DM (F).

Note: *** indicates $P < 0.001$ when there is a comparison between two groups

miR-497 expression remarkably upregulated in ATCI patients with recurrence and poor prognoses

The predictive values of miR-497 for the recurrence and prognosis of ATCI patients were further explored. miR-497 expression was higher in the serum of the patients with recurrence and poor prognoses ($P < 0.001$). According to the

plotted ROC curves for predicting recurrence, the four ROC parameters of this miR were 0.924 (95%CI: 0.881-0.966), 86.87%, 88.89% and 2.694, respectively. According to the curves for predicting poor prognoses, the parameters were 0.937 (95%CI: 0.898-0.975), 89.53%, 87.76% and 2.911, respectively (Fig. 3, Table 5).

Table 5: ROC parameters of miR-497 for predicting recurrence and poor prognoses

Objectives	AUC	95%CI	Standard error	Cut-off value	Sensitivity (%)	Specificity (%)
Predicting recurrence	0.924	0.881-0.966	0.022	2.694	86.87	88.89
Predicting poor prognoses	0.937	0.898-0.975	0.019	2.911	89.53	87.76

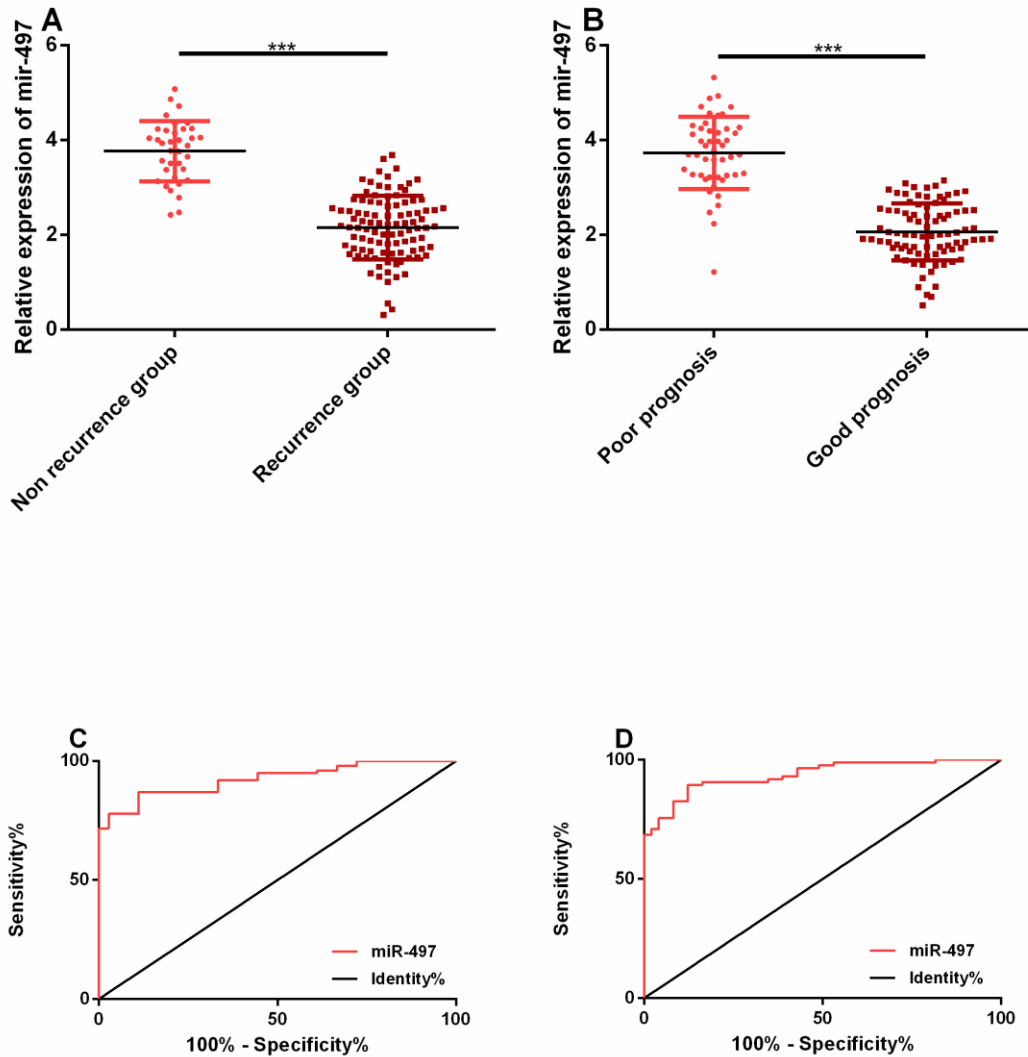


Fig. 3: miR-497 expression in and its predictive values for ATCI patients with recurrence and poor prognoses.

A: miR-497 remarkably upregulated in ATCI patients with recurrence.

B: miR-497 remarkably upregulated in ATCI patients with poor prognoses.

C: The ROC curve of miR-497 for predicting recurrence.

D: The ROC curve of miR-497 for predicting poor prognoses.

Note: *** indicates $P < 0.001$ when there is a comparison between two groups

Correlation of miR-497 with prognostic survival

All ATCI patients were followed up for 3 and 5 years. Their 3-year and 5-year overall survival rates (OSRs) were 73.33% (99/135) and 51.85% (70/135), respectively. With the median expression of serum miR-497 (3.643) as the threshold, the OSRs of patients with low expression were

remarkably higher than those of patients with high expression ($P=0.287$, $P=0.145$). According to the multivariate Cox regression, history of hypertension ($P=0.041$), history of smoking ($P=0.048$), history of DM ($P=0.036$) and miR-497 ($P=0.001$) were independent prognostic factors affecting the 3-year OSR of ATCI patients (Table 6 and 7 and Fig. 4).

Table 6: Assignment for COX regression analysis

<i>Factors</i>	<i>Variables</i>	<i>Assignment</i>
Gender	X1	Male = 1, female = 2
Age	X2	≥53 = 1, <53 = 2
BMI	X3	≥23 = 1, <23 = 2
History of hypertension	X4	Yes = 1, no = 2
History of drinking	X5	Yes = 1, no = 2
History of smoking	X6	Yes = 1, no = 2
History of DM	X7	Yes = 1, no = 2
Hyperlipemia	X8	Yes = 1, no = 2
Chol	X9	High expression = 1, low expression = 2
LDL	X10	High expression = 1, low expression = 2
HDL	X11	High expression = 1, low expression = 2
miR-497	X12	≥3.643 = 1, <3.643 = 2

Table 7: Univariate and multivariate Cox regression analyses on influencing factors of 3-year OSR

<i>Factors</i>	<i>Univariate</i>		<i>Multivariate</i>	
	HR (95CI%)	P	HR (95CI%)	P
Gender	1.053 (0.548-2.033)	0.137		
Age	1.105 (0.893-1.538)	0.112		
BMI	1.346 (0.378-2.196)	0.198		
History of hypertension	0.694 (0.347-1.384)	0.037	0.533 (0.266-1.066)	0.041
History of drinking	1.528 (1.078-3.973)	0.107		
History of smoking	0.619 (0.309-1.238)	0.039	0.604 (0.302-1.208)	0.048
History of DM	0.579 (1.289-1.158)	0.023	0.597 (0.298-1.194)	0.036
Hyperlipemia	1.793 (1.047-2.182)	0.162		
Chol	0.881 (0.783-1.893)	0.127		
LDL	1.973 (1.062-1.839)	0.182		
HDL	1.172 (0.625-1.983)	0.263		
miR-497	0.473 (0.237-0.946)	0.003	0.267 (0.133-0.534)	0.004

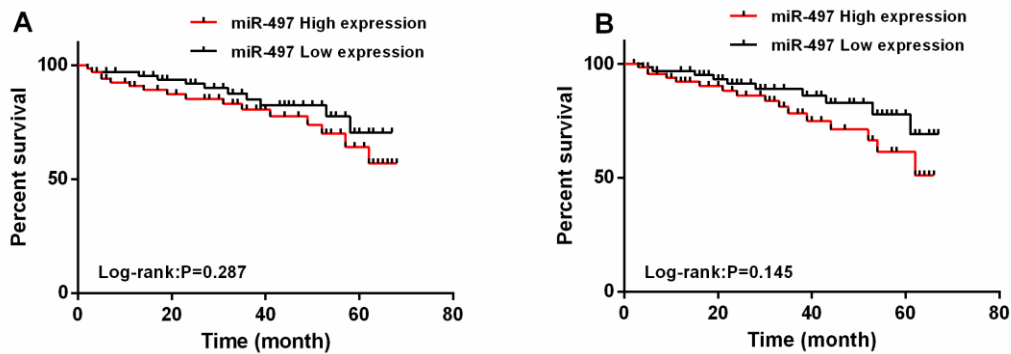


Fig. 4: Correlation of miR-497 with 3- and 5-year OS.

A: High miR-497 levels were associated with a lower 3-year OSR in ATCI patients.
 B: High miR-497 levels were associated with a lower 5-year OSR in ATCI patients

Discussion

As the pathogenic basis of many cardio-cerebrovascular diseases in clinical practice and a major incentive of ACI, atherosclerosis (AS) causes lipid accumulation in arterial vascular endothelium due to abnormal lipid metabolism in the body. As a result, the lipids form plaques, block arteries and produce lesions (15-17). As reported by clinical research, ATCI patients can suffer repeated attacks of ATCI, which aggravates their conditions and even endangers their life in serious cases (18). Therefore, improving the prognosis of patients is particularly important to study influencing factors of ATCI prognosis.

miRNAs are expressed in many malignant tumors, which include ATCI. miR-497 has a great effect on various tumors and ischemic diseases. According to Chen X and other researchers, it is highly expressed in the serum of patients with ischemic stroke, and plays a pivotal role in the pathogenesis of nervous system diseases (19). According to Yin KJ et al., its functional incapacitation leads to neuronal damage after focal cerebral ischemia, so knocking down it can reduce ischemic cerebral infarction and improve neurological functions (20). In our study, miR-497 had remarkably high expression in the serum of ATCI patients, which indicates that it may be involved in the pathological process of the disease. In the report of Wang YY et al., this miR is highly expressed in the serum of AS patients (21), which is similar to our research results. After further analyzing the diagnostic value of miR-497 for ATCI patients, we found that its AUC for distinguishing the patients was 0.868, which suggests that this miR may be a potential target for diagnosing and treating the disease. According to the analysis of its correlation with the patients' pathological parameters, this miR was correlated with histories of hypertension, smoking and DM. As reported by Luo L and other researchers, history of hypertension, history of smoking and history of DM have relatively high diagnostic values for ATCI (22), which is similar to our findings.

According to the ROC curves, miR-497 also had relatively high diagnostic values.

As reported by a previous study, both untimely treatment and various factors after treatment cause reinfarction in ATCI patients, thus increasing disability and mortality rates (23). In our study, the recurrence rate of 135 patients was 26.67%, similar to 25.12% from Yu LH and others (24). The 3- and 5-year OSRs of ATCI patients were 73.33% and 51.85%, respectively; the AUCs of miR-497 for predicting the recurrence and prognosis of ATCI were 0.924 and 0.937, respectively. These findings reveal that miR-497 has multiple predictive performance for ATCI, and that it can be used as a potential marker for predicting the recurrence and prognosis of the disease. In the research of Cui J et al., miR-497 upregulation is helpful to prompt the pathological condition of advanced AS, so this miR can be a potential therapeutic target for AS (25), which is similar to our results. According to the analysis of survival and prognostic factors, high miR-497 levels (>3.643) were correlated with lower 3- and 5-year OSRs and poor prognoses. According to results of Cox regression analyses, miR-497 and histories of hypertension, smoking and DM were independent prognostic factors for the poor prognosis of ATCI patients; the patients with high miR-497 (>3.643) and these histories had an increased risk of poor prognoses.

Our research has confirmed that miR-497 as a serological index has a relatively high value for the diagnosis and prognosis of ATCI patients. However, there are still some deficiencies. Firstly, we can supplement the analysis on the predictive value of this miR for efficacy in the patients. Secondly, we can analyze the risk factors that affect the curative effect on or the recurrence of ATCI. Additionally, we can focus on the molecular mechanism of this miR in ATCI or its influences on cell biological functions, so as to carry out basic research for exploring potential treatments for the disease. Therefore, this research will be gradually improved based on the above aspects.

Conclusion

miR-497 expression rises in ATCI patients, so this miR is expected to become a serum diagnostic marker for ATCI.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interest.

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