



# Valproic acid therapy decreases serum 25-hydroxyvitamin D level in female infants and toddlers with epilepsy – a pilot longitudinal study

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## Abstract

To evaluate if valproic acid (VPA) therapy is associated with vitamin D deficiency among infants and toddlers with epilepsy, a cross-sectional clinical study was conducted in 25 children with epilepsy taking VPA. Blood levels of calcium, phosphorus, alkaline phosphatase, and 25-hydroxy vitamin D [25(OH)D] and plasma VPA level were measured at 1- to 3-month intervals. At the initial and final measurements, vitamin D deficiency or insufficiency was recognized in 8 (32%) and 12 (42%), respectively. In girls, a decreasing trend in serum 25(OH)D levels ( $P<0.05$ ) was observed. Polytherapy had a significant negative effect on the longitudinal change of 25(OH)D ( $P<0.05$ ) in girls. In conclusion, our study indicates that a high proportion of girls after VPA therapy had hypovitaminosis D.

**Keywords:** valproic acid, vitamin D, hypovitaminosis D, epilepsy, infants and toddlers

## Introduction

Vitamin D plays a pivotal role in calcium homeostasis regulation, neuroprotection, brain development, and immune response<sup>[1]</sup>. The anticonvulsant effects of vitamin D have been verified in animal models<sup>[2]</sup>, and epidemiologic studies showed a link between vitamin D and epilepsy<sup>[3–4]</sup>.

Hypovitaminosis D is reported in epileptic children treated with antiepileptic drugs (AEDs), particularly

with enzyme-inducing AEDs (EIAEDs) such as carbamazepine and phenobarbital<sup>[5–6]</sup>. However, there are still controversies over the effects of valproic acid (VPA), a type of non-enzyme-inducing AEDs (non-EIAEDs). Some studies suggested that VPA monotherapy had a negative effect on vitamin D levels<sup>[7]</sup>, while others denied that vitamin D levels could be affected by VPA<sup>[8]</sup>. Our earlier meta-analysis provided evidence that long-term therapy with VPA causes a decrease in vitamin D levels in children with epilepsy<sup>[9]</sup>.

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Multiple factors such as age, gender, daily activities and sunlight exposure can affect vitamin D status. In the study of Fong *et al*, female gender and adolescence were significant risk factors for vitamin D deficiency<sup>[10]</sup>. However, few studies evaluate the vitamin D levels among infants and toddlers with epilepsy on VPA in accordance to diverse risk factors. This study aims to examine the longitudinal change of vitamin D levels during VPA treatment and the potential risk factors in infants and toddlers.

## Materials and methods

### Patients

A total of 25 infants and toddlers (defined as younger than 3 years old<sup>[11]</sup>) with epilepsy were included in the retrospective longitudinal cohort study. All participants underwent clinical examination and blood testing at the Children's Hospital of Nanjing Medical University from November 2017 to June 2018. The plasma VPA levels were monitored every quarter at least once during the VPA treatment. The exclusion criteria are shown in **Fig. 1**. The study was approved by the Research Ethics Committee of the Children's Hospital of Nanjing Medical University. The procedures followed the ethical standards of the responsible committee on human experimentation and the Helsinki Declaration.

### Clinical information and anthropometry

Epidemiologic and clinical data were recorded for every patient including sex, age, brain magnetic

resonance imaging (MRI), seizure outcomes, dosage, drug levels, and duration of therapy.

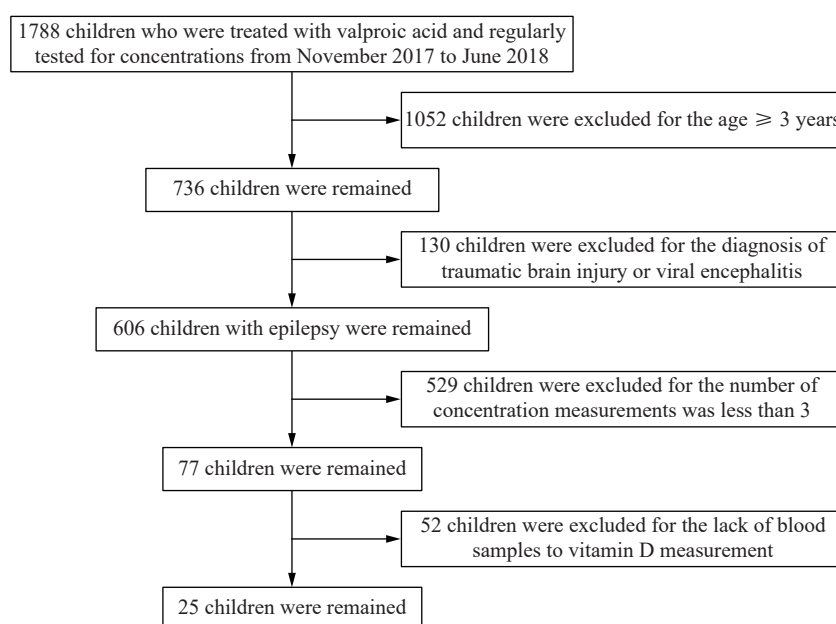
### Definitions

Patients taking VPA only were classified as monotherapy and those taking VPA simultaneously with levetiracetam and/or topiramate were defined as polytherapy.  $C_0/D$  was defined as trough concentration to dose. Seizure outcomes were classified as seizure free versus no seizure free.

### Biochemical analysis

Calcium, phosphorus, and alkaline phosphatase levels in plasma were measured during fasting by standardized methodologies.

The levels of serum 25(OH)D of all patients were measured at 1- to 3-months interval thereafter at least 3 times (every quarter once) from November 2017 to June 2018 by Triple Quad 4500MD liquid chromatography-tandem mass spectrometer equipped with an atmospheric pressure chemical ionization heated nebulizer ion source (AB Sciex, Toronto). In brief, the mass spectrometer was operated in the positive mode. The ion source gas, curtain gas, collision gas, nebulizer current, and source temperature were set at 60 psi, 35 psi, 6 psi, 5  $\mu$ A, and 550 °C, respectively. The precursor-product ion pairs used for multiple reaction monitoring of 25(OH)D<sub>2</sub>, 25(OH)D<sub>3</sub>, 25(OH)D<sub>2</sub>-d<sub>6</sub>, and 25(OH)D<sub>3</sub>-d<sub>6</sub> were  $m/z$  395.4→269.0 (declustering potential [DP], 50 V; entrance potential [EP], 10 V; collision energy [CE], 23 V; collision exit potential [CXP], 14 V),



**Fig. 1** Numbers of children who were eligible for the study.

383.4→257.2 (DP, 70 V; EP, 10 V; CE, 23; CXP, 13 V), 401.4→269.0 (DP, 76 V; EP, 10 V; CE, 28; CXP, 14 V), and 389.3→263 (DP, 70 V; EP, 10 V; CE, 23; CXP, 13 V), respectively. The assay precision (<10.7%) and accuracy (88.3% to 108.4%) was satisfactory.

The United States Endocrine Society criteria for classification of vitamin D status were applied<sup>[12]</sup>. Vitamin D deficiency was defined when 25(OH)D levels were lower than 20 ng/mL (<50 nmol/L), Vitamin D insufficiency when 25(OH)D levels were 21 to 29 ng/mL (50 to 75 nmol/L) and Vitamin D sufficiency when 25(OH)D levels reach or exceed 30 ng/mL (>75 nmol/L). The  $\Delta 1$  value of 25(OH)D [ $\Delta 25(\text{OH})\text{D}1$ ] was investigated as the value of the middle measurement minus that of the initial measurement. The  $\Delta 2$  value of 25(OH)D [ $\Delta 25(\text{OH})\text{D}2$ ] was investigated as the value of the final measurement minus that of the initial measurement.

### Statistical analysis

Statistical analysis was applied by SPSS 15.0 (SPSS Inc., USA) and the figures were drawn using GraphPad Prism 5.0 (GraphPad Software Inc., USA). The children's demographic characteristics in the girl and boy groups were compared using the chi-square test for categorical variables. Comparison of continuous data between two groups was analyzed by independent sample *t*-test or Mann Whitney *U*-test according to the nature of the data. Comparison of continuous data among more than two groups was analyzed by ANOVA or Kruskal-Wallis test. For the

$\Delta 25(\text{OH})\text{D}1$  and  $\Delta 25(\text{OH})\text{D}2$  comparison in girls, a paired *t*-test was used to evaluate the significant risk factors between the corresponding values. A general linear correlation model was used to examine the associations between VPA dose and concentration and to test the other relationships between two continuous variables. *P* values <0.05 were considered statistically significant.

## Results

### Characteristics of patients

This retrospective study included a total of 25 children (12 boys and 13 girls) with epilepsy, at a mean age of 15.28 months (SD, 5.89 months). The average duration of VPA therapy was (7.92±2.86) months. Among the study participants, 10 patients had brain lesions on MRI, and 15 patients had normal brain MRI findings. Ten children were receiving monotherapy, and 15 children were receiving polytherapy. The demographic profiles of the boys and girls are presented in **Table 1**.

**Table 2** shows the biochemical characteristics associated with longitudinal change of vitamin D status in the patients. There were no statistically significant differences in calcium, phosphorus and alkaline phosphatase levels between different groups.

### 25(OH)D status and the change from the initial measurement to the final measurement

The 25(OH)D levels were (37.32±11.99) ng/mL and (35.56±11.61) ng/mL at the initial and middle

Variable	Girl (n=13)	Boy (n=12)	<i>P</i>
Age (months)	13.5 (6–27)*	17.2 (7–29)*	0.318
Brain MRI			>0.999
Normal	8	7	
Abnormal	5	5	
AEDs			>0.999
Monotherapy	5	5	
Polytherapy	8	7	
Seizure outcomes			0.688
Seizure free	7	8	
No seizure free	6	4	
VPA dose (mg/kg)	25.4±6.4 <sup>#</sup>	29.8±8.4 <sup>#</sup>	0.012
Duration of VPA therapy (months)	8.23±3.35 <sup>#</sup>	7.58±2.31 <sup>#</sup>	0.810

\*These data are expressed as mean (range); <sup>#</sup>These data are expressed as mean±SD. MRI: magnetic resonance imaging; AEDs: antiepileptic drugs; VPA: valproic acid.

	Girl (n=13)				Boy (n=12)			
	Initial	Middle	Final	P	Initial	Middle	Final	P
Calcium (mmol/L)	2.45±0.08	2.44±0.08	2.45±0.07	0.957	2.44±0.09	2.43±0.07	2.37±0.08	0.147
Phosphorus (mmol/L)	1.76±0.23	1.82±0.11	1.78±0.12	0.670	1.70±0.27	1.84±0.23	1.80±0.18	0.282
ALP (U/L)	229.9±94.0	271.5±129.5	308.7±230.0	0.579	176.3±64.8	198.4±69.8	219.3±79.4	0.351
Serum VPA level (µg/mL)	75.9±31.7	69.0±25.0	73.2±21.4	0.797	52.7±18.7	63.0±10.8	70.0±19.8	0.053

All values in mean±SD. ALP: alkaline phosphatase; VPA: valproic acid.

measurements respectively. A decreasing trend was observed at the final measurement ([31.07±9.98] ng/mL) (**Fig. 2A**). The vitamin D deficiency or insufficiency status was 32%, 44%, and 48% at the initial, middle, and final measurements respectively (**Fig. 2B**).

Interestingly, the status of 25(OH)D levels showed a longitudinally significant decline in girls, but no significant changes in boys (**Fig. 3A** and **B**). The vitamin D deficiency or insufficiency status in these female patients was 23.1%, 53.8%, and 61.5% at the initial, middle, and final measurements respectively (**Fig. 2B**). The 25(OH)D status of two girls was decreased to deficiency (**Fig. 3B**, right).

Potential risk factors that might reduce vitamin D levels from the onset of VPA treatment in girls were

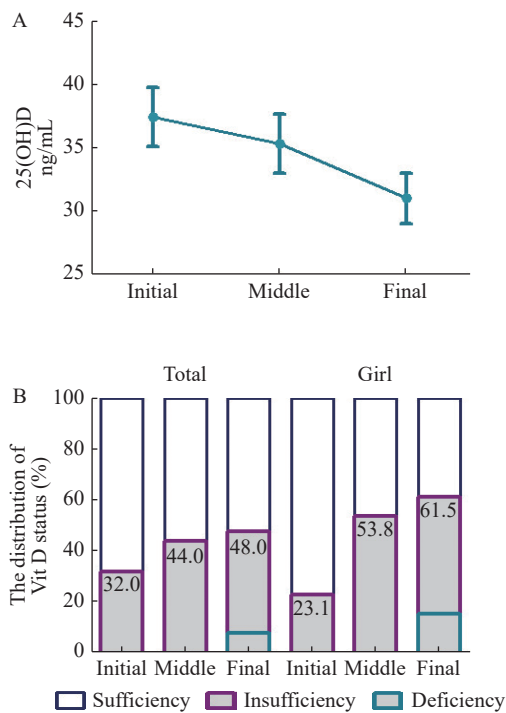
shown in **Table 3**. Polytherapy had a more negative impact on 25(OH)D levels than monotherapy. The remaining factors did not contribute significantly to the models (**Table 3**). In addition, there was a weak negative association between 25(OH)D levels and plasma trough concentration to dose ratio ( $C_0/D$ ) of VPA in the whole cohort (**Fig. 4A**, left). The correlation was stronger in girls, but no difference in boys (**Fig. 4A**, middle and right). The weak negative association was also found in 25(OH)D levels and duration of VPA therapy (**Fig. 4B**, left). The correlation was stronger in girls (**Fig. 4B**, middle) while no difference in boys as well (**Fig. 4B**, right).

## Discussion

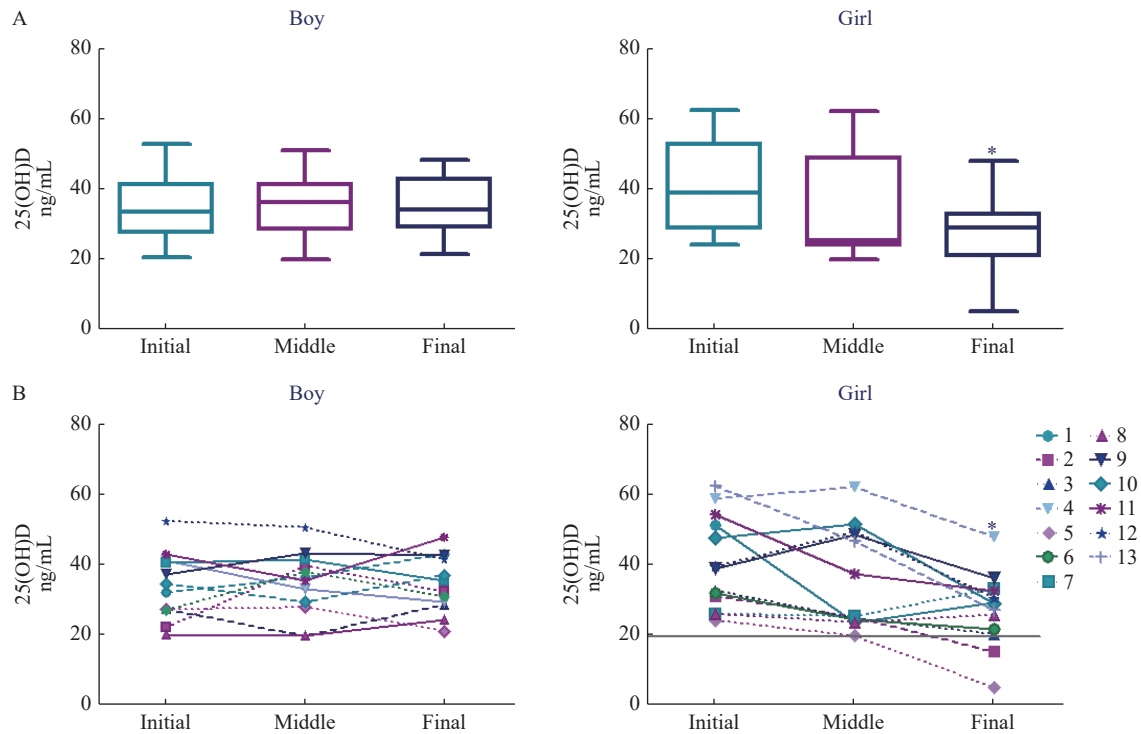
This study investigated the effect of VPA therapy on vitamin D status in pediatric patients with epilepsy. Unlike other studies conducted on this subject, our study is one of the scarce reports to record the continuous changes (3 measurements) occurring in the levels of vitamin D during VPA treatment in infants and toddlers (< 3 years old).

VPA, a type of non-enzyme-inducing antiepileptic drugs (non-EIAEDs), is a widely used primary medication in the treatment of epilepsy in children<sup>[13]</sup>. Studies on its adverse effects on vitamin D status in epileptic children are inconsistent. Several publications concluded that VPA had no effect on vitamin D levels compared to control children<sup>[7,14]</sup>. There was no longitudinal change of 25(OH)D levels before and after VPA treatment as well<sup>[8,15]</sup>. However, some other studies showed that VPA therapy was associated with an increased risk of vitamin D deficiency<sup>[16-17]</sup>. In this study, we observed vitamin D deficiency or insufficiency status within a 6-month longitudinal decrease by 6.24 ng/mL from the initial to the final measurements of 25(OH)D levels in pediatric epilepsy treatment with VPA.

Vitamin D levels are affected by age<sup>[18]</sup>. Earlier studies showed that adolescent age was a significant



**Fig. 2 25(OH)D status in infants and toddlers with epilepsy after taking VPA.** A: The mean 25(OH)D levels at the initial and middle measurements respectively. B: The vitamin D deficiency or insufficiency status in all children and girls.



**Fig. 3** The longitudinal change of the mean of 25(OH)D levels in boys and girls with epilepsy after taking VPA. A: The status of 25(OH)D levels showed a longitudinally significant decline in girls,  $P < 0.05$ . B: The vitamin D status in two female infants was decreased to deficiency at the final measurement during the treatment with VPA.

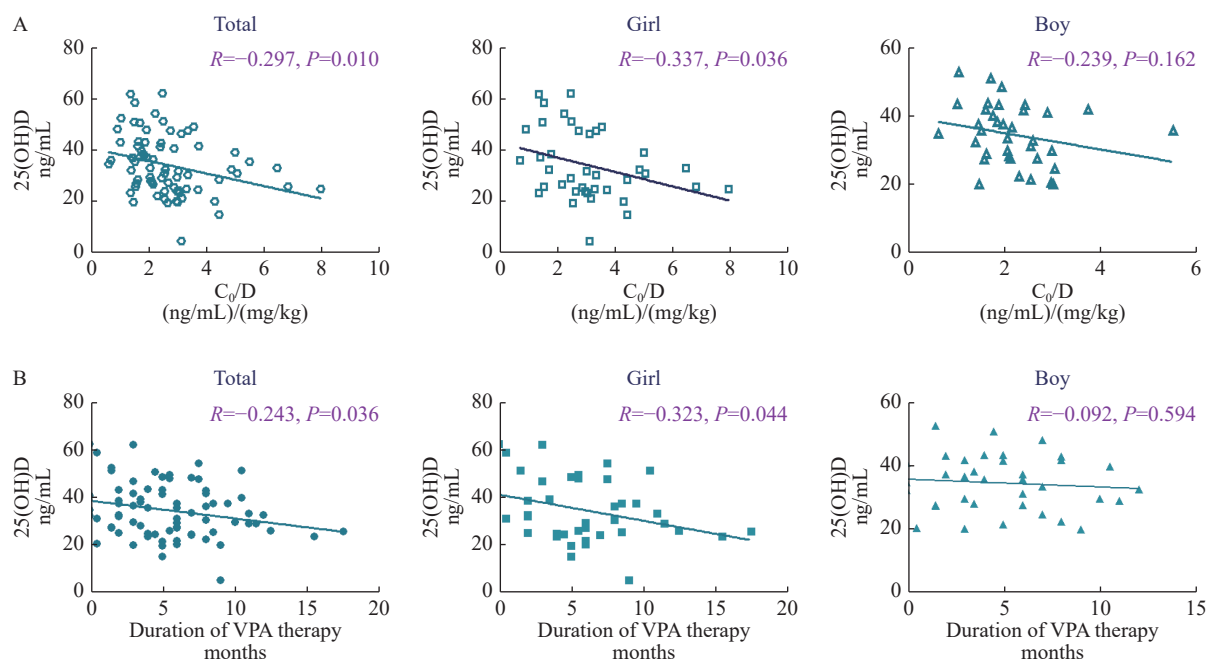
**Table 3** Comparisons of the coupled variables in the longitudinal decrease 25(OH)D in girls

	$\Delta 25(\text{OH})\text{D}1^*$ (mean, ng/mL)	$\Delta 25(\text{OH})\text{D}2^\#$ (mean, ng/mL)
<b>Brain MRI</b>		
Normal	-2.31	-11.85
Abnormal	-7.01	-13.24
<i>P</i>	0.482	0.787
<b>AEDs</b>		
Monotherapy	1.79	-2.95
Polytherapy	-7.81	-18.88
<i>P</i>	0.133	0.003
<b>Seizure outcomes</b>		
Seizure free	-3.03	-8.20
No seizure free	-5.06	-15.97
<i>P</i>	0.757	0.196
<b>Duration of VPA therapy (months)</b>		
$\leq 6$	-10.23	-17.72
$> 6$	1.11	-7.81
<i>P</i>	0.062	0.091

\* $\Delta 25(\text{OH})\text{D}1$  was investigated as the value of middle measurement minus that of the initial measurement.  $^\# \Delta 25(\text{OH})\text{D}2$  was investigated as the value of final measurement minus that of the initial measurement. MRI: magnetic resonance imaging; AEDs: antiepileptic drugs; VPA: valproic acid. Bold font presents  $P < 0.05$

risk factor for vitamin D deficiency in children with epilepsy taking anticonvulsants<sup>[10,19]</sup>. However, another study indicated that there was no difference in ages between cases with vitamin D deficient status and cases with normal vitamin D levels<sup>[17]</sup>. This study found that there was a decreasing trend of 25(OH)D levels in infants and toddlers (less than 3 years old) after VPA therapy. Considering the pivotal role of vitamin D in neuroprotection and brain development<sup>[3]</sup>, more attention needs to be paid to the side effects of vitamin D deficiency in epileptic infants and toddlers.

In addition, a longitudinally significant decline of 25(OH)D levels was found in girls in our study, but not in boys. In an earlier study assessing the vitamin D status of the US population, females, not males, had a relatively higher prevalence of low 25(OH)D concentrations<sup>[20]</sup>. Results from Fong *et al* showed that 25(OH)D deficiency was significantly greater in female patients (31.6%) than in male patients (16.4%) in Malaysian children with epilepsy because of lifestyle behavioral factors<sup>[10]</sup>. Moreover, the girls with polytherapy (VPA combined with levetiracetam and/or topiramate) had a significant negative impact on 25(OH)D levels compared to those with monotherapy. Previous studies confirmed that polytherapy was a risk factor for vitamin D deficiency in patients with epilepsy<sup>[21-23]</sup>. Additionally, a negative



**Fig. 4** Correlation analysis between 25(OH)D levels and VPA therapy. A: The association between 25(OH)D levels and plasma trough concentration to dose ratio ( $C_0/D$ ) of VPA. B: The association between 25(OH)D levels and duration of VPA therapy.

association between 25(OH)D and  $C_0/D$  of VPA was observed in girls in this study. This finding was consistent with a previous study showing a negative association between 25(OH)D and VPA levels<sup>[16]</sup>. All these studies confirmed that patients receiving treatment with VPA had a higher tendency towards vitamin D deficiency. On the other hand, it was well established that vitamin D has a regulatory role in endocrine alterations and vitamin D deficiency has been shown in a relatively high prevalence in patients with polycystic ovarian syndrome<sup>[24]</sup> (a common female endocrine disorder associated with VPA therapy). Therefore, VPA therapy decreased vitamin D levels in our study partially confirmed the broad spectrum of VPA on the endocrine disturbances<sup>[25]</sup>.

Furthermore, Dravet syndrome, one of the most severe epilepsy syndromes in early childhood, was diagnosed in two infants (one 9-month-old girl and one 12-month-old boy). Their decline of 25(OH)D was by more than 10 ng/mL from the initial measurement to the final measurement, especially the vitamin D levels in this girl decreased to deficiency. Thiel and colleagues suggested that nutritional interventions including vitamin D might be used as an alternative treatment to those with this type of epileptic disorders<sup>[26]</sup>. In addition, the vitamin D status in another female infant was from insufficiency at the initial measurement (24.4 ng/mL) to deficiency at the final measurement (5.39 ng/mL) during the treatment with VPA. She was diagnosed with epilepsy with brain hypoplasia. Increasing evidence showed vitamin

D can influence fundamental processes for brain development<sup>[27-29]</sup>, and in this case vitamin D insufficiency or deficiency might be a potential risk factor to aggravate brain hypoplasia.

The main limitation of this study is that the sample size was small and basal biochemical analysis was not measured before VPA therapy. In addition, comprehensive dietary assessments, daily activities and sunlight exposure were reported to play important roles in determining vitamin D levels. The lack of these data was another limitation of this pilot study. However, the study is valuable because it is a longitudinal study and was performed in a population of infants and toddlers.

In conclusion, this study indicates that a longitudinal decrease in 25(OH)D levels was found in infants and toddlers with epilepsy after VPA treatment, especially in female patients. Therefore, monitoring of vitamin D status should be performed regularly during VPA therapy and vitamin D supplementation should be warranted according to individual basis, which is important in children with developmental delay or mental retardation.

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