



Diagnostic Values of miR-221-3p in Serum and Cerebrospinal Fluid for Post-Stroke Depression and Analysis of Risk Factors

Yan Cui¹, Guodi Ma², Fanying Kong¹, *Lingli Song³

1. The Second Affiliated Hospital of Harbin Medical University, School of Nursing, Harbin 150001, P.R.China
2. Cardiology ICU, The Second Affiliated Hospital of Harbin Medical University, Harbin 150001, P.R.China
3. Department of Neurosurgery, The Second Affiliated Hospital of Harbin Medical University, Harbin 150001, P.R.China

*Corresponding Author: Email: skis4518@163.com

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Abstract

Background: We aimed to explore the diagnostic values of miR-221-3p in serum and cerebrospinal fluid (CSF) for post-stroke depression (PSD) and to analyze the risk factors of the disease.

Methods: Admitted to the Second Affiliated Hospital of Harbin Medical University, Harbin, China from May 2013 to May 2020, 136 stroke patients were enrolled, among which 76 PSD patients were taken as a PSD group and 60 non-depressed patients were taken as a Non-PSD group. miR-221-3p expression in serum and CSF and concentrations of inflammatory cytokines (IL-6, TNF- α) in serum were detected, to analyze the diagnostic and prognostic values of the indicators for PSD. Correlations of miR-221-3p in serum with that in CSF, with the National Institute of Health Stroke Scale (NIHSS) score and the Hamilton Depression Rating Scale (HAMD) score, and with inflammatory cytokines were analyzed, so as to analyze the risk factors affecting the occurrence of PSD.

Results: Compared with the Non-PSD group, miR-221-3p remarkably upregulated in serum and CSF in the PSD group, and its areas under the curves (AUCs) for PSD identification were 0.900 and 0.925, respectively. According to the correlation analysis, miR-221-3p in serum was remarkably positively correlated with that in CSF, NIHSS score, HAMD score, IL-6 and TNF- α . In addition, a history of mental illness, NIHSS score, HAMD score, IL-6, TNF- α and miR-221-3p were risk factors of PSD.

Conclusion: miR-221-3p in serum and CSF can be used as the diagnostic and risk warning indicators of PSD.

Keywords: Post-stroke depression; miR-221-3p; Risk factors; Inflammatory cytokines

Introduction

Stroke is an age-related nervous system disease that may cause permanent brain injury due to insufficient blood supply to the brain (1,2). As a common complication of stroke patients, post-stroke depression (PSD) is a subtype of depression and may increase the risks of disability and even death (3). Both serum and cerebrospinal

fluid (CSF) can reflect the pathological changes of PSD. Although the latter reflects the molecular changes more, the detection of the former is more convenient (4). Therefore, analyzing indices for molecular screening and risk factors of PSD based on serum and CSF is of great significance for diagnosing, treating and preventing PSD.



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miRNAs are a small non-coding RNA molecular regulator for post-transcriptional levels, exerting a decisive function in many pathophysiological processes (5). At present, miRNAs in serum have different degrees of correlations with post-stroke complications (such as post-stroke cognitive impairment and PSD). For example, miR-132 up-regulates in the serum of patients with post-stroke cognitive impairment, and has a certain correlation with the severity of cognitive impairment. Highly expressed in early PSD patients, serum miR-92a-3p is related to white matter damage and the early occurrence of PSD (6,7). miR-221-3p as a member of miRNAs is also closely associated with stroke, found to abnormally upregulate in the CSF of patients with acute ischemic stroke (8). In addition, its abnormally high expression has also been reported in patients with advanced PSD, suggesting that this miR may be involved in the pathological mechanism of PSD (9). However, the diagnostic value of miR-221-3p as a serum indicator for PSD has been rarely reported. According to previous studies, inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), are also related to the progression of PSD. They may mediate inflammatory pathways of the body and be regulated by molecular networks of miRNAs, thereby regulating the severity of the disease (10-12).

In this study, the diagnostic values of miR-221-3p in serum and CSF for PSD, the correlation of serum miR-221-3p with inflammatory cytokines, and the risk factors of the disease were analyzed, in order to provide clinical reference for diagnosing and preventing PSD.

Materials and Methods

General information

Admitted to the Second Affiliated Hospital of Harbin Medical University, Harbin, China from May 2013 to May 2020, 136 stroke patients were enrolled, among which 76 PSD patients were taken as the PSD group and 60 non-depressed patients were taken as the Non-PSD group.

Those in the PSD group consisted of 48 males and 28 females, with an average age of (46.02 ± 10.13) years. Those in the Non-PSD group consisted of 30 males and 30 females, with an average age of (44.96 ± 9.25) years.

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University, Harbin, China. We provided the written informed consent form for the patients or their guardians. The patients were informed and signed the informed consent form, voluntarily involved in this study.

Inclusion and exclusion criteria

Inclusion criteria: Patients initially confirmed with stroke (13); patients who had not taking hormone drugs, anti-inflammatory drugs and other drugs in the past 3 months; patients without cognitive impairment or communication disorders.

Exclusion criteria: Those complicated with other brain diseases, malignant tumors or infectious diseases; those previously with mental diseases; those with incomplete pathological data.

Detection methods

Elbow venous blood (5 mL) was drawn from all subjects on an empty stomach in the morning, placed in vacuum blood collection tubes without anticoagulants, and centrifuged at $15000 \times g$ (4°C , 10 min), so as to separate the upper serum, which was stored in a refrigerator (-70°C) for later use. RT-qPCR was performed for detecting the relative expression of serum miR-211-3p. From the serum, total RNA was extracted using Trizol reagents (Simgen, Hangzhou, China, 5301100), with its concentration and purity detected by a spectrophotometer (Hangzhou Bigfish Bio-tech Co., Ltd., Hangzhou, China, BFMUV-2000). cDNA was synthesized using reverse transcription kits (Shanghai Zeye Biological Technology Co., Ltd., Shanghai, China, ZY1011R). U6 was the internal reference gene of miR-211-3p. Primer sequences were designed and synthesized by Shanghai Daixuan Biotechnology Co., Ltd. A real-time fluorescence quantitative PCR instrument (Beijing

Image Trading Co., Ltd., Beijing, China, 100133) was used for PCR amplification. $2^{-\Delta CT}$ was used to represent the relative expression of miR-211-3p.

Enzyme-linked immunosorbent assay (ELISA) (14) was performed for detecting IL-6 and TNF- α in the serum, with the steps strictly carried out in accordance with the instructions of IL-6 and TNF- α ELISA kits (Shanghai Yansheng Industrial Co., Ltd., Shanghai, China, YS-ELISA3732, YS-ELISA1826).

Statistical analysis

SPSS22.0 (Easy Bio System Inc., Beijing, China) was used for statistical analysis. Count data were expressed by the number of cases/percentage (n/%), compared between groups by chi-square test. When the theoretical frequency in the chi-square test was less than 5, the comparison was conducted by chi-square test with correction for continuity. Measurement data were expressed by mean \pm SEM, and independent samples t test was used for the comparison of the data between groups. Receiver operating characteristic (ROC) curves were plotted to analyze the value of miR-211-3p for PSD identification. Pearson Correlation Coefficient was used to conduct correlation analysis on miR-211-3p. Multivariate Logistic re-

gression was performed to analyze the risk factors affecting PSD. $P < 0.05$ indicated a statistically significant difference.

Results

Baseline data

There were significant differences between the PSD and Non-PSD groups in a history of mental illness, the National Institute of Health Stroke Scale (NIHSS) score (15) and the Hamilton Depression Rating Scale (HAMD) score (16) ($P < 0.05$), not in gender, age, average age, body mass index (BMI), history of drinking, history of smoking, marital status, educational background and stroke location. The NIHSS score was 0-42 points, proportional to the severity of neurological impairment. The HAMD score was 0-64 points, proportional to the severity of depression (Table 1).

miR-221-3p upregulated in PSD

To investigate whether miR-221-3p has abnormal imbalance in PSD, we detected its expression in PSD patients. Compared with the Non-PSD group, this miR remarkably upregulated in serum and CSF in the PSD group ($P < 0.001$) (Fig. 1).



Fig. 1: miR-221-3p expression in serum and CSF of PSD patients

A. miR-221-3p expression in serum was remarkably higher in the PSD group than that in the Non-PSD group.

B. miR-221-3p expression in CSF was remarkably higher in the PSD group than that in the Non-PSD group.

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

Table 1: Baseline data [n(%), mean±SD]

<i>Factors</i>	<i>n</i>	<i>PSD group(n=76)</i>	<i>Non-PSD group(n=60)</i>	χ^2/t	<i>P</i>
Gender				2.373	0.123
Male	78	48 (63.16)	30 (50.00)		
Female	58	28 (36.84)	30 (50.00)		
Age (yr)				0.477	0.490
<45	68	40 (52.63)	28 (46.67)		
≥45	68	36 (47.37)	32 (53.33)		
Average age (yr)	136	46.02±10.13	44.96±9.25	0.629	0.530
BMI	136	22.25±2.84	22.57±2.50	0.687	0.493
History of drinking				1.821	0.177
No	82	42 (55.26)	40 (66.67)		
Yes	54	34 (44.74)	20 (33.33)		
History of smoking				2.000	0.157
No	91	47 (61.84)	44 (73.33)		
Yes	45	29 (38.16)	16 (26.67)		
A history of mental illness				9.819	0.002
No	97	46 (60.53)	51 (85.00)		
Yes	39	30 (39.47)	9 (15.00)		
Marital status				3.000	0.083
Married	77	48 (63.16)	29 (48.33)		
Unmarried / widowed / divorced	59	28 (36.84)	31 (51.67)		
Educational background				2.252	0.133
Above senior high school	55	35 (46.05)	20 (33.33)		
Below senior high school	81	41 (53.95)	40 (66.67)		
Stroke location				1.729	0.421
Left hemisphere	56	33 (43.42)	23 (60.00)		
Right hemisphere	61	35 (46.05)	26 (40.00)		
Bilateral hemisphere	19	8 (10.53)	11 (54.00)		
NIHSS score	136	4.84±2.30	13.48±4.29	15.041	<0.001
HAMD score	136	6.32±2.03	16.62±4.63	17.403	<0.001

miR-221-3p had high diagnostic performance for PSD identification

ROC curves of miR-221-3p were plotted to identify PSD. The area under the curve (AUC) of miR-221-3p in serum for PSD identification was 0.900 (95% CI: 0.850-0.950), the best cut-off val-

ue was 3.21, and the sensitivity and specificity were 78.95% and 91.67%, respectively. The parameters of miR-221-3p in CSF were 0.925 (95% CI: 0.884-0.966), 2.96, 81.58% and 90.00%, respectively (Fig. 2, Table 2).

Table 2: ROC parameters of miR-221-3p for PSD identification

<i>Sources</i>	<i>AUC</i>	<i>95%CI</i>	<i>Standard error</i>	<i>Cut-off value</i>	<i>Sensitivity (%)</i>	<i>Specificity (%)</i>
Serum	0.900	0.850-0.950	0.026	3.21	78.95	91.67
CSF	0.925	0.884-0.966	0.021	2.96	81.58	90.00

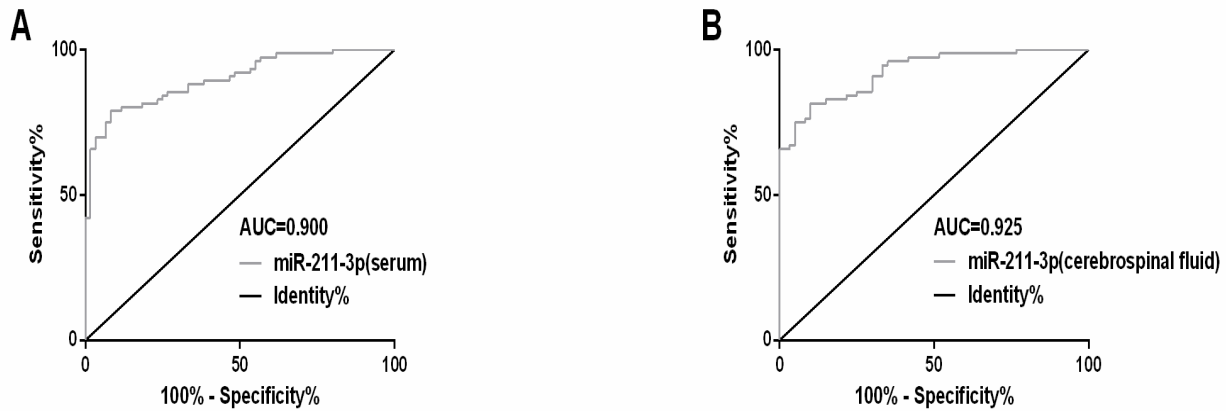


Fig. 2: ROC curves of miR-221-3p for PSD identification

- A. The AUC of miR-221-3p in serum for PSD identification was 0.900, and the best cut-off value was 3.21.
- B. The AUC of miR-221-3p in CSF for PSD identification was 0.925, and the best cut-off value was 2.96

miR-221-3p in serum was positively correlated with that in CSF, NIHSS score and HAMD score

For exploring the correlations of miR-221-3p in serum with that in CSF, NIHSS score and HAMD score, we conducted correlation analysis.

According to the analysis, miR-221-3p expression in serum was remarkably positively correlated with that in CSF ($r=0.637, P<0.001$), NIHSS score and HAMD score ($r=0.620, P<0.001$; $r=0.608, P<0.001$) (Fig. 3).

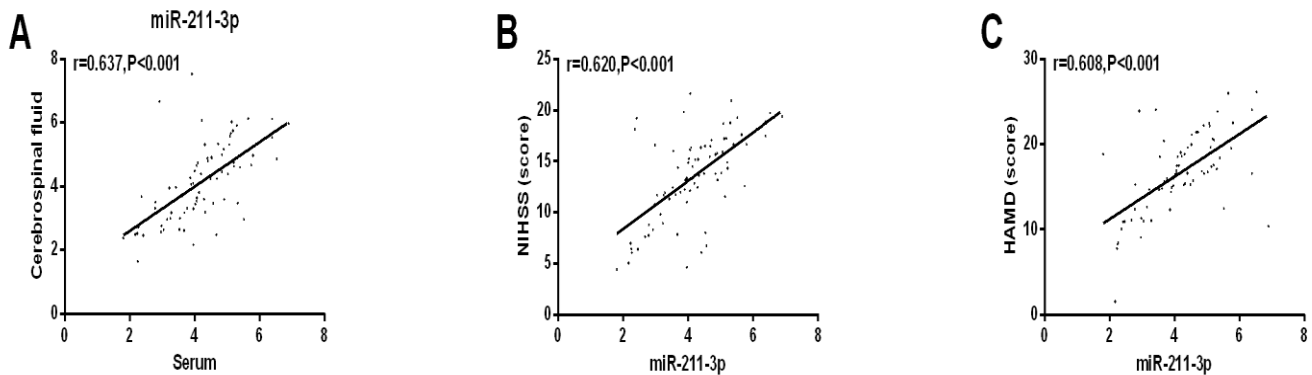


Fig. 3: Correlation analysis of miR-221-3p

- A. miR-221-3p expression in serum was positively correlated with that in CSF ($r=0.637, P<0.001$).
- B. serum miR-221-3p was positively correlated with the NIHSS score of PSD patients ($r=0.620, P<0.001$).
- C. serum miR-221-3p was positively correlated with the HAMD score of PSD patients ($r=0.608, P<0.001$)

Serum miR-221-3p was positively correlated with inflammatory cytokines

For exploring the correlations of serum miR-221-3p with inflammatory cytokines, we analyzed its

correlations with IL-6 and TNF- α . Serum miR-221-3p was remarkably positively correlated with IL-6 and TNF- α ($r=0.606, P<0.001$; $r=0.654, P<0.001$) (Fig. 4).

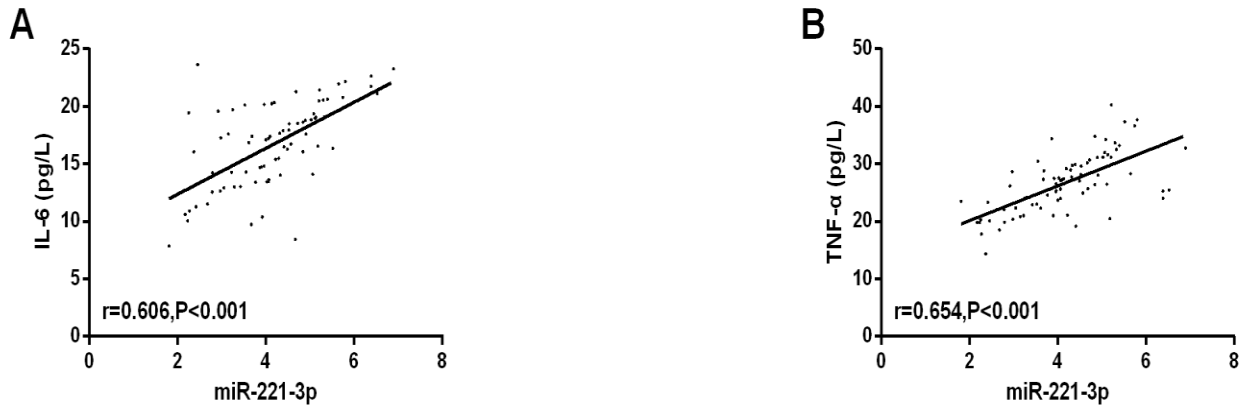


Fig. 4: Correlation analysis of serum miR-221-3p with inflammatory cytokines
 A. serum miR-221-3p was remarkably positively correlated with IL-6 ($r=0.606, P<0.001$).
 B. serum miR-221-3p was remarkably positively correlated with TNF- α ($r=0.654, P<0.001$)

Analysis of prognostic factors affecting PSD occurrence

We included miR-221-3p, IL-6 and TNF- α into the analysis, and took them as dependent variables for assignment. With the presence or absence of PSD occurrence taken as a dependent variable, a Logistic regression model was used for

conducting multivariate analysis on factors with differences (such as a history of mental illness, NIHSS score, HAMD score). A history of mental illness ($P=0.032$), NIHSS score ($P=0.003$), HAMD score ($P=0.002$), IL-6 ($P=0.037$), TNF- α ($P=0.009$) and miR-221-3p ($P=0.001$) were independent risk factors for PSD (Table 3, 4).

Table 3: Assignment for multivariate Logistic regression analysis

<i>Factors</i>	<i>Variables</i>	<i>Assignment</i>
A history of mental illness	X1	No = 0, Yes = 1
NIHSS score	X2	A continuous variable
HAMD score	X3	A continuous variable
IL-6	X4	A continuous variable
TNF- α	X5	A continuous variable
miR-221-3p	X6	A continuous variable

Table 4: Multivariate analysis of risk factors affecting PSD

<i>Factors</i>	β	<i>S.E</i>	<i>Wald</i>	<i>P</i>	<i>OR</i>	<i>95% CI</i>
A history of mental illness	0.489	0.231	4.482	0.032	1.623	1.035-2.548
NIHSS score	1.126	0.384	8.620	0.003	3.079	1.454-6.520
HAMD score	1.005	0.347	8.594	0.002	2.726	1.397-5.332
IL-6	0.613	0.291	4.315	0.037	1.849	1.035-3.279
TNF- α	0.757	0.293	4.413	0.009	2.214	1.121-3.775
miR-221-3p	0.762	0.345	8.299	0.001	3.368	2.329-6.879

Discussion

As a complication of stroke patients, PSD that has an incidence of approximately 25-79% seriously and negatively affects patients' rehabilitation and quality of life (17,18). There is more evidence that serum miRNAs are involved in blood-brain barrier disruption, microbleeds and other pathophysiological processes of stroke patients, so they can be used as biological indicators for PSD diagnosis (19,20). In this study, the potential application value of miR-221-3p for PSD was mainly analyzed to provide reference for diagnosing and preventing the disease, which is of great significance for improving the quality of life of patients.

As reported by previous studies, miR-221-3p is involved in the pathogenesis of many cerebrovascular diseases including stroke. For instance, in cerebral ischemic stroke, it regulates endothelial cell functions by regulating the signal transduction of PTEN-PI3K-AKT pathway. In the mouse model of Parkinson's disease, it is regulated by HOTAIR and then specifically regulates downstream factor NPTX2, further regulating the proliferation and autophagy of dopaminergic neurons in the substantia nigra pars compacta (21,22). Additionally, it remarkably upregulates in liver cancer (23), gastric cancer (24) and acute lung injury (25). In our study, miR-221-3p had higher levels in serum and CSF in the PSD group than those in the Non-PSD group, suggesting that this miR mediates pathological changes of PSD in serum and CSF. Then, we plotted ROC curves of miR-221-3p in serum and CSF to identify PSD. Their AUCs were 0.900 and 0.925, respectively. The sensitivity and specificity of the former were 78.95% and 91.67%, respectively, while those of the latter were 81.58% and 90.00%, respectively. miR-221-3p in both serum and CSF showed excellent performance in PSD identification. According to the correlation analysis, miR-221-3p expression in serum was positively correlated with that in CSF. This indicates that changes of serum miR-221-3p are related to those of miR-221-3p in CSF, so serum miR-221-

3p as a simple and convenient biological indicator may replace that in CSF. Moreover, serum miR-221-3p was remarkably positively correlated with NIHSS and HAMD scores, revealing that serum miR-221-3p can reflect the severity of neurological impairment and depression in PSD patients. As reported by Feng et al, this indicator has a positive correlation with depression, so it can be used as a biological indicator of the disease in patients during the perioperative period. The mechanism of action may be related to its mediation of IRF2-IFN- α -NF- κ B neuroinflammatory signaling cascade reaction (26). This may be helpful to partially explain the abnormal imbalance of serum miR-221-3p in PSD in our study.

The pathogenesis of PSD involves inflammatory cytokines-mediated inflammatory responses and cytokine-nerve-endocrine-immune network (27). Therefore, we explored the correlations of miR-221-3p with inflammatory cytokines. This miR was remarkably positively correlated with IL-6 and TNF- α , suggesting that it may be associated with inflammatory responses related to IL-6 and TNF- α in PSD. This miR activated the pro-inflammatory function of M2-macrophages by regulating JAK3-STAT3 signaling pathway, thus regulating the release of IL-6 and other inflammatory cytokines (28). It could affect the apoptosis of osteoarthritis synovial cells via affecting the secretion level of TNF- α (29). All of these studies demonstrate that miR-221-3p has some pathological correlations with IL-6 and TNF- α . Finally, we analyzed the risk factors affecting PSD. NIHSS score, HAMD score, a history of mental illness, IL-6, TNF- α and miR-221-3p were the risk factors, which suggests that high NIHSS and HAMD scores, high levels of IL-6, TNF- α and miR-221-3p, and a history of mental illness can increase the risk of PSD in stroke patients. Inflammatory cytokines such as IL-6 and TNF- α were independent risk factors for PSD (27), which was consistent with our research results.

This study has confirmed that miR-221-3p in serum and CSF can be used as indicators of PSD, and have high diagnostic values for the disease. However, there is still room for improvement. Firstly, we can supplement basic research on this

miR and explore its potential molecular mechanism in PSD. Secondly, we can investigate its diagnostic value for severe PSD and expand its potential application value. In addition, we can study its relationship with pathological parameters of PSD patients, so as to further supplement its potential clinical significance. We will gradually improve this research based on the above points in the future.

Conclusion

To sum up, miR-221-3p in serum and CSF can be used as the diagnostic and risk warning indicators of PSD. In addition, serum miR-221-3p is positively correlated with PSD severity and inflammatory cytokines. Therefore, this miR in serum may provide new clues for diagnosing and preventing PSD.

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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