

# Efficacy of levetiracetam combined with sodium valproate on pediatric epilepsy and its effect on serum miR-106b in children

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**Abstract.** Efficacy of levetiracetam (LEV) combined with sodium valproate (SV) on pediatric epilepsy and its effect on serum miR-106b in children were investigated. One hundred and twenty children with epilepsy in Xuzhou Children's Hospital from July 2015 to July 2017 were enrolled, and divided into control group (n=60) and observation group (n=60) according to random sampling. Additionally, 100 children undergoing normal physical examination were collected as normal group. Patients in the control group were treated with SV, and patients in the observation group were treated with SV and LEV. RT-qPCR was used for detecting the relative expression of serum miR-106b in children. The clinical efficacy was evaluated. After treatment, the relative expression of serum miR-106b in the control group was significantly higher than that in the observation group ( $P<0.05$ ). The difference in the control group was smaller than that in the observation group ( $P<0.05$ ). According to the ROC curve analysis, when the cut-off value was 1.442, the sensitivity, specificity and area under curve (AUC) of miR-106b in the diagnosis of pediatric epilepsy were 94.00, 64.17 and 0.833 respectively. The clinical efficacy in the observation group was significantly better than that in the control group ( $P<0.05$ ). Spearman's test showed that the expression of miR-106b gradually decreased with the continuous improvement of the clinical efficacy ( $P<0.05$ ). The AUC of miR-106b was 0.833, 95% CI: 0.779 to 0.887, the cut-off was 1.442. LEV combined with SV is effective in the treatment of children with epilepsy, and does not increase the clinical ADR. The expression of serum miR-106b in children can be used as a clinical prognostic indicator and a potential diagnostic indicator.

## Introduction

Epilepsy is a chronic brain dysfunction syndrome caused by highly synchronous abnormal discharge of neurons in the brain (1). This common disorder in clinical neurology has sudden onset. Patients with epilepsy, mainly the elderly or children, are often accompanied by varying degrees of transient disturbance of consciousness, and patients with more severe epilepsy may have a general convulsion (2). It has been reported that (3) there are 50 patients diagnosed with epilepsy in 100,000 people in North America each year. Another study shows that (4) more than half of the 50 million patients with epilepsy in the world are in Asia. Childhood is a critical period for the development of human brain function, and the long-term abnormal discharge in the brain has a very adverse effect on the development of the brain function (5). The treatment of epilepsy in children needs to completely control the onset of the disease and eliminate the cause, as well as ensure the learning and daily living ability of children as much as possible. Therefore, how to actively and effectively treat children with epilepsy has become one of the problems that clinicians need to solve now.

New anti-epileptic drugs have emerged, bringing new hope for the treatment of epilepsy. The main component of levetiracetam (LEV) is a derivative of pyrrolidone. By binding to SV2A in the brain, synaptic vesicle protein 2A, LEV effectively inhibits the high-voltage-activated N-type Ca ion channel in the vertebral neurons of the hippocampus CA1 region, and thereby plays an anti-epileptic role (6). By improving the activity of glutamic acid decarboxylase, sodium valproate (SV) as a conventional therapeutic drug for epilepsy promotes the synthesis of  $\gamma$ -aminobutyric acid in the brain, inhibits  $\gamma$ -aminobutyrate transaminase and prevents the degradation of  $\gamma$ -aminobutyric acid, thereby improving the epileptic symptoms in patients (7).

MicroRNA (miR), a hot research topic in recent years, is a small (19-22 nucleotides) non-coding single-stranded RNA molecule. It causes the translational inhibition of target mRNA or the cleavage of mRNA by regulating gene expression (8). Studies in recent years have shown that miR is differentially expressed in tumor, cardiovascular and nervous system diseases (9-11). In the study by Sun *et al* (12), the expression of miR-106b is significantly increased in the serum of adult patients with epilepsy. However, there is no study showing

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the expression of miR-106b in pediatric epilepsy. Whether it can be a potential prognostic indicator has not been reported previously.

Therefore, the efficacy of LEV combined with SV in the treatment of pediatric epilepsy was analyzed in this study, and whether miR-106b could be used as a potential prognostic indicator for the treatment of children with epilepsy was also analyzed, in order to provide a reference for clinicians.

### Patients and methods

A total of 120 children with epilepsy treated in Xuzhou Children's Hospital (Xuzhou, China) from July 2015 to July 2017 were enrolled, and divided into the control group ( $n=60$ ) and the observation group ( $n=60$ ) according to random sampling. The control group consisted of 31 males and 29 females, and the observation group consisted of 35 males and 25 females. Additionally, 100 children undergoing normal physical examination were collected as the normal group for this study.

This study was approved by the Ethics Committee of Xuzhou Children's Hospital, Xuzhou Medical University. Patients who participated in this research had complete clinical data. The family members of the children were informed of this study and signed an informed consent form.

*Inclusion and exclusion criteria.* Inclusion criteria: All children met the diagnostic criteria for pediatric epilepsy developed by The Basic and Clinical Aspects of Pediatric Neurological Diseases (13). Children had normal blood routine indicators, coagulation function and liver and kidney function.

Exclusion criteria: Children with a history of encephalitis, brain trauma and meningitis; children with immunodeficiency; children complicated with other malignant tumors; children treated with anti-epileptic drugs 1 month before enrollment; children with abnormalities in CT and MRI detection and children with an allergy to therapeutic drugs or a corresponding contraindication.

*Drugs and source of kits.* SV (Hangzhou Sanofi Pharmaceutical Co., Ltd., SFDA approval number: H20010595), LEV (UCB Pharma S.A., approval number: H20160251), TransScript miRNA first-strand cDNA synthesis superMix and easypure miRNA kit (TransGen Biotech, AT351-01, ER601-01), PCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc., 7500). The primers were designed and synthesized by Sangon Biotech (Shanghai) Co., Ltd.

*Treatment programs.* Patients in the control group were treated with SV, and the specific treatment was as follows: the initial dose was 5-10 mg/(kg x day). If epilepsy was not controlled 1 week after medication, the dose would be added with 5-10 mg/(kg x day) per week based on the initial dose, until the seizure did not occur [the maximum dose was controlled at 30-40 mg/(kg x day)].

Patients in the observation group were treated with LEV and SV, and the specific treatment was as follows: the initial dose was 10 mg/(kg x day), which was added once a week. The target dose was controlled at 20-60 mg/(kg x day) on the 3rd to 4th week, the daily dose was divided into 2 doses.

*RT-qPCR detection.* Venous blood (5 ml) was collected from the child in the morning, allowed to stand at room temperature for 15-30 min, and centrifuged at 3,000 x g for 10 min at 4°C. Serum was collected and centrifuged at 10,080 x g for 10 min using an ultra-speed centrifuge at 4°C to remove the cell debris. A portion of the serum was taken for subsequent experiments, and the rest was stored at -80°C. The EasyPure miRNA kit was used for the extraction of total RNA from the serum, UV spectrophotometer and agarose gel electrophoresis for detecting the purity, concentration and integrity of the total RNA extracted, TransScript® miRNA RT Enzyme mix and 2X TS miRNA reaction mix (belonging to the PCR kit) for the reverse transcription of cDNA. The reverse transcription was carried out in strict accordance with the instructions, and cDNA was taken for subsequent experiments. PCR system was as follows: 1  $\mu$ l of cDNA, each of 0.4  $\mu$ l of upstream and downstream primers, 10  $\mu$ l of 2X TransStart® Top/Tip green qPCR supermix, passive reference dye (50X) (optional), and finally nuclease-free water supplemented to 20  $\mu$ l. A two-step method was used for detection. PCR reaction conditions were: pre-denaturation at 94°C for 30 sec, denaturation at 94°C for 5 sec, annealing and extension at 60°C for 30 sec, for a total of 40 cycles. U6 was used as an internal reference gene in this experiment, and  $2^{-\Delta\Delta Cq}$  was used to express the relative expression of miR-106b. The primer sequences are shown in Table I (14).

*Outcome measures.* Main outcome measures: The clinical efficacy in the children was evaluated according to The Diagnosis and Curing Criteria of Clinical Diseases in 2002 (Table II). The difference in the expression of serum miR-106b was observed in children in the control group and the observation group before treatment and subjects in the normal group. The relative expression of serum miR-106b in children was observed before and 12 weeks after treatment, and that of serum miR-106b in children with different efficacy was observed.

Secondary outcome measures: The clinical data of children in the groups were observed. The ROC curve was plotted according to the expression of serum miR-106b in control, observation and normal group. The incidence of adverse drug reaction (ADR) was compared between the two groups.

*Statistical analysis.* In this study, SPSS20.0 software package was used for the statistical analysis of the data, GraphPad Prism 7 for plotting the figures. Count data were expressed as rate (%), tested by Chi-square and denoted by  $\chi^2$ . Measurement data were expressed as mean  $\pm$  standard deviation (means  $\pm$  SD). Independent sample t-test was used for comparison between the groups, paired t-test for comparison in the group before and after treatment and denoted by *t*. Grade data were tested by rank sum test and denoted by *Z*. Analysis of variance was used for comparison between multiple groups, LSD t-test for pairwise comparison in the group. The ROC curve was plotted according to the expression of serum miR-106b in the control, the observation and the normal group. The Spearman's test was used to analyze the relationship between the expression of miR-106b and the clinical efficacy.  $P<0.05$  was considered to indicate a statistically significant difference.

Table I. Primer sequences.

Genes	Upstream primer	Downstream primer
miR-106b	5'-TGCCTCCTCATTGTCTTCA-3'	5'-GCCATCTCAAATACCTCCC-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'

Table II. Clinical efficacy grading.

Efficacy grade	Performance
Cured	No epileptic seizure in children
Markedly effective	No epileptic seizure or seizure frequency reduced by >75%
Effective	Seizure frequency reduced by 25-75%
Invalid	Seizure frequency reduced by <25%, or no significant reduction, or increase in seizure

Table III. Clinical data of children.

Factor	Control group (n=60)	Observation group (n=60)	Normal group (n=100)	t/ $\chi^2$ /F value	P-value
Sex					
Male	31 (51.67)	35 (58.33)	58 (58.00)	0.742	0.690
Female	29 (48.33)	25 (41.67)	42 (42.00)		
Age (years)	7.2±1.8	7.5±1.9	7.0±1.5	1.624	0.200
BMI (kg/m <sup>2</sup> )	15.12±1.25	14.82±1.42	14.98±1.62	0.623	0.537
Average course of disease (years)	1.25±0.35	1.28±0.29		0.511	0.610
Seizure frequency (times/years)	2.48±1.22	2.52±1.35		0.170	0.865
Epileptic seizure type					
Simple partial seizure	23 (38.33)	25 (41.67)		1.110	0.893
Complex partial seizure	11 (18.33)	9 (15.00)			
Tonic-clonic seizure	10 (16.67)	11 (18.33)			
Tonic seizure	7 (11.67)	9 (15.00)			
Lennox-Gastaut syndrome	9 (15.00)	6 (10.00)			
Place of residence					
Urban	35 (58.33)	30 (50.00)	58 (58.00)	1.170	0.557
Rural	25 (41.67)	30 (50.00)	42 (42.00)		

## Results

**Clinical data of children.** The clinical data of children in the three groups were analyzed. There were no statistically significant differences in sex, age, BMI and place of residence between the three groups ( $P>0.05$ ). The children in the control group and the observation group were compared. In the control group, the average course of disease was 1.25 years and the seizure frequency was 2.48 times/year; there were 23 children with simple partial seizure, 11 children with complex partial seizure, 10 children with tonic-clonic seizure, 7 children with tonic seizure and 9 children with Lennox-Gastaut syndrome. In the observation group, the average course of disease was 1.28 years and the seizure frequency was 2.52 times/year;

there were 25 children with simple partial seizure, 9 children with complex partial seizure, 11 children with tonic-clonic seizure, 9 children with tonic seizure and 6 children with Lennox-Gastaut syndrome. There were no statistically significant differences in other data between the two groups ( $P>0.05$ ) (Table III).

**Relative expression of serum miR-106b in three groups of children.** The expression of serum miR-106b in children before treatment was detected. Before treatment, the expression of serum miR-106b in the control group and the observation group was significantly higher than that in the normal group, with a significant difference ( $P<0.05$ ); there was no statistically significant difference between the control group and the

Table IV. Relative expression of miR-106b in two groups of children before and after treatment.

Group	Relative expression of miR-106b		t value	P-value
	Before treatment	After treatment		
Control group (n=60)	1.627±0.456	1.454±0.302	7.508	<0.001
Observation group (n=60)	1.586±0.466	1.244±0.256	12.205	<0.001
t value	0.487	4.109		
P-value	0.627	<0.001		

Table V. Comparison of difference in relative expression of miR-106b between the two groups before and after treatment.

Score	Control group (n=110)	Observation group (n=110)	t value	P-value
Difference in relative expression of miR-106b	0.172±0.128	0.342±0.217	5.227	<0.001

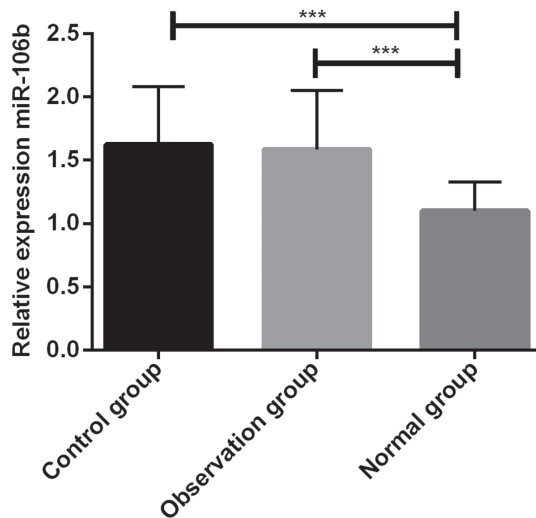


Figure 1. Relative expression of serum miR-106b in groups of children. RT-qPCR showed that there was a difference in the expression of serum miR-106b in children between the groups. The expression of serum miR-106b in the normal group was significantly lower than that in the control group and the observation group ( $P<0.05$ ). There was no significant difference between the control group and the observation group ( $P>0.05$ ). \*\*\* $P<0.001$  indicates that the two groups were compared.

observation group ( $P>0.05$ ) (Fig. 1). The expression of serum miR-106b in the two groups after treatment was significantly lower than that before treatment ( $P<0.05$ ); after treatment, the relative expression of serum miR-106b in the control group was significantly higher than that in the observation group, with a statistically significant difference ( $P<0.05$ ). The difference of miR-106b during the treatment was compared. The difference in the control group was smaller than that in the observation group, with a statistically significant difference ( $P<0.05$ ) (Tables IV and V).

**Diagnostic value of miR-106b in children with epilepsy.** The serum miR-106b before treatment was detected in healthy children and in children with epilepsy. The area under curve (AUC)

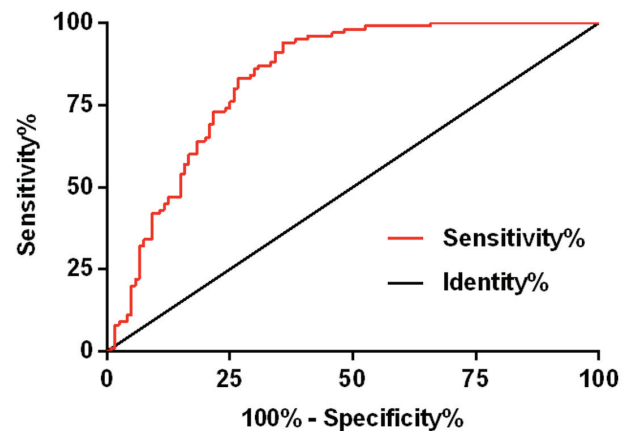


Figure 2. Diagnostic value of miR-106b in children with epilepsy. The ROC curve showed that the AUC of miR-106b was 0.833, 95% CI: 0.779-0.887. The maximum sensitivity and specificity obtained at 1.442 were 94.00% and 64.17%, respectively. The Youden index was 58.17%. AUC, area under curve.

of miR-106b was 0.833, 95% CI: 0.779 to 0.887 the cut-off: 1.442. The sensitivity, the specificity and the Youden index were 94.00, 64.17, and 58.17%, respectively (Fig. 2).

**Relationship between clinical efficacy and miR-106b in two groups of children.** The clinical efficacy in the two groups of children was compared. In the control group, there were 8 children cured, 20 children with marked effect, 18 children with effect and 14 children with invalidity. In the observation group, there were 15 children cured, 27 children with marked effect, 10 children with effect and 8 children with invalidity. The results of comparison showed that the clinical efficacy in the observation group was significantly better than that in the control group, with a statistically significant difference ( $P<0.05$ ) (Table VI). The relationship between miR-106b and different clinical efficacy was analyzed. Spearman test showed that the expression of miR-106b gradually decreased with the continuous improvement of the clinical efficacy ( $P<0.05$ ) (Table VII).

Table VI. Clinical efficacy in two groups of children.

Groups	Cured	Markedly effective	Effective	Invalid	Z value	P-value
Control (n=60)	8 (13.33)	20 (33.33)	18 (30.00)	14 (23.33)	-2.525	0.012
Observation (n=60)	15 (25.00)	27 (45.00)	10 (16.67)	8 (13.33)		

Table VII. Relationship between clinical efficacy and miR-106b.

Efficacy grade	n	Relative expression of miR-106b	r value	P-value
Cured	23	1.124±0.394		
Markedly effective	47	1.319±0.284	0.784	<0.001
Effective	28	1.239±0.298		
Invalid	22	1.658±0.207		

Table VIII. ADR statistics.

Groups	Nausea	Vomiting	Hypersomnia	Anorexia	Dizziness	Liver function damage
Control (n=60)	3 (5.00)	4 (6.67)	2 (3.33)	5 (8.33)	3 (5.00)	3 (5.00)
Observation (n=60)	2 (3.33)	3 (5.00)	2 (3.33)	3 (5.00)	2 (3.33)	2 (3.33)
$\chi^2$ value	0.209	0.152	0	0.536	0.209	0.209
P-value	0.648	0.697	1	0.464	0.648	0.648

**ADR statistics.** According to the ADR statistics, in the control group, there were 3 children with nausea, 4 children with vomiting, 2 children with hypersomnia, 5 children with anorexia, 3 children with dizziness and 3 children with liver function damage. In the observation group, there were 2 children with nausea, 3 children with vomiting, 2 children with hypersomnia, 3 children with anorexia, 2 children with dizziness and 2 children with liver function damage. The results of comparison showed that there was no statistically significant difference in the ADR between the two groups ( $P>0.05$ ) (Table VIII).

## Discussion

Epilepsy is a common syndrome in neurology (15). It has been reported that approximately 9 million people are affected by epilepsy in China, the population of which is mainly children and the elderly (16,17). Compared with the elderly, the child's brain is at the developmental stage. Recurrent and transient tonic and clonic seizures for a long time easily cause hypoxia in the brain. Without timely intervention, the neurons of the child are damaged as the disease progresses, seriously affecting the intelligence and the daily life of the child. Therefore, the treatment of pediatric epilepsy is increasingly valued (18).

In this study, LEV and SV were used to treat children with epilepsy, and their clinical efficacy was observed. SV is the most commonly used broad-spectrum antiepileptic drug for the clinical treatment of epilepsy in clinical practice (19). Although the drug is effective, it is metabolized by the liver and

may cause liver function damage in children. Besides, the anti-epileptic treatment process is long and prone to various adverse reactions, which increases the treatment pain and reduces the treatment compliance of the child, resulting in unsatisfactory expected effect (20). Guo *et al* (21), reported that the long-term use of SV has a serious impact on growth and osteopenia in children with epilepsy. LEV is a new broad-spectrum anti-epileptic drug for the treatment of epilepsy (22). There is a study showing that it has a lower effect on liver enzymes and creatine kinase in children (23). The clinical efficacy in the two groups of children after treatment was first observed. The improvement of the disease condition in the observation group was significantly better than that in the control group. In the study by Tan and Appleton (24), 26 children with epilepsy aged under 10 years were treated with LEV alone, and 61% of them had a good response to LEV, with the seizure frequency reduced by at least 50%. In addition, 2 children with refractory epilepsy had no seizure after medication. In this study, the effective rate of LEV combined with SV for the treatment of children was 93.64%, and the efficacy was significantly better than that of single medication. This suggests that the combined medication can effectively improve the condition of the child. Then, the adverse events during the treatment were compared between the two groups. There was no difference in the ADR between the two groups. This suggests that LEV and SV have good efficacy in the treatment of children with epilepsy, and have small adverse reactions.

miR-106b is an important member of the miR-17 family and differentially expressed in tumor and cardiovascular

diseases (25,26). According to Cava *et al* (27), highly expressed in the serum of patients with epilepsy, miR-106b is expected to become a new diagnostic indicator. However, there is no relevant research on whether it can be a prognostic indicator in the treatment of epilepsy. Therefore, in this study, whether miR-106b can be used as a prognostic indicator in the treatment of children with epilepsy was investigated. The expression of serum miR-106b in children with epilepsy was first detected. The expression of serum miR-106b in the control group and the observation group was significantly higher than that in the normal group, consistent with the results of Pitkänen *et al* (28). Subsequently, the ROC curve was plotted. In the study by An *et al* (29), the AUC of miR-106b was 0.786, 95% CI: 0.693-0.824, while the AUC was 0.882, 95% CI: 0.839-0.926 in the study by Wang *et al* (30), consistent with the results of this study. In the present study, the AUC of miR-106b was 0.833, 95% CI: 0.779-0.887. These findings suggest that miR-106b can be used as a potential diagnostic indicator for children with epilepsy. Finally, the correlation analysis between the clinical efficacy and the expression of miR-106b in children was performed. The expression of miR-106b in children after treatment gradually decreased with the improvement of the disease, suggesting that miR-106b is expected to be a potential prognostic indicator for children with epilepsy after treatment.

This study showed the clinical efficacy of LEV combined with SV in children with epilepsy. The combination of the two drugs effectively improves the condition of the child, and does not increase the clinical ADR. The expression of serum miR-106b in children was detected. miR-106b was highly expressed in children with epilepsy. The expression of serum miR-106b in children can be used as a prognostic indicator. However, there are still limitations in this study. First, the children were not followed up for a long time, and the specific improvement of them remains unclear. Secondly, the specific mechanism of LEV combined with SV on improving of the expression of miR-106b in children needs further investigation.

In summary, LEV combined with SV is effective in the treatment of children with epilepsy, and does not increase the clinical ADR. The expression of serum miR-106b in children can be used as a clinical prognostic indicator and a potential diagnostic indicator.

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### Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

### Authors' contributions

JZ wrote the manuscript. JZ and YS were responsible for PCR. YZ and DZ assisted with the clinical efficacy analysis. JC and

XL were responsible for the statistical analysis. All the authors read and approved the final manuscript.

### Ethics approval and consent to participate

The study was approved by the Ethics Committee of Xuzhou Children's Hospital, Xuzhou Medical University (Xuzhou, China). Patients who participated in this research, had complete clinical data. The family members of the children were informed of this study and signed an informed consent form.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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