Original Research Article

Effects of Levetiracetam Monotherapy on Vitamin D Status and Serum Calcium in Children with Epilepsy

Abstract

Background: Epilepsy is a common neurological disorder that affects people of all ages, races, and social classes, but it can be especially devastating to a growing child. Adults and children experience fractures 2-6 times more frequently than the general population due to the adverse effects of antiepileptic drugs on bone mineral density or vitamin D (Vit. D) levels. **Aim:** This study aimed to investigate the effect of levetiracetam monotherapy on Vit. D status and minerals of bone metabolism (serum calcium (S.Ca.), phosphorus (P), and alkaline phosphatase (Alk. P.) in epileptic children. **Patients:** Fifty children with epilepsy (27 boys and 23 girls, mean age 7.78 ± 3.66 years), who had generalized (19) or partial seizures (31) and had been treated with levetiracetam monotherapy for at least six months or more, were enrolled in this study. Another thirty healthy children (16 boy and 14 girl, mean age 6.37 ± 3.45), were chosen to serve as a control group. **Results:** The mean S.Ca. , P, and 25-OH Vit. D levels were significantly lower in epileptic children treated with levetiracetam than in healthy control children (p-value <0.05), but the mean serum Alk. P. level was significantly higher (p-value < 0.01). This study also showed that there were significant inverse correlations between the serum 25-OH Vit. D levels and both the serum levels (p-value < 0.001) and the duration (p-value < 0.013) of levetiracetam treatment in the epileptic children.

Conclusion: Vit. D deficiency is common in epileptic children without abnormal underlying conditions who have received levetiracetam as a monotherapy for at least six months.

Keywords: Levetiracetam, Monotherapy, Vitamin D, Serum Calcium, Epilepsy.

Introduction

Epilepsy is a common neurological illness that affects people of all ages, ethnicities, and social levels, but can be especially detrimental to a child's development. Epilepsy

can have implications that extend beyond the incidence of seizures [1].

The implications of Vit. D insufficiency on bone health in epileptic children are becoming a major health care issue. Additionally, Vit. D insufficiency has been linked to cancer, multiple sclerosis, asthma, cardiovascular disease, and type 1 diabetes [2].

Antiepileptic drugs (AEDs), particularly when used for long periods of time, have been linked to significant metabolic effects, including decreased bone mass and increased bone fractures. AEDs are a class of drugs that can cause Vit. D catabolism and hypocalcemia. As a result, hypovitaminosis D with antiepileptic drug treatment is a widespread issue. [3].

Vit. D is an important component that regulates calcium and P levels in the body. Recent findings implicating Vit. D in cell proliferation and differentiation, as well as its central role in immunological and nervous system responses, have brought to light the significance of this nutrient [4].

Children and adolescents treated with antiepileptic drugs are known to have bone metabolism problems, lose bone mineral density, and have a fracture risk that is 2-3 times higher than healthy children [5].

Among recently developed AEDs is levetiracetam (LEV), which is safe, well tolerated, and effective in the control of several types of seizures. However, the effect of LEV on Vit. D and bone mineral density is unclear, and there have been few studies reporting the effect of LEV monotherapy on Vit. D status and bone mineral metabolism in epileptic children [6].

Patients and methods:

This cross-sectional study was conducted over one year, from December 2020 to December 2021, at the Neurology Unit, Pediatric Department, Tanta University Hospital. The Ethical Committee of the Faculty of Medicine at Tanta University approved the study, and all participants' carers provided written, informed consent.

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Patients:

Fifty children with epileptic seizures (19 with generalized seizures and 31 with partial seizures) who had been treated with levetiracetam monotherapy for at least six months

or more were chosen from the Pediatric Neurology OPD and enrolled in this study. The diagnosis of epilepsy had been made according to the criteria of the International League against Epilepsy [7]. This study also included 30 healthy children who served as the control group. Those children had no history of metabolic bone disease, renal, hepatic, or endocrine diseases, as well as no history of drug use such as Vit. D, calcium, or any vitamin supplements at least 6 months prior to the study.

Inclusion criteria:

1. Epilepsy in children treated with levetiracetam monotherapy for at least sixmonths.
2. Patients with generalized seizures, partial seizures or partial seizure with secondarygeneralization.
3. Age ranged from 3 to 16 years with no sexpredilection.

Exclusion criteria:

1. Patients with degenerative or neurometabolicdiseases.
2. Metabolic bonediseases.
3. Patients receiving corticosteroids or excessive doses ofvitamins.
4. Children with any endocrinaldiseases.
5. Patients with braintumors.
6. Patients with cerebral palsy.

Methods of the study:-

All the patients were subjected to the followings:

1. **Complete medical history**

   Personal, familial, perinatal, developmental, medical, and vaccine histories were all included in the whole history. A thorough history was obtained, including the age of onset, first presentation, and frequency of seizures, as well as any past medication usage, fractures, and family history of metabolic bone problems.

2. A comprehensive physical examination, including anthropometric measurements and local chest, heart, abdomen, and neurological tests.

Routine neuroimaging and electrophysiological examination for epileptic children

a. Magnetic resonance imaging (MRI)

Epileptic children had MRI to rule out structural reasons such as tumours, abscesses, previous strokes, and mesiotemporal sclerosis.

b. EEG was conducted on epileptic children to evaluate whether the seizure was epileptic or another paroxysmal occurrence, whether it was focal or generalized, and whether it related to a certain diagnosis.

4. Lab. investigation

Blood samples were taken to measure biochemical markers of bone metabolism, including S.Ca. , P, Alk. P., and 25-hydroxyVit. D, for all the children who participated (epileptic and healthy). In addition, a blood sample was collected to evaluate the serum concentration of levetiracetam in epileptic children.

Sampling:

A random 5-ml venous blood sample was withdrawn from each child under complete aseptic precautions. The blood was put into a plain vacutainer tube and was left to clot for 30 minutes. Serum was separated by centrifugation for 15 minutes and divided into two aliquots, one for the immediate assay of S.Ca., P, and Alk. P.. The remaining sera were stored at -20 c until the time of 25-OH Vit. D estimation by the ELISA method and the levetiracetam assay by high-performance liquid chromatography (HPLC).

Methods:

1. **Quantitative determination of calcium in human serum** was supplied by the SPINREACT Company.

Principle of the method:

The measurement of calcium in the sample is based on the formation of a color complex between calcium and o-cresolphtalein in an alkaline medium:

$$Ca^{++} + \text{o} - \text{Cresolphtalein} \xrightarrow{OH^-} \text{colored complex}$$

The intensity of the formed color is proportional to the calcium concentration in the

sample.

2. **Quantitative determination of phosphorus in human serum** was supplied by Biotecnica Instruments SpA Company.

Principle of the method:

Phosphate reacts with ammonium molybdate in sulfuric acid solution to form a yellow P molybdate complex. At 340 nm, the maximum complex absorption occurs. It is proportional to the concentration of inorganic phosphate in the sample.

3. **Quantitative determination of alkaline phosphatase in human serum** was supplied by Biotecnica Instruments SpA company.

Principle of the method:

$$\mathbf{p-Nitrophenylphosphat + H_2o \xrightarrow{ALP} p-Nitrophenol + phosphate}$$

Under alkaline circumstances, colourless p-nitrophenol is transformed to the yellow 4-nitrophenoxide, which has a highly strong hue. Increase in absorbance is proportional to the sample's Alk. P. activity.

4. **Assay of Serum 25 -hydroxyvitaminD:**

Quantitative Determination of 25-OH vit.D level by Enzyme-linked immunosorbent assay (ELISA)method was supplied by PishtazTeb Diagnostics Company.

Principle of the Assay:

The test is based on the ELISA method for competitive inhibition. This method employs monoclonal anti-Vit. D antibody (mAb anti-25-OH Vit.D) coated in microtiter wells. The addition of patient serum and standards, followed by a specific amount of extraction buffer, liberates Vit. D from its binding protein complex (DBP complex). After the first incubation and washing, a consistent quantity of biotinylated 25-OH Vit.D and HRP-conjugated streptavidin are applied to the wells concurrently. The additional reagents (biotinylated 25-OH Vit.D conjugated to streptavidin HRP) compete with the anti-Vit. D antibody binding sites [8, 9].

After incubation, the wells are thoroughly washed to eliminate unbound reagents, then a chromogen substrate solution is added and incubated for 15 minutes, resulting in the formation of a blue hue. The addition of stop solution halts the development of colour. The colour is altered to yellow and spectrophotometrically measured at 450 nm [(10)].

In the test sample, the colour intensity is proportional to the quantity of biotinylated 25-OH Vit.D and inversely proportional to the amount of endogenous 25-OH Vit.D. The content of 25-OH Vit.D in the unknown sample is calculated using a series of Vit. D standards measured in the same manner [(11)].

5. Assay of serum levetiracetam level:

The high-performance liquid chromatography (HPLC) test for levetiracetam was given by BIO-RAD Company with product code 1956690.

Principal of the procedure:

As an internal standard, the serum samples were deproteinized using methanol and spiked with gabapentin. HPLC was done at a flow rate of 1.0 mL/min on a Venusil XBP C18, 250 4.6 mm, 5 m column with a mobile phase of 50 mm potassium dihydrogen phosphate-acetonitrile at a pH of 5.5.

At 205 nm, the UV detector was calibrated, and 10 L of material were injected. The time was 15 mins. in total. Calibration curves were linear (correlation coefficient = 0.9999) for the concentration range of 1–60 g/mL. Inter- and intra-day precision and accuracy exhibited relative standard deviation values of 5% for the concentration range. For routine therapeutic drug monitoring, this method is uncomplicated, quick, cost-effective, reliable, and accurate, and requires minimal sample preparation [(12)].

Statistics

The present study was statistically presented and analysedutilising the mean, standard deviation, student t-test, chi-square, and linear correlation coefficient functions of SPSS V20.

Results

Our study included 50 epileptic children (27 boys and 23 girls) aged 3 to 16 years, with a mean age of 7.78 ± 3.66 years, who had been treated with levetiracetam

monotherapy for at least six months, and another thirty healthy children (16 boys, 14 girls) aged 3 to 15 years, with a mean age of 6.36 ± 3.45 years, who had not received any treatment for at least 6 months before the study, serving as controls. There was no significant difference between the epileptic children and the healthy controls regarding sex and age (P-value > 0.1) **(Table 1).**

Table (1):Comparison between epileptic children treated with levetiracetam and healthy control children as regardssex and age

		Epileptic children	Control children	
Sex	**Male**	27 (54 %)	16 (53.33 %)	0.954
	Female	23 (46 %)	14 (46.67 %)	
Age (Years)		7.78 ± 3.66	6.37 ± 3.45	0.092

Types of seizures among the epileptic group

Regarding types of seizures among the epileptic children, 31 (38%) of them had partial seizures and 19 (62%) of them had generalized seizures **(Table 2).**

Table (2): Types of seizures among the epileptic group

Type of seizures	N (%)
Generalized	19 (38 %)
Partial	31 (62 %)

Laboratory results in the studied groups

The mean S.Ca., P, and 25-OH Vit.D levels were significantly lower in the epileptic children treated with levetiracetam than in the healthy controls (p-value< 0.05). The mean serum Alk. P. level was significantly higher in epileptic children treated with levetiracetam than in healthy controls (p-value <0.01) **(Table 3).**

Table (3):Mean values of serum calcium, phosphorus, alkaline phosphatase, and 25-OH vitamin D levels in the epileptic children treated with levetiracetam and the healthy controls

laboratory data	Cases	Controls	
Ionized calcium (mg/dl)	4.014 ± 0.322	5.148 ± 0.424	**<0.001***
Phosphorus (mg/dl)	4.591 ± 0.472	5.509 ± 0.415	**<0.001***
Alkaline phosphatase (U/l)	506.340 ± 243.621	335.833 ± 53.553	**<0.01***
25-OH vitamin D (ng/ ml)	22.600 ± 9.460	42.178 ± 7.519	**<0.01***

*Statistically significant (P<0.05)

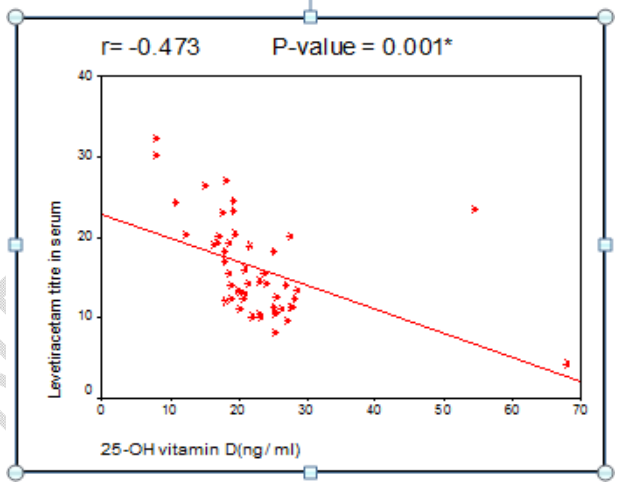
Correlation between serum 25-OH vitamin D level and serum level and duration of levetiracetam treatment in children with epilepsy

Our results revealed that there were significant inverse correlations between serum 25-OH Vit.D level and both serum level (r = 0.473), p-value = 0.001) and duration (r = 0.35, p-value = 0.013) of levetiracetam treatment in the epileptic children **(Table 4, Figures 1 and 2).**

Table (4):Correlation between serum 25-OH vitamin D level and serum level and duration of levetiracetam treatment in children with epilepsy

Levetiracetam	**25-OH vitamin D level (ng/ ml) in the epileptic group (n=50)**	
	R	**P-value**
Serum levetiracetam titre (ug\ml)	-0.473	0.001*
Duration of levetiracetam treatment (months)	-0.350	0.013*

*Statistically significant (P<0.05)



Figure(1) : Correlation between serum 25-OH vitamin D level and serum level of levetiracetam in the epileptic children

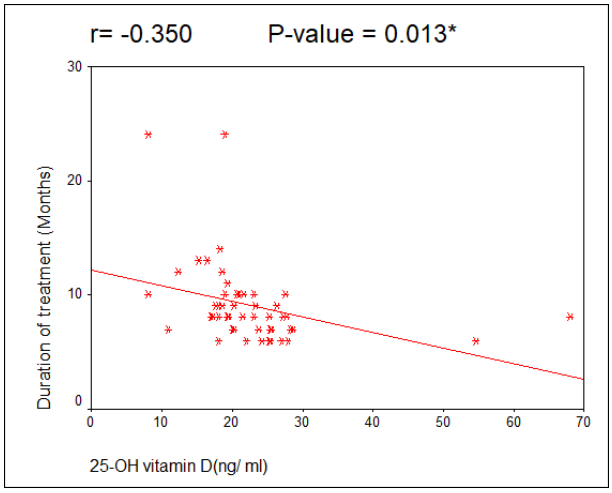


Figure (2):Correlation between serum 25-OH vitamin D level and duration of levetiracetam treatment in the epileptic children

Discussion

The purpose of this study was to determine the effect of levetiracetam monotherapy on Vit. D status and other minerals involved in bone metabolism in epileptic children.

Vit. D is involved in a number of health functions. Aside from its well-known role in bone health, it has a variety of other functions, including cardiovascular health, tumour prevention, immunological function, and glucose metabolism [(13)].

Our study included 50 epileptic children diagnosed in accordance with the criteria of the International League againstEpilepsy [(14)], treated with levetiracetam for at least 6 months. The results showed that the mean serum levels of calcium, P, and 25-OH Vit.D were significantly lower in epileptic children than in healthy control children, and the mean serum Alk. P. level was significantly higher. Furthermore, significant inverse correlations were found between serum 25-OH Vit.D levels and both serum levels and duration of levetiracetam treatment in epileptic children in this study.

The results of our study agreed with the results obtained by Duygu Aksoy et al. [(15),] which showed that the mean S.Ca. and Vit. D levels were significantly lower in the patient group receiving levetiracetam than in the healthy control group.

Vit. D levels were significantly lower in patients receiving carbamazepine, valproic acid, oxcarbazepine, and levetiracetam monotherapy, according to Oya Ztürk et al. [16].

However, Paticheep et al. reported that there were no statistically significant differences in serum 25-hydroxy Vit.D level, P, parathyroid hormone, or Alk. P. between epileptic children on AED monotherapy (levetiracetam, phenobarbital, phenytoin, or valproate) or polytherapy and the healthy control children [17] .

The children involved in this trial were being treated with levetiracetam as monotherapy for focal or generalized epilepsy. It was sought to make both groups (group receiving levetiracetam and control group) as comparable as possible to eliminate confounding variables that may have led to an inaccurate interpretation of the results. In reality, none of the patients exhibited motor deficiencies, mental retardation, or cerebral palsy, nor any ailment that may hinder development, body composition, food intake, or physical activity.

Although previous studies indicated that non-enzyme-inducing AED monotherapy did not result in Vit. D insufficiency [18], all of the participants in our research who received non-enzyme-inducing AED (levetiracetam) had a greater incidence of Vit. D deficiency than the healthy controls.

Currently, there are insufficient clinical data about the Vit. D status associated with the use of newer AEDs (gabapentin, tiagabine, lamotrigine, oxcarbazepine, levetiracetam, lacosamide, topiramate, vigabatrin, stiripentol, zonisamide, etc.), particularly in children [13].

The results of this study confirm that LEV monotherapy for at least six months is linked with considerably reduced blood calcium and 25-OH Vit.D levels, as well as a high incidence of Vit. D insufficiency.

The specific mechanism by which levetiracetam medication affects Vit. D levels in epileptic youngsters is unknown. Several hypotheses have been proposed, including renal tubular dysfunction resulting increased loss of urinary calcium, a direct effect of the drug on bone cell function by inhibiting osteocalcin secretion and the proliferation of osteoblasts, and a reduced intestinal calcium absorbtion that can be attributed to the

decrease in active forms of Vit. D resulting in hypocalcemia, increased bone resorption, and ultimately decreased bone mineral density and accelerated bone loss. These may partially explain why epilepsy patients have a greater fracture risk than the general population [19, 20].

Some authors hypothesised that genetic differences associated with Vit. D receptor polymorphism may affect Vit. D status in epilepsy patients treated with levetiracetam [21].

Limitations

The primary limitation of this research is that it was a cross-sectional study, and no baseline biochemical analysis was performed before to anticonvulsant (levetiracetam) administration.

Moreover, bone mineral density was not assessed. Dual-energy X-ray absorptiometry is the "gold standard" for determining bone mineral density, but the short duration of antiepileptic medication and the risk-benefit analysis do not support measuring bone mineral density in the patients under investigation.

Conclusion

Vit.D deficiency is common in epileptic children who have been on levetiracetam monotherapy for at least six months. Vit. D status should be assessed in epileptic children receiving levetiracetam treatment. The higher the level and the longer the duration of the drug, the more the 25-OH Vit.D level decreases.

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