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Short Communication

Clinical Evaluation of miR-155 and miR-21 Alone and in Combination for Diagnosis of Acute Progressive Cerebral Infarction

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LZ, HY and YW conducted the experiments in this study. JB, CB and YD contributed to the design and interpretation of the current study and wrote the article. All authors read, revised, and approved the final manuscript.

Key words
miR-155, miR-21, Detection, Diagnosis, Acute progressive cerebral infarction, Clinical

ABSTRACT

The objective of this study was to clinical evaluate miR-155 and miR-21 alone and in combination for the diagnosis of acute progressive cerebral infarction (APCI). One hundred twenty-nine patients with acute cerebral infarction (ACI) were collected as observation objects and divided into an APCI group and no progression group according to the progress of the patients' cerebral infarction, and 40 healthy people were selected as a control group. The levels of miR-155 and miR-21 in all subjects were determined by qRT-PCR. The miR-155 and miR-21serum levels in the APCI group and the progression-free group were higher than in control group, meanwhile those in the APCI group were higher than progression-free group (P<0.05). MLR analysis showed that miR-155 and miR-21 were influencing factors in APCI patients (P<0.05). ROC curve analysis showed that the AUC of miR-155 for APCI diagnosis was 0.869, the sensitivity was 86.13%, and the specificity was 81.75%.The AUC of miR-21 for APCI diagnosis was 0.815, the sensitivity was 83.49%, and the specificity was 78.43%; The AUC of the two combined for APCI diagnosis was 0.925, the sensitivity was 92.10%, and the specificity was 93.71%. It was concluded that the expression of miR-155 and miR-21 in the serum of APCI patients is significantly high, which are influencing factors in APCI patients. The combination of the two can be widely used in clinical practice.

Acute cerebral infarction (ACI) is mainly due to atherosclerosis and thrombosis in the arteries supplying blood to the brain, and blood supply obstacles in the local brain tissue area leading to ischemic hypoxic lesions and necrosis of brain tissue, which in turn produces corresponding neurological deficits clinically (Yang *et al*., 2022; Bong *et al*., 2017; Wang *et al*., 2021). Acute progressive cerebral infarction (APCI) is a severe clinical subtype of ACI, accounting for about 26%-43% of all cerebral infarctions (Aoyama *et al*., 2019; Yao *et al*., 2022). After the onset of APCI, the symptoms of localized cerebral ischemia and neurological deficits gradually progress and become aggravated in a stepwise manner (Yao *et al*., 2022). The narrow vascular cavity slows down or even stops blood flow, further aggravates the stenosis of the lumen, or even occlusion, and eventually leads to the gradual expansion of the infarction with the clinical manifestations of progressive deterioration of neurological function (Ueno *et al*., 2018). Therefore, early diagnosis and targeted treatment of APCI for improving the treatment effect and the quality of life of patients is important.

MicroRNA (miRNA) has temporal and spatial specificity during body development and is involved in many physiological processes (Aizawa *et al*., 2018; Russi *et al*., 2019). miR-155 is a member of the miRNA family, which not only promotes the formation of atherosclerosis, but also leads to a significant increase in cerebral infarction volume and apoptotic cells (Dong *et al*., 2016). The expression of miR-21 in the serum of patients with ACI is significantly up-regulated, which is related to the transformation of smooth muscle cells and the regulation of atherosclerosis (Gu *et al*., 2020). The present study was conducted on patients with ACI to analyze the clinical value of miR-155 and miR-21 alone and in combination in

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the diagnosis of APCI.

Materials and methods

The 129 patients (18-80 years) with ACI who were treated in our hospital from January 2019 to January 2020 were used as observation objects.They met the diagnostic criteria of ACI in the Chinese Guidelines for the Diagnosis and Treatment of Ischemic Stroke (Dong, 2016) and atherosclerosis confirmed by head MRA or CTA. The patients were divided into APCI group and non-progressive group. The diagnostic criteria of APCI were (i) Progressive worsening of neurological deficits within 1 week of disease onset in patients, (ii) with ≥ 1-point increase in National Institutes of Health Stroke Scale (NIHSS) score.

The patient with (i) concomitant impaired consciousness, (ii) transient ischaemic attack or haemorrhagic stroke, (iii) recently received arteriovenous thrombolysis, (iv) severe dysfunction of important organs, including the heart, liver, and kidney, (v) with syphilis or autoimmune disease, (vi) and those who declined this experiment or discontinued this experiment for other reasons were excluded. The control group comprised subjects of 18-80 years with no specific past medical history such as hypertension, coronary heart disease, diabetes and atrial fibrillation.

The blood samples were analyzed by an automated blood cell analyzer for WBC, and high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglycerides (TG) .

Collect 4ml of morning fasting venous blood from all patients with cerebral obstruction within 24 hours of admission. Collect 4ml of morning fasting venous blood from healthy individuals during physical examination, centrifuge at 3000r/min for 10 minutes, and collect serum. Use Trizol extraction kit (Biyuntian Co., Ltd., Shanghai, China; product number: R0017S) to extract total RNA from each group. The operation process strictly follows the operating instructions; Adopting BeyoRT ™ The III cDNA synthesis kit (Biyuntian Co., Ltd., Shanghai, China; product number: D7185M) was used for reverse transcription. The SYBR Green Master Mix kit (Dalian TaKaRa Co., Ltd., Dalian, China; product number: 3735A) was used for real-time quantitative PCR analysis of mRNA. The cDNA synthesis reaction conditions were: 50 minutes at 42℃, incubation at 95℃ for 5 min, inactivation of the reverse transcriptase, and PCR amplification of the prepared cDNA after the reaction was completed. Using GAPDH as the internal reference gene, the Ct values of each target gene were obtained. The RT-PCR reaction conditions were 95℃ and pre denaturation for 10 min, 95℃ for 15 sec, 60℃ for 45 sec, and 72℃ for 2 min, followed by 40 cycles and 4℃ storage. The data was analyzed using the $2^{-\triangle\triangle}$CT formula.

The data were analyzed by SPSS20.0 software. All measurement data comparison was expressed as ($\bar{x}\pm s$), Comparisons among multiple groups were performed by one-way ANOVA test, and the LSD-t method was used for pairwise comparisons; the count data were all represented by percentage, and the comparison between groups was by χ^2 test. Multivariate logistics regression was used to analyze the influencing factors of APCI patients, and the ROC curve was used to analyze the value of miR-155 and miR-21 in the diagnosis of APCI. The statistical result was considered as statistically significant at $P<0.05$ and an indicated that compared with the control group, $^aP<0.05$, and b indicated that compared with the no progressive group, $^bP<0.05$.

Results

Table I shows sense and ntisense primer sequences for miR-155, miR-21, and GAPDH.

Table II indicates descriptive data for miR-155 and miR-21 levels of subjects in each group. The serum levels of miR-155 and miR-21 in the APCI and no progression groups were higher than control group, and those in the APCI group were higher than no progression group (P<0.05). According to the analysis of influencing factors of APCI patients in Table III, MLR analysis showed that miR-155 and miR-21 were both dangerous factors of APCI patients ($P<0.05$).

According to the cutoff value analysis of miR-155 and miR-21 in the diagnosis of APCI in Table IV. ROC curve analysis showed that the AUC of miR-155 for APCI diagnosis was 0.869, the sensitivity was 86.13%, and the specificity was 81.75%. The AUC of miR-21 for APCI diagnosis was 0.815, the sensitivity was 83.49%, and the specificity was 78.43%. The AUC of the two combined

Table I. Primer sequences for miR-155, miR-21, and GAPDH.

Prime	Sense	**Antisense**
miR-155	5’-ACACTCCAGCTGGCTTAATGCTAATCCTGATA-3’	5’-TAATGCTAATCGTGATAGGGG-3’
miR-21	5’-GGGTAGCTTATCAGACTGATGTT-3’	5’-CAGTGCAGGGTCCGAGGT-3’
GAPDH	5’-CGAGCCACATCGCTCAGACA-3’	5’-GTGGTGAAGACGCCAGTGGA--3’

for APCI diagnosis was 0.925, the sensitivity was 92.10%, and the specificity was 93.71%.

Table II. miR-155 and miR-21 levels of subjects in each group using qRT-PCR (x±s).

Indicators	APCI group (n=68)	No progression group (n=61)	Control group (n=40)	F	P value
miR-155	0.18±0.09[ab]	0.08±0.04[a]	0.03±0.01	83.14	<0.001
miR-21	0.10±0.03[ab]	0.07±0.02[a]	0.04±0.01	87.54	<0.001

Table III. Analysis of influencing factors of APCI patients.

Variable	β	Wald χ^2 value	P value	OR	95%CI
miR-155	1.854	0.394	0.001	3.306	1.045-10.658
miR-21	1.573	0.367	0.014	1.772	1.346-16.342

OR, odds ratio.

Table IV. Analysis of the value of miR-155 and miR-21 in the diagnosis of APCI.

Variable	AUC	95%CI	Sensitivity	Specificity
miR-155	0.869	0.812-0.912	86.13%	81.75%
miR-21	0.815	0.764-0.869	83.49%	78.43%
Combined detect	0.925	0.874-0.981	92.10%	93.71%

AUC, area under the curve.

Discussion

ACI is a multi-morbidity and common disease that endangers the public health, second only to heart disease and tumor (Hiraoka *et al*., 2017). APCI is a kind of refractory cerebrovascular disease. Failure to treat in time or improper treatment can lead to irreversible neurological damage. More patients have severe disability and brain herniation, which has higher disability rate and fatality rate (Sato *et al*., 2018). Therefore, analysis of the pathogenesis of APCI, early diagnosis and screening of APCI are important for prolonging the patient's life cycle and quality. The clinical diagnosis of APCI mainly relies on the changes in the degree of the patient's nerve defect from mild to severe (Li and Zhou, 2016). The changes in some serum indexes of APCI patients have special significance for the assessment of brain nerve damage, which help to early diagnosis and early treatment.

miRNAs are involved in cell growth and development, organ formation, cell carcinogenesis and apparent regulation. The over expression or down-regulation of miRNA can reflect various pathological processes. miRNA can exist in a variety of body fluids stably, such as plasma, cerebrospinal fluid, urine, etc. (Pan *et al*., 2019). Not only is miRNA highly conservative in evolution, but can maintain a complete nucleotide structure in extreme environments such as abundant RNase and repeated freezing and thawing, and has a long half-life. It can be accurately detected by qRT-PCR in clinical practice and is expected to become an ideal biological markers and have great application prospects in diagnosing, monitoring diseases and judging prognosis.

As a kind of oncogenic miRNA, miR-155 participates in cell growth and development, differentiation, inflammation, and metabolism in cancer. The level of miR-155 in patients with ACI is higher than healthy people, which may become a new relevant biomarker for predicting acute cerebral infarction (Takeuchi *et al*., 2020). miR-21 is a multifunctional miRNA that is widely found in vascular endothelial cells and smooth muscle cells. The level of miR-21 in the serum of APCI patients is significantly increased, which may provide corresponding test support in the diagnosis of APCI (Zarch *et al*., 2020). In our study, the serum levels of miR-155 and miR-21 in the APCI group and the no-progressive group were higher than control group, and those in the APCI group were higher no progressive group (P<0.05). It is suggested that the abnormal expression of miR-155 and miR-21 in the serum of APCI patients may provide support for the diagnosis of APCI.

Next, we found that miR-155 and miR-21 were influential factors affecting APCI patients through MLR analysis (P<0.05). ROC curve analysis showed that the AUC of miR-155 for APCI diagnosis was 0.869, the sensitivity was 86.13%, and the specificity was 81.75%; the AUC of miR-21 for APCI diagnosis was 0.815, the sensitivity was 83.49%, and the specificity was 78.43%; The AUC of the two combined for APCI diagnosis was 0.925, the sensitivity was 92.10%, and the specificity was 93.71%, which is suggested combined detection has a better predictive value in the diagnosis of APCI than a single index detection, which helps to diagnose APCI early and take corresponding measures and has extremely significance for effective treatment of patients.

Conclusion

MiR-155 and miR-21 are highly expressed in the serum of APCI patients, both of which are influencing factors in APCI patients. The two have a certain value in the diagnosis of APCI. The combined of the two has a higher value and can be used in widely used clinically.

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IRB approval

This study was approved by the Advanced Studies Research Board of 3201 Hospital, Hanzhong 723000, Shaanxi Province, China. All participants were duly informed and were required to sign the consent form.

Ethical approval

The study was carried out in compliance with guidelines issued by Ethical Review Board Committee of 3201 Hospital, China. The official letter would be available on fair request to corresponding author.

Statement of conflict of interest

The authors have declared no conflict of interest.

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