Relationships between plasma expression levels of microRNA-146a and microRNA-132 in epileptic patients and their cognitive, mental and psychological disorders

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ABSTRACT
We aimed to explore the relationships between the plasma expression levels of microRNA (miR)-146a and miR-132 in epileptic patients and cognitive, mental and psychological disorders. Eighty epileptic patients and seventy healthy subjects as controls were evaluated with Montreal Cognitive Assessment (MoCA), Hamilton Anxiety Rating (HAMA) and Hamilton Depression Rating (HAMD) scales, and plasma samples were collected. MiR-146a and miR-132 levels were detected by real-time quantitative PCR. The total incidence rate of cognitive dysfunction, anxiety and depression in epilepsy group was 62.5%. Cognitive dysfunction was correlated positively with educational level, but negatively with disease course, duration and type of administration. The frequency and duration of seizures were positively correlated with anxiety. Depression was correlated negatively with educational level, whereas positively with course of disease and number of used drugs. Epileptic patients had significantly higher miR-146a and miR-132 levels than those of healthy controls. The miR-146a and miR-132 levels of patients with complications were significantly higher than those of cases without complications. Their expressions were correlated negatively with total MoCA scale score, but positively with type of complications. MiR-132 expression was positively correlated with the total scores of HAMA and HAMD scales. Plasma miR-146a and miR-132 expressions increased in epileptic patients, and miR-132 expression reflected the severity of epilepsy and predicted the risks of complications.

Introduction
Epilepsy is one of the common diseases of the nervous system, from which about 65 million people worldwide are suffering [1]. Cognitive dysfunction, anxiety and depression are the most common complications of epileptic patients, with the prevalence rates of 30–40%, 20–40% and 20–60%, respectively [2]. These complications reduce the tolerance of patients to antiepileptic drugs and self-management compliance, and even increase the risks of suicide and accidental death, which may seriously affect mental health and economically burden families and the society. Cognitive dysfunction, anxiety and depression have been mostly attributed to neurotransmitter abnormalities, impaired ion channels, glial cell proliferation, synaptic abnormalities, genetic factors and immune inflammation.

MicroRNAs (miRNAs) predominantly participate in various processes of living organisms. Some specific miRNAs play important roles in the pathogenesis of epilepsy. For instance, miR-146a in the brain tissue of epileptic patients is closely related to inflammatory response [3,4]. After seizures, the expressions of inflammatory factors such as IL-1\textbeta, IL-6, TNF and COX-2 in brain tissue increase. Maroso et al. found that inflammatory factors bound TLR4 to enhance the expressions of related inflammatory factors by amplifying cascade signals, and the excitability of neurons and recurrent seizures, thereby causing damage to hippocampal neurons [5].
In a rat model of epilepsy induced by pilocarpine, miR-132 expression increased 8 h after onset, which was also up-regulated in the brain with epileptic seizures [6–8]. In addition, miR-132 was overexpressed in the brain of patients with temporal lobe epilepsy [6,9]. Besides being involved in the regulation of neuronal dendrites and synaptic plasticity, miR-132 also plays a key role in the pathogenesis of epilepsy. Yu et al. used miRNA microarray and real-time quantitative PCR (qRT-PCR) to compare the expressions of miRNAs in peripheral blood mononuclear cells from patients with schizophrenia and normal controls, and proved that miR-132 was a potential diagnostic biomarker [10].

Cognitive dysfunction refers to abnormalities in advanced intelligent processing related to learning, memory, thinking and judgment, accompanied by pathological processes such as aphasia, misuse, agnosia and misbehavior. Moreover, the expression of miR-146a in the brain of patients with Alzheimer’s disease (AD) is significantly up-regulated. Cui et al. reported that elevated expression of polymorphic AA allele miR-146a rs57095329 in the peripheral blood mononuclear cells of AD patients increased the risk of cognitive impairment [11]. Additionally, the expression of miR-132 is significantly up-regulated in the early stage of AD but down-regulated in the later stage [12]. Therefore, both miR-146a and miR-132 are closely related to cognitive function.

Anxiety and depression are common mental disorders in epileptic patients. Depression is an emotional disorder with significant and lasting emotion or mood changes, and anxiety is typified by tension, fear, worry, scare, etc. Kawashima et al. confirmed that miR-132 was up-regulated by BDNF stimulation, being involved in the onset and progression of depression [13]. The lack of Mec P2, a target gene protein negatively regulated by miR-132, is closely related to persistent neurodegenerative changes upon Rett syndrome. In contrast, its overexpression leads to cognitive deficiency and mental disorders [14,15].

In short, miR-146a and miR-132 play crucial regulatory roles in the onset and progression of epilepsy, cognitive dysfunction, anxiety and depression, but the mechanisms have not been clarified. The expression levels of miR-146a and miR-132 are significantly up-regulated after epileptic seizures. However, the changes of plasma miR-146a and miR-132 levels in epileptic patients with cognitive dysfunction, anxiety and depression have not been studied.

Thereby motivated, the aim of this study was to explore the changes of plasma miR-146a and miR-132 levels after seizures by qRT-PCR, and to analyze their relationships with cognitive dysfunction, anxiety and depression. The results pave the way for developing new interventions and improving the quality of life of patients with epilepsy.

Materials and methods

Subjects

Eighty epileptic patients treated in our hospital from January 2017 to June 2018 were selected. Meanwhile, 70 volunteers who received physical examinations in our hospital were enrolled as a control group. The epilepsy group included 58 males (72.5%) and 22 females (27.5%) aged 20–72 years old, (51.48 ± 15.88) on average. The control group consisted of 48 males (68.6%) and 22 females (31.4%) aged 20–70 years old, (52.71 ± 16.64) on average. This study has been approved by the ethics committee of our hospital, and written consent has been obtained from all patients.

Inclusion and exclusion criteria

Epilepsy group

Inclusion criteria: 1) Epilepsy was diagnosed by attending and senior physicians engaged in the diagnosis and treatment of epilepsy for many years according to current and history of medical records, family history, physical examination, laboratory examination, electroencephalogram, brain CT and MRI; 2) diagnosis and type of epilepsy met the classification criteria of the International League Against Epilepsy; 3) gender was not limited, and older than 18 years old; 4) patients voluntarily participated in this study.

Exclusion criteria: 1) Epileptic patients with neurological and other systemic diseases that may affect serum detection results; 2) history of other mental illnesses such as schizophrenia and bipolar
disorder, history of abuse of psychoactive substances and long-term heavy drinking history; 3) patients recently taking drugs that affected cognitive function, anxiety and depression; 4) patients with visual and hearing impairment or aphasia unable to participate in scale assessment.

**Control group**

Inclusion criteria: 1) Without history of neurological diseases, mental illnesses or other systemic diseases; 2) able to correctly understand the relevant contents of scale assessment; 3) total score of Montreal Cognitive Assessment (MoCA) scale (cognitive function) ≥26 points, total score of Hamilton Anxiety Rating (HAMA) scale (anxiety) <14 points and total score of Hamilton Depression Rating (HAMD) scale (depression) <17 points; 4) subjects agreed and volunteered to participate in the study. Exclusion criteria: 1) Patients with history of neurological or mental diseases; 2) patients with history of abusing psychoactive substances and long history of heavy drinking; 3) patients recently taking drugs that affected cognitive function and anxiety and depression; 4) patients with visual and hearing impairment and aphasia unable to participate in scale assessment; 5) total score of MoCA scale <26 points, total score of HAMA scale ≥14 points and total score of HAMD scale ≥17 points; 6) patients with autoimmune diseases and neoplastic process.

**Collection of clinical data**

(1) Before the study started, all subjects were trained for scale assessment. The objective of this study, precautions and the principle of confidentiality of psychological assessment results were explained to the subjects who received psychological assessments under voluntary circumstances, and filled in or answered truthfully according to the actual situation. The clinical data of the epilepsy group were collected jointly by researchers, patients and their family members, and the contents filled in should be ensured as true and reliable as possible.

(2) The sociodemographic data of all subjects and the general clinical data of the epilepsy group were collected through a self-made general situation questionnaire prepared by the authors. The epilepsy group was evaluated by using MoCA, HAMA and HAMD scales in the morning after admission, within 2 h after plasma collection, at the time of onset and 2 h after onset [16,17]. Before the control group was enrolled, they were assessed by MoCA, HAMA and HAMD scales, respectively, and those who met the inclusion criteria entered the next phase of this study. Total score of MoCA scale <26 points: cognitive dysfunction; total score of HAMA scale ≥14 points: anxiety; total score of HAMD scale ≥17 points: depression.

(3) The subjects were evaluated by neurological physicians and psychiatrists. The researchers were familiar with the guidelines and entries of all scales, and could correctly interpret the meaning of the items. The test was conducted after confirming that the subjects understood all the requirements. The diagnosis was performed according to the criteria of various members of the medical staff.

**Collection of plasma samples**

Briefly, 2 mL of fasting peripheral blood was collected from the epilepsy group in the next morning after admission and 2 h after onset, and 2 mL was taken from the control group in the morning and then injected into an EDTA-K2 anticoagulant tube. The blood samples were centrifuged at 4°C and 3000 rpm for 10 min to separate the plasma. The supernatant was subpackaged into new 1.5 mL RNase-free EP tubes (600 μL each tube), and stored in a −80°C refrigerator prior to use [18].

**qRT-PCR**

qRT-PCR was conducted with commercially available and validated methods. All equipment was regularly calibrated using reference samples. Before detection, U6 snRNA and β-actin were both used as internal references for correction.

Plasma total RNA was extracted with Trizol reagent (Aidlab, Beijing, China), and reverse-transcribed into cDNA using a two-step method (M-MLV reverse transcriptase, Gene Copoeia,
USA). Reverse transcription and RT-q-PCR were performed by using SYBR-green PCR kit (Nanjing Vazyme Biotech Co., Ltd., China). U6 snRNA was utilized as the internal reference. The relative expression levels of miR-146a and miR-132 of each sample were measured in triplicate using the \(2^{-\Delta\Delta C_t}\) method. All measurements were carried out by an experienced laboratory physician blinded for study aims [19].

The sequences of miR-146a and miR-132 were obtained from miRBase (http://www.mirbase.org/). The reverse transcription primers for miR-146a, miR-132 and U6 as well as the upstream and downstream primers for qRT-PCR were designed and synthesized by GenePharma (Shanghai, China) (Table 1).

### Bioinformatics analysis

The targets for miR-146a and miR-132 were predicted with TargetScan version 5.1 (http://www.targetscan.org/).

### Statistical analysis

All data were analyzed by SPSS 22.0 software. Continuous variables were subjected to normality test using the one-sample K-S method, the distribution of which was expressed as mean ± standard deviation. The continuous variables with normal distribution and variance homogeneity were subjected to the two-independent-sample t test and one-way analysis of variance, while those without normal distribution or variance homogeneity were subjected to the non-parametric Mann-Whitney U test and Kruskal-Wallis H test. Categorical data were analyzed by the Chi-square test. Correlations were studied based on nonparametric Spearman rank-order correlation coefficients. Multiple linear regression analysis was performed. All tests were two-tailed. \(P < 0.05\) was considered statistically significant.

### Results

#### Baseline clinical data

#### Social and demographic data of epilepsy and control groups

The age, gender ratio and educational level of epilepsy and control groups were similar \((P > 0.05)\) (Table 2).

#### Clinical characteristics of epilepsy group

The clinical characteristics of epilepsy group, including age of first onset, disease course, type of seizure, frequency of seizure, duration and number of used antiepileptic drugs, are listed in Table 3.

| Table 1. Primer sequences of miR-146a, miR-132 and Hsa-U6. |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gene | Sequence |  |
| hsa-miR-146a-5p (RT-PCR) | Loop primer | 5′-GTGTGATTCCAGTGGGGTCTCGAGGTAGG  
ATTCGCACTCGGATGACGTACCTGGAAATCGGGTCTCGAGGT |  |
| hsa-miR-132-3p (RT-PCR) | Loop primer | 5′-GTGTGATTCCAGTGGGGTCTCGAGGTAGG  
ATTCGCACTCGGATGACGTACCTGGAAATCGGGTCTCGAGGT |  |
| hsa-miR-146a-5p (qPCR) | F primer | 5′-TGCGCTGAGAACTGAATTCCAT-3′  
5′-TGCGCTAACAGTCTACAGCCA-3′ |  |
| R primer | 5′-TGCGCTGGCGAGGCGGTCTCGAGGTAGG  
ATTCGCACTCGGATGACGTACCTGGAAATCGGGTCTCGAGGT |  |
| hsa-miR-132-3p (qPCR) | F primer | 5′-TGCGCTGAGAACTGAATTCCAT-3′  
5′-TGCGCTAACAGTCTACAGCCA-3′ |  |
| R primer | 5′-TGCGCTGGCGAGGCGGTCTCGAGGTAGG  
ATTCGCACTCGGATGACGTACCTGGAAATCGGGTCTCGAGGT |  |
| Hsa-U6 (qPCR) | F primer | 5′-TGCGCTGAGAACTGAATTCCAT-3′  
5′-TGCGCTAACAGTCTACAGCCA-3′ |  |
| R primer | 5′-TGCGCTGGCGAGGCGGTCTCGAGGTAGG  
ATTCGCACTCGGATGACGTACCTGGAAATCGGGTCTCGAGGT |  |

| Item | Epilepsy (n = 80) | Control (n = 70) | \(t/\chi^2\) | \(P\) |
|---|---|---|---|---|
| Age (year) | 51.48 ± 15.88 | 52.71 ± 16.64 | 0.463 | 0.644 |
| Gender | | | 0.278 | 0.598 |
| Male | 58 (72.5%) | 48 (68.6%) | | |
| Female | 22 (27.5%) | 22 (31.4%) | | |
| Educational level (year) | | | 0.129 | 0.938 |
| <6 | 26 (32.5%) | 22 (31.4%) | | |
| 6–12 | 42 (52.5%) | 36 (51.4%) | | |
| >12 | 12 (15.0%) | 12 (17.1%) | | |
Table 3. Clinical characteristics of epilepsy group (%).

| Item                          | n (%) | Item                          | n (%) |
|-------------------------------|-------|-------------------------------|-------|
| Age of first onset (year)     | <18   | Age of first onset (year)     | ≥18   |
|                               | 8 (10%) | 72 (90%) | Frequency of seizure | Single | 20 (25%) |
| Disease course (year)         | <1    | Disease course (year)         | ≥1    |
|                               | 44 (55%) | 36 (45%) | Duration (min) | Frequent | 60 (75%) |
| Type of seizure               | SPS   | SPS                           | CPS   |
|                               | 10 (12.5%) | 10 (12.5%) | Number of used antiepileptic drugs | 0 |
|                               | GTCS  | GTCS                          |       | 1 |
|                               | 60 (75%) |                      |       | 2 |

Table 4. Total scores of MoCA, HAMA, and HAMD scales [M (P25 – P75)].

| Item       | MoCA (point) | HAMA (point) | HAMD (point) |
|------------|--------------|--------------|--------------|
|            | [26.00 (22.25–27.00)] | [12.00 (8.55–17.00)] | [16.00 (14.00–18.00)] |
|            | [27.00 (26.00–28.00)] | [9.00 (8.00–11.00)] | [9.00 (8.00–11.00)] |

Total scores of MoCA, HAMA and HAMD scales, and incidence rates of cognitive dysfunction, anxiety and depression

The total score of MoCA scale of epilepsy group was significantly lower than that of control group (Z = −3.533, P = 0.001), and the total scores of HAMA and HAMD scales of epilepsy group were significantly higher than those of control group (Z = −3.317, P = 0.001; Z = −6.598, P = 0.001) (Table 4).

The total incidence rate of complicated cognitive dysfunction, anxiety and depression in the epilepsy group was 62.5%.

Correlations between clinical characteristics and cognitive dysfunction, anxiety and depression

The nonparametric Spearman rank-order correlation analysis revealed that cognitive dysfunction was positively correlated with educational level, but negatively correlated with course of disease, duration and type of administration.

The frequency and duration of seizures were positively correlated with anxiety.

Depression was negatively correlated with educational level, whereas positively correlated with course of disease and number of used drugs (Table 5).

Plasma miR-146a and miR-132 levels as well as correlations with clinical characteristics

The expression levels of miR-146a and miR-132 in epileptic patients, particularly miR-132, were significantly higher than those of healthy controls (Table 6).

Educational level was negatively correlated with miR-146a and miR-132 expressions, and course of disease and duration of seizures were positively correlated with miR-132 expression (Table 7).

Table 5. Correlations between clinical characteristics and cognitive dysfunction, anxiety and depression.

| Item                          | MoCA r | MoCA p | HAMA r | HAMA p | HAMD r | HAMD p |
|-------------------------------|--------|--------|--------|--------|--------|--------|
| Age (year)                    | −0.036 | 0.833  | −0.090 | 0.595  | 0.012  | 0.946  |
| Gender                        | 0.000  | 1.000  | −0.077 | 0.647  | −0.212 | 0.241  |
| Educational level (year)      | 0.412**| 0.010  | −0.202 | 0.222  | −0.341*| 0.030  |
| Age of first onset (year)     | 0.112  | 0.534  | −0.157 | 0.343  | −0.096 | 0.535  |
| Disease course (year)         | −0.404**| 0.010 | 0.243  | 0.131  | 0.345* | 0.033  |
| Type of seizure               | −0.054 | 0.743  | 0.033  | 0.843  | −0.031 | 0.873  |
| Frequency of seizure          | −0.207 | 0.207  | 0.361* | 0.022  | 0.307  | 0.062  |
| Duration (min)                | −0.433**| 0.006 | 0.321* | 0.043  | 0.285  | 0.077  |
| Number of used drugs          | −0.459**| 0.003 | 0.469**| 0.002  | 0.407**| 0.008  |

*P < 0.05; **P < 0.01.
Correlations of plasma miR-146a and miR-132 levels with cognitive dysfunction, anxiety and depression

The area under the ROC curve for the positive diagnostic results of epilepsy complicated with cognitive dysfunction by using miR-146a was 0.808 (95%CI: 0.654 ~ 0.951, P < 0.001). The optimal cutoff value was 0.65, the sensitivity was 87.8%, and the specificity was 68.2%. Additionally, the area under the curve for the positive diagnostic results of epilepsy complicated with cognitive dysfunction by using miR-132 was 0.791 (95%CI: 0.672 ~ 0.923, P < 0.001). The optimal cutoff value was 0.40, the sensitivity was 87.8%, and the specificity was 68.2% (Figure 1).

The plasma expression levels of miR-146a and miR-132 in patients with cognitive dysfunction, anxiety and depression significantly exceeded those of cases without complications, accompanied by increase in the type of complications (Table 8).

The expressions of miR-146a (miR-146a ≥0.40) and miR-132 (miR-132 ≥ 0.65) were negatively correlated with the total score of MoCA scale, and positively correlated with the type of complications. The expression of miR-132 was positively correlated with the total scores of HAMA and HAMD scales (Table 9).

Multiple linear regression analysis results

Multiple linear regression analysis showed that elevated plasma miR-132 expression was a risk factor for cognitive dysfunction (F = 106.215, P < 0.001), anxiety (F = 17.811, P < 0.001) and depression (F = 11.525, P = 0.001) upon epilepsy.

Table 6. Plasma miR-146a and miR-132 levels [M (P25 – P75)].

| Item          | Epilepsy          | Control          |
|---------------|-------------------|------------------|
| miR-146a      | [0.38 (~0.03–0.75)]** | [0.00 (~0.50–0.25)] |
| miR-132       | [0.47 (~0.10–0.82)]*** | [0.43 (~0.72–0.15)] |

Compared with control group, *P < 0.05; **P < 0.01.

Table 7. Correlations of plasma miR-146a and miR-132 levels with clinical characteristics.

| Item                | miR-146a r | p     | miR-132 r | p     |
|---------------------|------------|-------|-----------|-------|
| Age (year)           | 0.043      | 0.778 | 0.019     | 0.915 |
| Gender               | 0.253      | 0.130 | 0.055     | 0.744 |
| Educational level (year) | −0.417** | 0.006 | −0.367*   | 0.012 |
| Age of first onset (year) | −0.192 | 0.241 | −0.124    | 0.433 |
| Disease course (year)  | 0.243      | 0.160 | 0.412**   | 0.008 |
| Type of seizure       | 0.079      | 0.631 | 0.034     | 0.832 |
| Frequency of seizure  | 0.005      | 0.972 | 0.251     | 0.143 |
| Duration (min)        | 0.132      | 0.441 | 0.407**   | 0.006 |
| Number of used drugs  | 0.288      | 0.070 | 0.471**   | 0.002 |

*P < 0.05; **P < 0.01.

Table 8. Plasma miR-146a and miR-132 levels in patients with cognitive dysfunction, anxiety and depression [M (P25-P75)].

| Item            | miR-146a level | Z/\chi^2/P | miR-132 level | Z/\chi^2/P |
|-----------------|----------------|------------|---------------|------------|
| MoCA ≥26 points | [0.18 (~0.10–0.38)] | −3.289     | [0.15 (~0.25–0.37)] | −5.413     |
| MoCA <26 points | [0.77 (~0.40–0.90)] | 0.001      | [0.80 (~0.65–0.97)] | 0.000      |
| HAMA ≥14 points | [0.34 (~0.05–0.63)] | −1.490     | [0.33 (~0.20–0.61)] | −3.221     |
| HAMA <14 points | [0.60 (~0.04–0.88)] | 0.141      | [0.72 (~0.45–0.93)] | 0.001      |
| HAMD ≥17 points | [0.62 (~0.14–0.92)] | 0.024      | [0.70 (~0.63–0.95)] | 0.000      |
| Comorbidity      |                 |            |               |            |
| None             | [0.30 (~0.05–0.37)] | 0.03 (~0.35–0.34) | 0.000      |
| 1 type           | [0.20 (~0.05–0.58)] | 8.048      | [0.41 (~0.13–0.90)] | 23.732     |
| 2 types          | [0.55 (~1.44–1.68)] | 0.042      | [0.65 (~0.44–0.83)] | 0.000      |
| 3 types          | [0.75 (0.51–0.92)] |            | [0.84 (0.71–0.99)] |            |

Figure 1. ROC curve analysis of diagnostic values of miR-146a and miR-132 for epilepsy complicated with cognitive dysfunction.
Prediction of targets for miR-146a and miR-132

An online tool TargetScan was employed to predict the targets for miR-146a and miR-132. There were targeted binding sites between miR-146a and IRAK1 (Figure 2), as well as between miR-132 and SIRT1 (Figure 3).

Discussion

Epilepsy is a chronic recurrent transient brain dysfunction syndrome and one of the common neurological diseases. Cognitive dysfunction, anxiety and depression are the most common complications of patients with epilepsy. The prevalence of cognitive dysfunction in patients with epilepsy is 30–40%, and those of anxiety and depression are 20–40% and 20–60%, respectively [2].

In this study, the MoCA, HAMA and HAMD scales were used to assess the mental health status of patients with epilepsy. The incidence rate of psychological abnormalities in patients with epilepsy was 62.5%, including 47.5% of cognitive dysfunction, 40.0% of anxiety and 42.5% of depression. The differences between the incidence rates of cognitive dysfunction and depression, and total score of MoCA scale were statistically significant (P < 0.05), but the incidence rate of anxiety and the total scores of HAMA and HAMD scales were similar (P > 0.05). The cognitive dysfunction, anxiety and depression incidence rates as well as total scores of MoCA, HAMA and HAMD scales in epileptic patients of different ages and genders were significantly different (P > 0.05). There was a positive correlation and a negative correlation between educational level and total score of MoCA scale and total HAMD scale score, respectively. The epileptic patients with higher educational level had higher total score of MoCA scale, milder cognitive impairment, and lower total score of HAMD scale, explaining the lower incidence rate of depression.

Each miRNA can regulate dozens or even hundreds of target mRNAs, and one mRNA can be regulated by multiple miRNAs [20]. Aronica et al. established a rat model of temporal lobe epilepsy by repeated perforation of electric shock [21,22]. After 1 week (developmental phase) and 3 months (chronic phase) of status epilepticus, miR-146a was up-regulated in the CA3 region of the hippocampus, being in accordance with the results in this study.

Cognitive dysfunction is a crucial clinical manifestation of AD. The expression of miR-146a in the brain of AD patients is significantly up-regulated. MiR-132 affects synaptic plasticity.

Table 9. Correlations of plasma miR-146a and miR-132 levels with cognitive dysfunction, anxiety and depression.

| Item       | miR-146a | miR-132 |
|------------|----------|----------|
|            | r        | p        | r        | p        |
| MoCA       | -0.462** | 0.001    | -0.781***| <0.001   |
| HAMA       | 0.178    | 0.272    | 0.569*** | <0.001   |
| HAMD       | 0.208    | 0.198    | 0.341*   | 0.044    |
| Complication | 0.432** | 0.005    | 0.611*** | <0.001   |

*P < 0.05; **P < 0.01; ***P < 0.001.
under the regulation of CREB, thereby dynamically regulating advanced cognitive functions such as learning and spatial memory [23], similar to the results of Suzuki et al [24].

Anxiety and depression are common mental disorders in patients with epilepsy. Leptin regulates synapse formation and activity in the hippocampus via the miR-132/p250GAP pathway, mitigates depression and anxiety, and improves cognitive function [25]. We herein also confirmed that miR-146a and miR-132 were closely related to cognitive dysfunction, anxiety and depression in the case of epilepsy, and reflected the severity of complications, further suggesting that they were potential biomarkers for epilepsy. Regression analysis showed that elevated plasma miR-132 expression was a risk factor for cognitive dysfunction, anxiety and depression upon epilepsy. Compared with miR-146a, miR-132 was more predictive of the risk of epilepsy.

Furthermore, TargetScan showed that there were targeted binding sites between miR-146a and IRAK1, as well as between miR-132 and SIRT1. Yan found that the brain tissue of patients with refractory temporal lobe epilepsy had higher IRAK1 expression than that of normal control group [26]. Therefore, IRAK1 plays an essential role in the onset of epilepsy. Kumar et al. used SIRT1 to monitor the early occurrence of AD, because the SIRT1 expression in AD patients was lower than that of normal control group [27]. Besides, SIRT1 can promote axonal growth and memory production in hippocampal neurons, and dominantly participate in neurogenesis and synaptic plasticity [28].

After a seizure, the expression levels of inflammatory factors such as IL-1β, IL-6, TNF and COX-2 increase in brain tissues. Maroso et al. found that inflammatory factors bound TLR4 to elevate the expression levels of related inflammatory factors through the amplification of cascade signals, thereby causing the high excitability of neurons, recurrent seizures and hippocampal neuron damage [5]. As a regulatory miRNA in the immune system, miR-146a is an endogenous regulator of TLR and cytokine receptor signaling pathways, and closely related to immune and inflammatory responses [29]. MiR-132 is highly expressed in pyramidal neurons and granular cells in brain tissues, which gradually increases along with the maturation of neurons. When neurons receive external stimuli, miR-132 expression is significantly up-regulated [7]. The miR-132 expression in a rat model of pilocarpine-induced epilepsy increased 8 h after onset. MiR-132 expression was also up-regulated in the individuals with status epilepticus, and miR-132 was overexpressed in human brains with temporal lobe epilepsy [8].

**Conclusion**

In summary, the expressions of miR-146a and miR-132 increased in epileptic patients, and that of miR-132 reflected the severity of epilepsy. MiR-146a and miR-132 expressions can reflect the severity of cognitive dysfunction, anxiety and depression in the case of epilepsy, and that of miR-132 can predict the risks of complications. The findings provide a theoretical basis for the clinical diagnosis, risk assessment and outcome evaluation of complications for epilepsy. Nevertheless, this study still has limitations. First, this is a single-center study with small sample size, so multicenter studies with larger sample sizes are in need. Second, the aim of this study was to explore the target proteins of miR-146a and miR-132 (IRAK1 and SIRT1). Further studies regarding their regulatory effects on proteins related to other psychiatric comorbidities are ongoing in our group. Third, the results herein should be validated by performing cell and animal experiments.

**Highlights**

- MiR-146a and miR-132 expressions increase in epileptic patients.
- MiR-132 expression reflects the severity of epilepsy.
- MiR-132 expression predicts the risks of complications.

**Disclosure statement**

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**References**

[1] Moshé SL, Perucca E, Ryvlin P, et al. Epilepsy: new advances. Lancet. 2015;385:884–898.

[2] Verrotti A, Carrozzino D, Milioni M, et al. Epilepsy and its main psychiatric comorbidities in adults and children. J Neurol Sci. 2014;343:23–29.

[3] Iori V, Aronica E, Vezzani A. Epigenetic control of epileptogenesis by miR-146a. Oncotarget. 2017;8:45040–45041.

[4] Wilson EH, Weninger W, Hunter CA. Trafficking of immune cells in the central nervous system. J Clin Invest. 2010;120:1368–1379.

[5] Maroso M, Balosso S, Ravizza T, et al. Toll-like receptor 4 and high-mobility group box-1 are involved in icotogenesis and can be targeted to reduce seizures. Nat Med. 2010;16:413–419.

[6] Jimenez-Mateos EM, Bray I, Sanz-Rodriguez A, et al. miRNA Expression profile after status epilepticus and hippocampal neuroprotection by targeting miR-132. Am J Pathol. 2011;179(5):2519–2532.

[7] Risbud RM, Porter BE. Changes in microRNA expression in the whole hippocampus and hippocampal synaptoneurosome fraction following pilocarpine induced status epilepticus. PLoS One. 2013;8:e53464.

[8] Peng J, Omran A, Ashhab MU, et al. Expression patterns of miR-124, miR-134, miR-132, and miR-21 in an immature rat model and children with mesial temporal lobe epilepsy. J Mol Neurosci. 2013;50:291–297.

[9] Shaltiel G, Hanan M, Wolf Y, et al. Hippocampal microRNA-132 mediates stress-inducible cognitive deficits through its acetylcholinesterase target. Brain Struct Funct. 2013;218:59–72.

[10] Yu HC, Wu J, Zhang HX, et al. Alterations of miR-132 are novel diagnostic biomarkers in peripheral blood of schizophrenia patients. Prog Neuropsychopharmacol Biol Psychiatry. 2015;63:23–29.

[11] Cui L, Li Y, Ma G, et al. A functional polymorphism in the promoter region of microRNA-146a is associated with the risk of Alzheimer disease and the rate of cognitive decline in patients. PLoS One. 2014;9:e89019.

[12] Reiman EM, Chen K, Liu X, et al. Fibrillar amyloid-β burden in cognitively normal people at 3 levels of genetic risk for Alzheimer’s disease. Proc Natl Acad Sci U S A. 2009;106:6820–6825.

[13] Kawashima H, Numakawa T, Kumamaru E, et al. Glucocorticoid attenuates brain-derived neurotrophic factor-dependent upregulation of glutamate receptors via the suppression of microRNA-132 expression. Neuroscience. 2010;165(4):1301–1311.

[14] Hernandez-Rapp J, Smith PY, Filali M, et al. Memory formation and retention are affected in adult miR-132/212 knockout mice. Behav Brain Res. 2015;287:15–26.

[15] Zhang R, Huang M, Cao Z, et al. MeCP2 plays an analgesic role in pain transmission through regulating CREB/miR-132 pathway. Mol Pain. 2015;11:19.

[16] Hoops S, Nazem S, Siderowf AD, et al. Validity of the MoCA and MMSE in the detection of MCI and dementia in Parkinson disease. Neurology. 2013;73:1738–1745.

[17] Wang Y, Ma Y, Sun Y, et al. Application of correlation analysis HAMA in patients with venous leg ulcers of the lower extremities. J Vasc Endovasc Surg. 2019;5:34–39.

[18] Bernstein JA, Wedner HJ, Busse PJ, et al. Refined method for collection of Plasma Samples to Evaluate the Role of Plasma Kallikrein in various disease states. J Allergy Clin Immunol. 2016;137:AB248.

[19] Solayman M, Langae T, Patel A, et al. Identification of suitable endogenous normalizers for qRT-PCR analysis of plasma microRNA expression in essential hypertension. Mol Biotechnol. 2016;58:179–187.

[20] Schmidt M, Oldenburger A, Poppinga W, et al. Cigarette smoke and A-kinase anchoring proteins (AKAPs) in human airway smooth muscle function. FASEB J. 2011;25:864–866.

[21] Aronica E, Crino PB. Inflammation in epilepsy: clinical observations. Epilepsia. 2011;52:26–32.

[22] Aronica E, Fluitzer K, Iyer A, et al. Expression pattern of miR-146a, an inflammation-associated microRNA, in experimental and human temporal lobe epilepsy. Eur J Neurosci. 2010;31(6):1100–1107.

[23] Scott HL, Tamagnini F, Narduzzo KE, et al. MicroRNA-132 regulates recognition memory and synaptic plasticity in the perirhinal cortex. Eur J Neurosci. 2012;36:2941–2948.

[24] Suzuki A, Fukushima H, Mukawa T, et al. Upregulation of CREB-mediated transcription enhances both short-and long-term memory. J Neurosci. 2011;31:8786–8802.

[25] Dhar M, Zhu M, Impey S, et al. Leptin induces hippocampal synaptogenesis via CREB-regulated microRNA-132 suppression of p250GAP. Mol Endocrinol. 2014;28:1073–1087.

[26] Yan J. Doctoral dissertation: expressions of IRAK1 in hippocampus of epileptic patients and rats and the effect of IRAK1 inhibition on seizures in epileptic rats. Southwest Medical University; 2013.

[27] Kumar R, Chaterjee P, Sharma PK, et al. Sirptuin1: a promising Serum Protein marker for early detection of Alzheimer’s disease. PLoS One. 2013;8:e61560.

[28] Herskovits AZ, GUarente L. SIRT1 in neurodevelopment and brain senescence. Neuro. 2014;81:471–483.

[29] Otaegui D, Baranzini SE, Armananzas R, et al. Differential micro RNA expression in PBMC from multiple sclerosis patients. PLoS One. 2009;4:e6309.