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ORIGINAL ARTICLE

Bone mineral density loss in ambulatory children with epilepsy in spite of using supplemental vitamin D in Southern Iran: a case–control study

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Abstract

Epilepsy might have adverse effect on bone density due to underlying disease, drugs, vitamin D deficiency, immobilization and malnutrition. We investigated the bone mineral density in ambulatory vitamin-D supplemented children with epilepsy. This case–control study was conducted on 90 epileptic children aged 11.4 ± 3.3 years, and age and gender matched controls in pediatric neurology clinics of Shiraz, in Southern Iran, 2016. Anthropometric measurements, puberty, sun exposure, physical activity and biochemical variables were assessed. Bone mineral density was evaluated by dual-energy X-ray absorptiometry method. Data were analyzed by SPSS.v21. Prevalence of low bone mass in femur was more in patients (27%) than the controls (9%) (P value = 0.002). Age, weight Z score and height Z score were the most significant associated factors on lumbar BMD, BMAD, and femur BMD. Seizure duration and how it responded to anticonvulsants were the most associated factors with both lumbar and femur bone density. Sodium valproate and carbomazepin usage had negative association with lumbar Z score (beta = − 0.216, P = 0.017 and beta = − 0.336, P = 0.027, respectively). We hypothesized that epilepsy per se could affect bone density by an unknown pathophysiology, which was independent from vitamin D deficiency, effects of anticonvulsant and physical activity.

Keywords Bone mineral density · Epilepsy · Vitamin D · Children

Abbreviations

AED	Antiepileptic drugs
BMI	Body mass index
BMAD	Bone mineral apparent density
BMD	Bone mineral density
DXA	Dual-energy X-ray absorptiometry
25OHD	25-hydroxy vitamin D

Introduction

One of the most common chronic neurological conditions in children is epilepsy. Its prevalence is about 4–10/1000, with two incidence peaks during childhood and old age [1]. Childhood is an essential period in acquiring peak bone mineral density (BMD); hence, factors affecting bone mass have the greatest impact during this period [2, 3]. Previous reports revealed that low bone mass and fractures are relatively common among children with epilepsy [2, 4–6]. Many factors have been proposed for bone mineral loss in children with epilepsy [1–3, 6–10]. Epilepsy itself and its underlying diseases, drugs, vitamin D deficiency, immobilization, and malnutrition might have adverse effect on bone density [7], which leads to abnormal bone architecture, and higher risk of bone fractures both during seizures and other times [2]. Among these factors vitamin D deficiency and antiepileptic drugs (AED) were the two most investigated, which could lower both the lumbar and femur BMD [9, 11]. Vitamin D deficiency could be due to immobilization, low sun exposure, and using drugs that induced cytochrome P450 liver

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enzyme [9]. In nearly all the previous reports investigating the children's BMD with epilepsy, the effect of epilepsy itself might have been confused with the effect of other factors, such as vitamin D deficiency, low physical activity, low sun exposure, and vitamin D deficiency secondary to AEDS used to treat epilepsy [6]. To reduce these confounding factors, for the first time we merely included ambulatory children with seizure that were given supplementary 50,000 units of 25-hydroxy vitamin D on monthly bases, without concomitant disease or malnutrition [12]. Also, this study is the first case–control study in southern Iran, which has investigated the bone mineral density of children with epilepsy. Consequently, in this case–control study we aim to investigate the bone mineral density in ambulatory children with epilepsy who were given monthly vitamin D in southern Iran.

Materials and methods

This study is a case–control study, which was performed in the pediatric neurology clinics affiliated to Shiraz University of Medical Sciences, Southern Iran, during 2016. Children with epilepsy who were defined by having at least two unprovoked seizures more than 24 h apart were enrolled in this study. Other inclusion criteria were age 6–18 years and receiving vitamin D supplements. Patients with paralysis and other chronic disorders (e.g. renal failure, liver diseases, endocrinopathies, diabetes mellitus, and other chronic inflammatory disease), history of physical impairment that restricted normal ambulation, and preexisting metabolic bone disease (e.g. Rickets, and hypoparathyroidism) were excluded from the study. Initially, 100 patients were invited to participate in this study, but due to the exclusion criteria, 4 children were excluded, and 6 parents/children refused to participate in this research. Finally, 90 children with epilepsy and 90 age/gender-matched healthy controls participate in this study. Considering the age and patients' gender, the controls were recruited from local schools in Shiraz through an age/gender-stratified randomly selected sampling method. All the children's parents or their guardians, both in case and control groups signed a written informed consent. All children with epilepsy in this study were taking vitamin D_3, 50,000-unit capsule, monthly for at least 6 months prior to the study [12].

Ethical statement

Shiraz University of Medical Sciences local ethics committee and vice-chancellor of research both approved the present research with the grant number of 95-01-49-13302. Written informed consent was signed by all the children's parents/guardians.

Body measurements, body mass index (BMI), pubertal stage, physical activity, and sun exposure

An expert physician evaluated the weight, height, and pubertal stage of all participants. Height was measured, while the child stood without shoes near a standard wall-mounted meter and the height was rounded to the nearest of 0.5 cm. Weight was measured, while the child wore light clothes on a standard scale (Seca, Germany), and the weight was rounded to the nearest of 0.1 kg. BMI was calculated according to the below formula:

$$\text{BMI}\left(\text{kg/m}^2\right) = \text{Weight (kg)}/\left[\text{Height (m)}\right]^2.$$

Puberty was assessed according to the tanner standard staging system [13]. We considered tanner stage 1 as the prepubertal stage, tanner stages 2 and 3 as early pubertal and tanner stage 4 and 5 as late-pubertal stage [13, 14]. Children physical activity was categorized through the recommendation by the American College of Sport Medicine. Sufficient physical activity was defined by having at least 3 days of physical activity per week [15, 16]. Sufficient sun exposure was defined as having more than 30 min/day exposure to sun light [17]. A self-report questionnaire was given to obtain the amount of physical activity and sun exposure, which was filled by the parents/older children themselves.

Biochemical laboratory data

One trained technician took 5 ml venous blood in Shiraz Endocrinology and Metabolism Research Center during the summer of 2016. All the participants were asked to fast 8–12 h before samples were taken. Colorimetric method was used to assay serum calcium, phosphorous, and alkaline phosphatase, using an auto analyzer (Biosystem SA, Barcelona, Spain). Normal serum alkaline phosphatase level in adults are approximately 20–140 IU/L, though they are significantly higher in children with upper ranges approaching 240 U/L for females, 270 U/L for males, during pubertal age. Serum 25-hydroxy vitamin D (25OHD) was measured by electrochemiluminescence method, using Cobas E411 (Roche, Germany). According to the latest global consensus recommendations on prevention and management of nutritional rickets, 25OHD serum level higher than 50 nmol/l (20 ng/ml) was considered as vitamin D sufficient [17, 18].

Bone densitometry

Bone mineral density was evaluated with a Hologic system dual-energy X-ray absorptiometry (DXA) (Discovery QDR, USA). The interpretation of BMD was done using the normative database of Hologic system DXA for children ages 5–23 years, gathered during BMD evaluation in childhood

study (BMDCS) [19]. The coefficient of variation in our center was 0.5% for the lumbar spine and 2.5% for the femur, according to the measurements in ten children. We define low bone mass for chronological age as having BMD Z score less than − 2 for patient's age and gender. To reduce the effect of growth and bone size on bone mineral density, we calculated the bone density per unit volume [20, 21]. Hence, bone mineral apparent density (BMAD) in femoral neck and lumbar area was calculated trough the following formula:

$$\text{Lumbar BMAD} = \text{BMC of } L_2\text{–}L_4/area^{1.5}.$$

$$\text{Femoral neck BMAD} = \text{BMC of femoral neck}/\text{area}^2.$$

Statistical analysis

We used SPSS software, version 21 to analyze our data. Descriptive data were written as mean ± standard deviation and percentage. Student's *t* test and Mann–Whitney test were used to compare quantitative data, and Chi square test and fisher exact test were used to compare qualitative data in two groups. Pearson correlation test was used to evaluate the correlation between the two quantitative variables. Analysis of covariance (ANCOVA) was used to compare each DXA outputs between case and controls adjusted for serum vitamin D level. Binary logistic regression was used to compare prevalence of low bone mass in lumbar or femur regions between case and controls, considering serum vitamin D level. Graphs were prepared using Microsoft office Excel, 2010. Multiple linear regression models with forward stepwise method were used to determine the association between DXA data and some factors. *P* value less than 0.05 was considered to be statistically significant.

Results

Ninety children with epilepsy and mean age of 11.4 ± 3.3 years and 90 healthy age-gender-matched children aged 11.4 ± 3.2 years were included in this study. Total of 52% from each groups were male. Sixty-eight percent of the patients had generalized tonic–clonic convulsion. Prevalence of partial seizure and absence seizure were 13.3% and 7.8%. The most used anticonvulsants were sodium valproate (62%) and carbomazepin (32%). There was no difference in type of seizure and anticonvulsants between both genders ($P = 0.56$ and $P = 0.27$, respectively). Eighty-two percent of the patients used one type anticonvulsant medication, 15.6% used two anti-consultants, and 2% used three or more anticonvulsants, simultaneously. Epilepsy was controlled with medications in 80% of patients, and 18.9% had uncontrolled seizures in spite of using more than one anticonvulsants, which was not associated with gender ($P = 0.395$). Other general characteristics of both the patients and controls are shown in Table 1. Vitamin D deficiency was seen less (27.6%) in patients than the controls (76%), ($P < 0.001$). Table 2 shows the DXA outputs of femur and spine in both patients and the controls, and the results of analysis of covariance (ANCOVA) for each DXA data adjusted for serum vitamin D level. It shows that femur *Z* score in patients was lower that the controls, considering serum vitamin D

Table 1 General characteristic of both patient and control groups and the related comparisons

Variables	Children with seizure disorders (patient group), $n = 90$		Control group, $n = 90$		*P* value
Age (years)	11.4 ± 3.27		11.43 ± 3.2		0.945
Gender (male/female %)	52/48		52/48		1.000
Weight (kg)	37.6 ± 15.9		36.9 ± 13.1		0.739
Weight *Z* score	− 0.6 ± 1.3		− 0.7 ± 1.3		0.279
Height (cm)	143.4 ± 18.1		145.2 ± 15.9		0.466
Height *Z* score	− 0.3 ± 1.3		− 0.44 ± 0.99		0.421
BMI (kg/m^2)	17.7 ± 4.4		16.98 ± 3		0.162
Calcium (mg/dl)	9.9 ± 0.48		9.9 ± 0.41		0.994
Phosphorous (mg/dl)	4.76 ± 0.76		4.05 ± 0.54		< 0.001
Alkaline phosphatase (IU/l)	430 ± 206		367 ± 159		0.192
25OHD (ng/ml)	35.2441 ± 23		16.04 ± 5.6		< 0.001
Vitamin D deficiency (%)	27.6%		76.7%		< 0.001
Sun exposure (%)	Insufficient Sufficient	19% 81%	Insufficient Sufficient	28.0% 72.0%	0.159
Physical activity (%)	Insufficient Sufficient	15% 85%	Insufficient Sufficient	18% 82%	0.543
Puberty (%)	Pre pubertal Early pubertal Late pubertal	51.1% 18.9% 30%	Pre pubertal Early pubertal Late pubertal	48.9% 16.7% 34.4%	0. 8

Table 2 DXA characteristics of femur and lumbar area in both case and control groups and the results of analysis of covariance (ANCOVA) for each data adjusted for serum vitamin D level

Variable	Children with seizure disorders	Control group	Non-adjusted *P* value	Adjusted *P* value
Lumbar BMD (g/cm^2)	0.684 ± 0.17	0.672 ± 0.18	0.354	0.559
Lumbar BMC (g)	29.04 ± 13.2	32.4 ± 15.2	0.122	0.84
Lumbar *Z* score	− 0.99 ± 1.1	− 0.93 ± 1.1	0.729	0.474
Lumbar BMAD (g/cm^3)	0.099 ± 0.02	0.098 ± 0.019	0. 872	0.435
Femur BMD (g/cm^2)	0.688 ± 0.13	0.755 ± 0.12	< 0.001	0.059
Femur BMC (g)	17.56 ± 7.4	19.89 ± 7.7	0.040	0.066
Femur *Z* score	− 1.14 ± 1.07	− 0.67 ± 0.94	0.002	0.041
Femoral neck BMAD (g/cm^3)	0.136 ± 0.039	0.144 ± 0.025	0.107	0.075

BMD bone mineral density, *BMC* bone mineral content, *BMAD* bone mineral apparent density

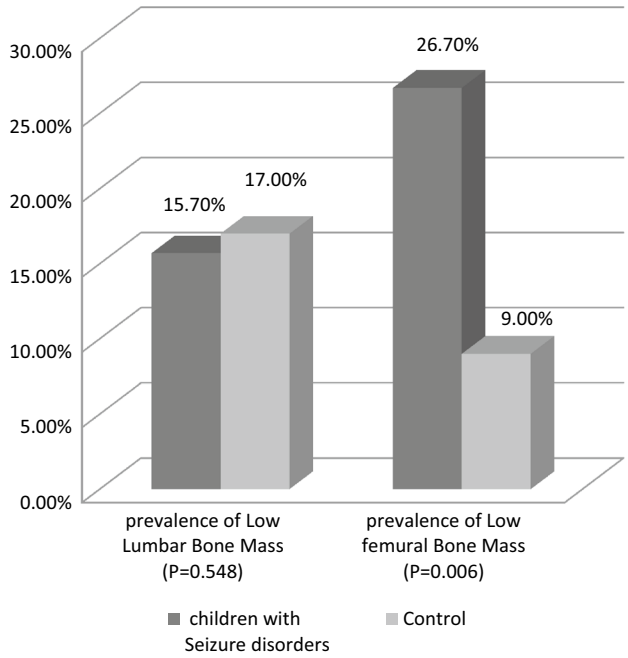

Fig. 1 Prevalence of low bone mass in femur and lumbar bones in both case and control groups, and their comparison adjusted for vitamin D level by binary logistic regression test

($P = 0.041$). Figure 1, shows the prevalence of low bone mass in femur and spine of patients and the controls, and their comparison were adjusted for vitamin D level by binary logistic regression test. It revealed that prevalence of low bone mass in femur area was more in patients (27%) vs. control (9%), regarding the serum vitamin D level with *P* value of 0.006. In addition, prevalence of low bone mass in femur of male patients (23.3%) was not significantly different with female patients (29.8%), ($P = 0.634$), and there was no significant difference between the prevalence of low lumbar bone mass in male patients (17.6%) and females (15.6%) ($P = 0.704$). However, lumbar BMAD in males were lower than females ($P = 0.007$, $b = 0.28$). Table 3 shows the results of association of DXA characteristics of femur and lumbar area and seizure disorder characteristics. It shows that duration of seizure disorder and response of seizures to the anticonvulsant therapy were the most associated factors with both lumbar and femur bone density of patients.

Table 4 summarizes the result of multiple linear regression of DXA outputs and its associated factors. In forward stepwise multiple linear regression model, age, height *Z* score, weight *Z* score, BMI, seizure duration, serum calcium, phosphorous, and vitamin D were entered as continuous variables. Categorical variables were coded 0 and 1 for gender (male = 1), sun exposure and physical activity (sufficient = 1), and using carbomazepin and sodium valproate (usage = 1). Puberty was given three levels; hence, prepubertal and early pubertal stages were converted to dummy coding and were compared to late pubertal stage. It revealed that age, weight *Z* score, and height *Z* score were the most important associated factors on lumbar BMD, BMAD, and femur BMD. Also, it showed that physical activity had positive association with femur *Z* score, and femur BMD was lower in prepubertal stage. Duration of seizure and serum calcium had negative association with lumbar BMAD.

Using sodium valproate and carbomazepin had negative association with lumbar Z score. All the *P* values and standardized coefficient beta of the previous mentioned analyses are shown in Table 4. Prevalence of low lumbar bone mass was higher in patients using sodium valproate ($P = 0.04$) and in patients using carbomazepin ($P = 0.017$); however, prevalence of low femur bone mass was not different in patients using sodium valproate ($P = 0.149$) or carbomazepin ($P = 0.451$).

Discussion

The present study revealed that 26.7% of ambulatory children with epilepsy, in spite of receiving 50,000 units of vitamin D on monthly basis, had low femoral bone mass. However, lumbar bone density in these patients was not less than the healthy controls. In addition, duration of seizure disorder

Table 3 Association of DXA characteristics of femur and lumbar area and characteristics of seizure disorder

Variables	Number of anticonvulsant medications			Response of seizures to the Therapy			Duration of seizure disorder			Type of seizure		
	1	≥ 2	*P* value	Controlled	Refractory	*P* value	< 3 years	≥ 3 years	*P* value	Partial	Generalized	*P* value
Lumbar BMD (g/cm^2)	0.65 ± 0.17	0.66 ± 0.2	0.82	0.67 ± 0.18	0.55 ± 0.13	0.007	0.55 ± 0.12	0.68 ± 0.18	0.004	0.67 ± 0.17	0.59 ± 0.17	0.09
Lumbar BMC (g)	28.6 ± 13.1	30.8 ± 13.6	0.55	30.2 ± 13.3	24 ± 11.8	0.08	20.4 ± 7.6	31.6 ± 13.5	< 0.001	30.3 ± 13.4	25.12 ± 12.12	0.108
Lumbar Z score	− 0.99 ± 1.14	− 1.01 ± 1.2	0.95	− 0.92 ± 1.12	− 1.3 ± 1.25	0.21	− .085 ± 1.01	− 1.04 ± 1.2	0.503	− 0.94 ± 1.06	− 1.18 ± 1.39	0.38
Lumbar BMAD (g/cm^3)	0.1 ± 0.02	0.09 ± 0.02	0.68	0.1 ± 0.03	0.08 ± 0.01	0.023	0.09 ± 0.03	0.1 ± 0.03	0.257	0.1 ± 0.02	0.09 ± 0.03	0.387
Femur BMD (g/cm^2)	0.68 ± 0.13	0.71 ± 0.16	0.51	0.7 ± 0.12	0.63 ± 0.16	0.06	0.62 ± 0.11	0.71 ± 0.13	< 0.001	0.7 ± 0.13	0.64 ± 0.12	0.055
Femur BMC (g)	17.58 ± 7.4	17.48 ± 7.1	0.96	18.2 ± 7.1	14.8 ± 8.1	0.09	12.9 ± 4.8	19.1 ± 7.4	0.006	18.3 ± 7.4	15.17 ± 7.01	0.08
Femur Z score	− 1.17 ± 0.96	− 1.02 ± 1.5	0.59	− 1.1 ± 0.95	− 1.3 ± 1.5	0.492	− 1.01 ± 0.86	− 1.2 ± 1.14	0.73	− 1.06 ± 1.12	− 1.41 ± 0.86	0.187
Femoral neck BMAD (g/cm^3)	0.135 ± 0.04	0.14 ± 0.03	0.55	0.14 ± 0.04	0.13 ± 0.03	0.20	0.13 ± 0.02	0.14 ± 0.04	0.369	0.14 ± 0.03	0.14 ± 0.06	0.434

BMD bone mineral density, *BMC* bone mineral content, *BMAD*: bone mineral apparent density

and response of seizures to the anticonvulsant therapy, were the most associated factors with both lumbar and femur bone density of patients. Also, we showed that carbomazepin or sodium valproate usage can be associated with low lumbar bone mass in epileptic children, in spite of using supplemental vitamin D. Age, gender, weight *Z* score, height *Z* score, duration of seizure and serum calcium, and puberty were the most associated factor with lumbar and hip bone density in these children. Also, we found that lumbar BMAD was lower in male epileptic children.

Previous studies showed that bone density in epileptic patients was lower than the normal population [3, 6, 22]. Many factors have been purposed to be associated with osteopenia in these patients, which includes vitamin D deficiency (e.g. secondary to medications, low sun exposure, and malnutrition), low physical activity and immobility, anticonvulsant medication, and the seizures itself [3, 6]. Also, previous reports suggested that epileptic children should receive extra vitamin D and calcium to increase their bone mineral density [8]. Previous studies in Iran revealed that prevalence of low bone mass in Iranian children aged 9–18 years was relatively high and it was 10.7 and 18.7% in the femoral and lumbar region, respectively [23]. Also, another study showed that 81.3% of the Iranian children aged 9–18 years were vitamin D deficient. They revealed that it might be related to insufficient sun exposure, low physical activity, and genetic factors [17]. Considering these reports, the prevalence of vitamin D deficiency and low bone mass in our control group was similar to the general population. Due to high prevalence of vitamin D deficiency in Iranian children [17], especially with epilepsy [10], we treated the epileptic children with supplemental 50,000 units of vitamin D, monthly. However, this study revealed that the patients in spite of having normal serum vitamin D level still had low bone mass in femur. In line with this study, Sheth et al. showed that epilepsy and its medications induced low bone mineral density, irrespective of vitamin D levels. They revealed that valproate was associated with low BMD in adults regardless of normal serum vitamin D level and alkaline phosphatase [24]. Also, Petty et al. study showed that treatment with calcium and vitamin D alone could not prevent fractures in epileptic patients and suggested further studies about specific bone therapies [9]. In the present study, we found that in ambulatory epileptic children on supplemental vitamin D, there was no decrease in lumbar bone mass; however, there was still a high prevalence of low bone mass in their femur. As a routine, in Epilepsy clinics of our university, all patients are treated for vitamin D deficiency, due to high prevalence of vitamin D deficiency in our general population and risk of vitamin D deficiency as a result of anticonvulsant therapy [10, 17]. This analysis was done when the patients were treated for vitamin D deficiency; hence we did not have access to the

Table 4 Results of multiple linear regressions of associated factors with lumbar BMD, BMAD, and *Z* score, and femur BMD, and *Z* score, femoral neck BMAD, conducted by stepwise method

Variables	Lumbar BMD[c] $R^2=(0.743)$[d]		Lumbar BMAD[b] $R^2=(0.669)$		Lumbar *Z* score $R^2=(0.230)$		Femur BMD[c] $R^2=(0.649)$		Femoral neck BMAD[b] $R^2=(0.149)$		Femur *Z* score $R^2=(0.257)$	
	Beta[a]	*P* value	Beta	*P* value	Beta	Beta	Beta	*P* value	Beta	*P* value	Beta	*P* value
Age	0.817	< 0.001	0.719	< 0.001	–	–	0.336	0.041	–	–	–	–
Gender	− 0.229	0.005	− 0.28	0.007	–	–	–	–	–	–	–	–
Weight *Z* score	–	–	0.23	0.018	0.480	0.001	0.409	< 0.001	–	–	–	–
Height *Z* score	0.384	< 0.001	–	–	–	–	–	–	–	–	0.344	0.01
Physical activity	–	–	–	–	–	–	–	–	–	–	0.356	0.008
Pre-pubertal	–	–	–	–	–	–	− 0.304	0.001	–	–	–	–
Duration of seizure	–	–	− 0.218	0.043	–	–	–	–	–	–	–	–
Calcium	–	–	− 0.253	0.015	–	–	–	–	− 0.386	0.007	–	–
Carbomazepin	–	–	–	–	− 0.216	0.017	–	–	–	–	–	–
Sodium valporate	–	–	–	–	− 0.336	0.027	–	–	–	–	–	–

Only significant data are presented

[a]Beta: Standardized Coefficient Beta

[b]Bone mineral apparent density

[c]Bone mineral density

[d]R squared

25OHD and DXA before the vitamin D treatment. However, this study showed that in spite of vitamin D therapy in epileptic patient, the femoral bone mass was still low. And this is an important alarming sign for the unknown role of epilepsy itself in bone mass reduction. The mean serum alkaline phosphatase in our case and control group were 430 ± 206 and 367 ± 159 IU/L, respectively. This relatively high serum alkaline phosphate level was due to vitamin D deficiency; however, we expected higher levels in vitamin D deficient patients. High prevalence of serum zinc deficiency in Iranian children [25] might be the cause for preventing serum alkaline phosphatase to increase dramatically in our patients [26]. It would be more accurate to measure serum zinc level of our participants, because some previous reports showed that low serum Zinc level seems to be a risk factor for osteoporosis [27] and adding oral administration of zinc preparations has a restorative effect on bone loss under various pathophysiologic conditions [28].

Traditionally, antiepileptic medications have been associated with low bone mineral density in children with epilepsy [1, 29–32]. We found that lumbar bone mineral density was negatively associated with using sodium valproate or carbomazepin, but they were not associated with low femoral BMD. Possible mechanisms of anticonvulsant related osteopenia might include hepatic induction of cytochrome P450 enzymes that leads to increased vitamin D metabolism (e.g. in treatment with carbamazepine), direct effect of anticonvulsant on osteoblasts, impaired calcium absorption, increased homocysteine, inhibition of response to PTH, hyperparathyroidism, reduced gonadal hormones (e.g. anti-androgenic effect of sodium valproate), and metabolic acidosis (e.g. valproate) [1, 29, 31, 33]. Sheth et al. showed that valproate, but not carbamazepine, could reduce axial and appendicular BMD in 26 children with epilepsy. However, patients in Sheth et al. study did not receive supplemental vitamin D [29]. Guo et al. revealed that anticonvulsant effects on bone mineral density was more in epileptic patients with low physical activity [32]; however, our patients were ambulatory and physically active. Also, we found that duration of seizure and therapy was negatively associated with lumbar BMAD. Boluk et al. showed a significant correlation between BMD and duration of valproate therapy [34]. Also, we found that lumbar BMD in male epileptic children with supplemental vitamin D was lower than that in females. Similar to our findings Sheth et al. showed that BMD in both males and females was reduced; however, young males having more than 6 years of epilepsy had the lowest BMD [35]. They also revealed that men appeared to be more susceptible to adverse effects of epilepsy on bones [3]. We showed that prevalence of low bone mass in both genders was higher in femoral area of physically active children given vitamin D supplements. We hypothesize that epilepsy per se could affect bone density by an unknown pathophysiology, which is independent of vitamin D deficiency, effects of anticonvulsant and physical activity. Sheth et al. suggested that both ictal and inter-ictal activities influence the hormonal pulse release, which might include growth hormone and sex hormones, which are both important in growth

and maturation [36]. Further studies are suggested to find the molecular mechanism of epilepsy affecting bone density.

This study has several strong points. To the best of our knowledge, this is the first study in Middle East and Asia that has evaluated the BMD of vitamin D supplemented children with epilepsy, who were physically active, to eliminate the effect of vitamin D deficiency and inactivity on bone mineral density, which are both common in epileptic children. Also, this is the first study in the Middle East to evaluate bone mineral density of children with epilepsy, considering puberty as a confounding factor (Tanner stage), height z scores and weight z scores to have a better estimation of bone density by considering growth and puberty on bones.

Despite these strong points, we had some limitations. We obtained our data via a cross-sectional protocol. Ideally, a longitudinal study design is a better choice to evaluate the effect of epilepsy on bone mineral density of growing children. In addition, to assess skin exposure to sunlight, not only is it necessary to determine the duration spent under the sun, but also the area of skin exposed to sunlight. However, we could not estimate this issue and dietary calcium intake accurately. We suggest to check 25OHD and DXA before vitamin D treatment as well as fracture risk should be taken into consideration in future studies for investigating bone health of children with epilepsy. Also, it would be more accurate to measure serum zinc level in future studies evaluating bone mineral density of Iranian children.

Conclusion

Our study showed that in spite of supplemental vitamin D and physical activity, children with epilepsy have lower bone mass in femur. We showed that anticonvulsant drugs have more effects on lumbar BMD. We hypothesized that epilepsy per se could affect bone density through an unknown pathophysiology, which should be investigated in future studies.

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Author contributions SI: Concept, design, data gathering. MP: Concept, design, data gathering. FS: Concept, design, data gathering, preparing the manuscript and correspondence. PK: design, data gathering of patients. HN: design, data gathering of patients. MHD: data gathering of controls. GHRO: data gathering of controls.

Compliance with ethical standards

Conflict of interest Forough Saki, Soroor Inaloo, MohammadPaktinat, pegah Katibe, Hamid Nemati, Mohammad Hossein Dabbaghmanesh, and Gholamhossein Ranjbar Omrani declare that they have no conflict of interest.

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