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RESEARCH

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Serum level of sclerostin and vitamin D in children with epilepsy

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Abstract

Background Epileptic children can pose an additional risk of poor bone health; this study aimed to evaluate the influence of anti-seizure medications (ASMs) on vitamin D level and sclerostin as a bone turnover biomarker in children with epilepsy.

Subject and methods This case–control comparative study was conducted on 180 children aged from 5–18 years diagnosed with epilepsy according to the definition of the International League Against Epilepsy on ASMs for more than 3 months and were classified into 90 epileptic children on ASM monotherapy and 90 epileptic children on ASM polytherapy, in addition to 90 healthy children age- and sex-matched who served as controls. After obtaining basic data, laboratory investigations were performed, including serum calcium, phosphorus, alkaline phosphatase, parathyroid hormone, vitamin D, and serum sclerostin.

Results When we compared epileptic patients to the control group, there was a statistically significant low level of vitamin D, calcium, and phosphorus and a high level of sclerostin among both epileptic groups with mono or polytherapy. Sclerostin has a statistically significant negative correlation with vitamin D, alkaline phosphatase and parathyroid hormone. Additionally, it has a negative correlation with serum phosphorus, but without a significant correlation. On the other hand, sclerostin has a statistically positive correlation with age and serum calcium, but without a significant correlation. Multiple linear regression analyses were conducted to predict the contributing factors of sclerostin. Only duration of treatment and BMI were significant predictors of high levels of sclerostin. In contrast, the other factors failed to show any significant contribution.

Conclusion The present study showed that ASMs modulate the serum levels of sclerostin and vitamin D hence, might be involved in their adverse effects on bone.

Keywords Epilepsy, Bone health, ASM, Vitamin D, Sclerostin

Introduction

The increased risk of osteoporosis and fractures in patients with epilepsy has long been associated with the use of antiseizure medications (ASMs), leading to increased bone turnover resulting in decreased bone mineral density (BMD) evaluated by dual- energy x-ray absorption (DEXA) [1]. However, the deficiency in BMD is insufficient to explain the increased risk of fracture especially with new drugs that do not affect the cytochrome P450 [2].

The bone's ability to resist fractures comprises both bone quantity, the BMD, and quality, which comprises the mineral composition and the micro-architectural preparation, which cannot be measured by DEXA scan [3].

The Wingless-related integration site (Wnt) signaling pathways are a group of signal transduction pathways that begin with protein that pass signals into a cell through cell surface receptors. The Wnt includes a large

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group of glycoproteins that play a vital role in bone development, metabolism, and homeostasis. Wnt enhance mesenchymal stem cell (MSC) differentiation into osteoblast while suppressing their differentiation into osteoclasts or adipocytes [4].

Dysregulation of Wnt/B-catenin signaling is associated with many neurological disorders including autism spectrum disorders, psychiatric diseases, and epilepsy [5, 6]. Inactivation or mutation of genes implicated in this pathway result in hippocampal agenesis and overall brain malformation [7, 8]. Increased expression of the Wnt signaling component is associated with neurogenesis and neuronal death which observed after seizure [9].

The SOST gene, encodes for the sclerostin protein, is located on the long arm of chromosome 17 (17q12–q21) [10] and is mainly expressed in bone cells. Sclerostin appeared as one of Wnt canonical signaling inhibitors. It has a critical role in the differentiation and proliferation of stem cells by involvement in embryogenesis and morphogenesis [11]. Indeed, sclerostin inhibits the osteoblasts differentiation and reduces bone formation. Besides its antianabolic effects, sclerostin promotes the differentiation of osteoclasts and thus negatively affects bone resorption [12].

Interestingly, osteocytes can directly initiate the osteolytic process because sclerostin regulates the extracellular matrix acidity. Finally, sclerostin negatively modulates the mineralization process directly or indirectly by regulating fibroblast growth factor 23 [13, 14]. Thus, the objective of the present study was to evaluate the influence of ASMs on sclerostin as a marker of bone turnover in children with epilepsy. As well as, to study the correlation between serum sclerostin level with body mass index (BMI) and vitamin D. This is the first Egyptian research to evaluate serum sclerostin in epileptic children.

Patients and methods

Subjects included in the study

This case–control comparative study was conducted in the Epilepsy Clinic Neurology Department, Faculty of Medicine, Zagazig University Hospitals from January 2022 to January 2023. Children diagnosed as epileptic, according to the definition of the International League Against Epilepsy [15], on a different single or multiple ASMs for more than three months were included in this study. They were divided into 2 groups: Group I, ninety patients (49 males and 41 females) epileptic children on single ASM for more than three months; and Group II, ninety patients (45 males and 45 females) epileptic children on more than one ASMs for more than three months. Ninety healthy subjects (44 males and 46 females) matched for age, gender, and ethnicity served as the control group.

This study excluded children aged less than five years or more than 18 years, those with any disease known to impact bone metabolism (such as renal disease and hyperparathyroidism), severe diseases (including cancer, liver impairment), or significant physical disabilities. Additionally, children with congenital bone deformities and recent fractures, those taking medications affecting bone metabolism (like corticosteroids), those using dietary supplements containing calcium or vitamin D, or those following a ketogenic diet with vitamin D were excluded. Furthermore, children with cerebral palsy or experiencing delays in mental or motor milestones were not included in the study.

This study was approved by the local ethics committee of the Faculty of Medicine, Zagazig University, Egypt. Moreover, an informed consent was signed by all patients' guardians before their enrollment in the study.

Methods

All patients were subjected to full history taking including age, gender, and average sunlight exposure hours with stress on the characteristics of epilepsy disorder this involved details on the duration, types, and causes of seizures. Additionally, information was gathered on the ASMs used, including their names, numbers, doses, and duration of usage. Other aspects covered in the history-taking process included developmental history, family history, other neurological symptoms, and symptoms suggestive of involvement of other systems. A complete clinical examination with particular emphasis on anthropometric measurements and a complete neurological examination to exclude neurological diseases other than idiopathic epilepsy.

Brain magnetic resonance imaging (MRI) images were obtained for all patients by T1 weighted images of 1.5 mm slice thickness were acquired in the coronal oblique plane without any gaps in between. Coronal and axial FLAIR sequences with slice thicknesses ranging from 2 to 3 mm and an interslice gap of 0–1 mm), and the findings were documented along with the results of the EEG which was done using 22- channel Digital EEG (Nihon Kohden machine, Nihon Kohden Corporation, Japan.

Laboratory investigations

Venous blood samples were withdrawn from each participant and the blood was left to clot. The serum was then separated by centrifugation and divided into two aliquots. One aliquot was immediately assessed for calcium, phosphorous, alkaline phosphatase, vitamin D and parathyroid hormone. The second aliquot was stored at -20°C for the subsequent assay of sclerostin.

Vitamin D sufficiency was defined as a serum level of ≥ 30 ng/mL. vitamin D insufficiency was defined as a serum level of 20–29 ng/mL while vitamin D deficiency was indicated by a serum level of < 20 ng/ml [16].

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 20 (IBM, USA). Parametric data were compared using an independent student t- test for two groups and a one-way ANOVA test for multiple comparisons for the three groups. A chi-square test was implemented for categorical data. The correlation between variables was evaluated using the Pearson correlation coefficient test (2- tailed). Multiple linear regression analyses were conducted to predict contributors to sclerostin. The level of significance was identified at $P < 0.05$. The sample size was determined using EPI-info version 6 (EPI-info, Atalanta, USA) statistical packages at 80% power and 95% confidence interval.

Results

Table (1) showed that there were no significant differences between epileptic patients and control groups regarding age, gender, BMI or exposure to sun (Table 1). However, among epileptic patients, those with polytherapy had a highly statistically significant, more prolonged disease duration ($P < 0.0001$) and a higher percentage of MRI brain abnormalities (68.9%) ($P < 0.001$) than patients on monotherapy.

Significantly lower levels of vitamin D, calcium, and phosphorus, along with higher levels of sclerostin, were observed in both epileptic groups receiving mono and poly therapy compared to the control group. Additionally, there was a statistically significantly higher value for alkaline phosphatase and parathyroid hormone in the epileptic group with polytherapy compared to the control group. Among the epileptic patients, patients with polytherapy had a statistically significant high level of serum sclerostin, parathyroid hormone, and alkaline phosphatase when compared to epileptic patients on monotherapy (Table 2**).**

Among the epileptic patients' group with monotherapy, a statistically significant difference was detected between patients groups with different types of treatment, regarding the serum vitamin D level, with the lowest level in patients taking carbamazepine treatment (11.1 ± 4.1). Moreover, a highly statistically significant difference was observed between patients regarding serum sclerostin ($P = 0.0004$), with a higher level in patients taking lamotrigine (1050 ± 92.1). Conversely, no significant difference was observed between patient groups with different lines of treatment regarding serum calcium, phosphorus, alkaline phosphatase, or parathyroid hormone **(**Table 3**).**

Figure 1 shows a significant difference between epileptic pateints and controls regarding vitamin D level ($P = < 0.001$). In the control group, 77 people (85.55%) had normal vitamin D levels, while 9 children (10.1%) had insufficient vitamin D levels and only 4 children (4.44%) had deficient vitamin D levels. In the patient group, 35 patients (19.4%) had normal vitamin D level while 49 patients (27.2%) had insufficient levels and 96 patients (53.3%) had deficient vitamin D serum levels.

Table 1 Demographic data of patients and control groups

Demographic data	Children with mono ASM (N = 90)	Children with poly ASM (N = 90)	Control group (N = 90)	P1	P2	P3
Age (years)	10.3 ± 3.4	10.5 ± 3.5	10.1 ± 3.5	0.95	0.72	0.87
Gender				$X^2 = 0.62$, P = 0.73		
Male	49 (55%)	45 (50%)	44 (49%)			
Female	41 (45%)	45(50%)	46 (51%)			
Height (m)	1.34 ± 0.16	1.34 ± 0.15	1.33 ± 0.15	0.81	0.8	1
Weight (Kg)	33.4 ± 10.3	33.2 ± 11.05	32.1 ± 10.8	0.69	0.78	0.98
BMI(Kg\ m^2)	17.9 ± 2.4	17.6 ± 2.8	17.5 ± 2.7	0.54	0.9	0.81
Sun light exposure				$X^2 = 0.407$, P = 0.8		
More than 1 h	61 (67.8%)	59 (65.6%)	63 (70%)			
Less than 1 h	29 (32.2%)	31 (34.4%)	27 (30%)			
Duration of illness (months)	36.4 ± 5.51	61.5 ± 8.2	–	**P < 0.0001***		
MRI abnormality	4 (4.4%)	62 (68.9%)	–	$X^2 = 80.4$, **P < 0.001***		

Data are represented as mean ± SD or number (%)

Data are analyzed using one way ANOVA test followed by Tukey's test for multiple comparisons and chi square test; *P < 0.05 is considered significant. P > 0.05 is considered non-significant

BMI: body mass index; MRI: Magnetic resonance imaging; P1: Control Vs Mono ASM. P2: Control Vs Poly ASM, P3: Mono ASM VS poly ASM group

Table 2 Comparison between patients and control groups regarding serum biochemical parameters

Parameters	Children with monotherapy	Children with polytherapy	Control	P1	P2	P3
Vit D(ng/mL)	21.1 ± 11.9	20.8 ± 10.6	39.2 ± 8.7	**< 0.001***	**< 0.001***	0.98
Serum Sclerostin(pmol\L)	916.5 ± 81.2	950.2 ± 80.4	528.1 ± 77.7	**< 0.001***	**< 0.001***	**0.04***
Serum Calcium(mg/dl)	9.2 ± 0.69	9.1 ± 1.08	10.2 ± 0.71	**< 0.001***	**< 0.001***	0.68
Serum Phosphorus(mg/dl)	5.4 ± 0.45	5.4 ± 0.49	8.1 ± 0.44	**< 0.001***	**< 0.001***	0.79
Alkaline Phosphatase(iu/l)	86.7 ± 19.4	95.2 ± 18.4	85.1 ± 17.9	0.84	**0.006***	**0.02***
Parathyroid hormone(pg/ml)	29.8 ± 9.2	35.5 ± 9.3	30.2 ± 8.7	0.94	**0.008***	**0.003***

Data are represented as mean ± SD

Data are analyzed using one way ANOVA test followed by Tukey's test for multiple comparisons; *P < 0.05 is considered significant. P > 0.05 is considered non-significant

Vit D: Vitamin D; P1: Control VS Monotherapy; P2: Control VS polytherapy; P3: Monotherapy VS polytherapy group

Table 3 Relation between different types of treatment and serum laboratory markers of bone health in monotherapy group

Parameters	Children with monotherapy								P
	VPA (n = 26)	LCM (n = 6)	CBZ (n = 8)	LEV (n = 16)	TPM (n = 4)	LTG (n = 5)	ZNS (n = 12)	OXC (n = 13)	
Vit D(ng/mL)	26 ± 10.3	13 ± 6	11.1 ± 4.1	29.3 ± 9.4	27 ± 10.8	18.8 ± 5.5	12.4 ± 4.7	16 ± 7.4	**< 0.001***
Sclerostin(pmol\L)	909.1 ± 91.1	917.5 ± 91.5	925.5 ± 98.6	922.6 ± 80.7	851 ± 57.2	1050 ± 92.1	949.5 ± 64.8	917.6 ± 63.3	**0.0004***
Calcium(mg/dl)	9.3 ± 0.78	9.1 ± 0.68	9.4 ± 0.6	9.2 ± 0.77	9 ± 0.54	8.9 ± 0.3	9.1 ± 0.59	8.8 ± 2.3	0.9
Phosphorus(mg/dl)	5.5 ± 0.43	5.6 ± 0.38	5.3 ± 0.48	5.5 ± 0.5	5.6 ± 0.61	5.4 ± 0.25	5.2 ± 0.5	5.4 ± 0.36	0.4
Alkaline Phosphatase(iu/l)	91.6 ± 21.1	91.5 ± 15.2	95.5 ± 21.7	85.8 ± 19.1	99.7 ± 8.6	74 ± 20.1	83.2 ± 18.4	74.3 ± 12.6	0.05
Parathyroid hormone (pg/ml)	28.5 ± 8.2	30.3 ± 9.6	28.1 ± 10.4	29 ± 9.5	32 ± 12.8	33.2 ± 7.1	32.1 ± 10.7	29.8 ± 9.3	0.9

Data are represented as mean ± SD

Data are analyzed using one way ANOVA test followed by Tukey's test for multiple comparisons; *P < 0.05 is considered significant. P > 0.05 is considered non-significant

VPA: valopric acid; LCM: lacosamide; CBZ: carbamazepine; LEV: lvetiracetam; TPM: topiramate; LTG: lamotrigine; ZNS: zonisamide; OXC: oxacarbazepine

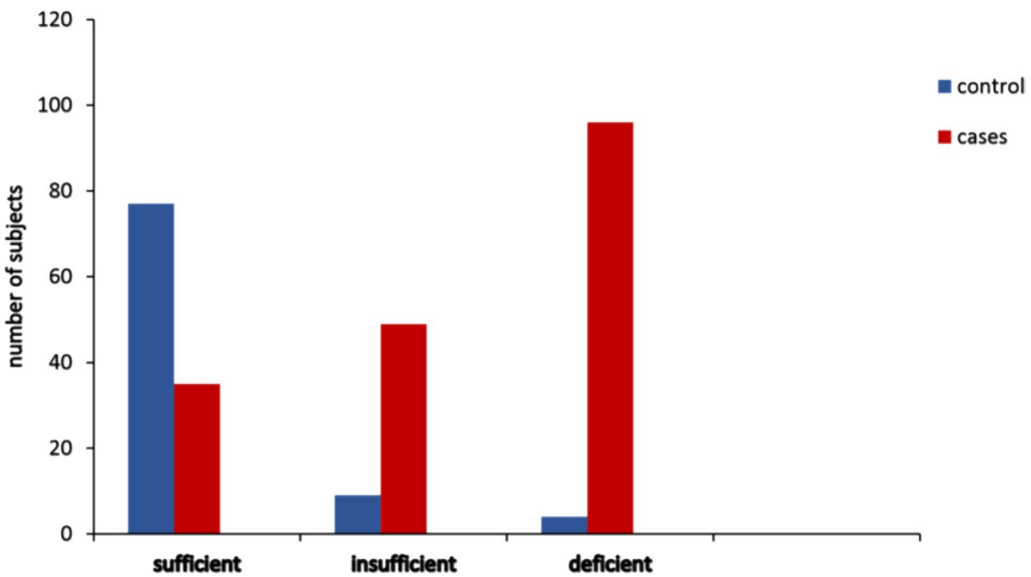

Fig. 1 Vit D distribution among patients and control groups

Table 4 Comparisons between male and female according to the demographics and serum biochemical parameters

Parameters	Male		P1	Female		P2	P3
	Patient (N = 94)	Control (N = 44)		Patient (N = 86)	Control (N = 46)		
Age(years)	10.3 ± 3.3	9.8 ± 3.4	0.47	10.5 ± 3.6	10.4 ± 3.5	0.85	0.69
Weight(Kg)	33.5 ± 10.8	31.1 ± 10.3	0.23	33.3 ± 10.6	33.1 ± 11.2	0.97	0.9
Height(m)	1.34 ± 0.15	1.32 ± 0.14	0.36	1.35 ± 0.16	1.34 ± 0.17	0.87	0.67
BMI(Kg\ m^2)	17.9 ± 2.7	17.3 ± 2.9	0.26	17.6 ± 2.48	17.6 ± 2.6	0.97	0.44
Vit D(ng/mL)	21.1 ± 11.2	39.4 ± 7.8	**< 0.001***	20.8 ± 11.4	39.06 ± 9.5	**< 0.001***	0.86
Serum sclerostin (pmol\L)	913.8 ± 82.4	531.4 ± 78.3	**< 0.001***	932.01 ± 75.5	524.9 ± 77.8	**< 0.001***	0.13
Serum calcium (mg/dl)	9.2 ± 1.06	10.5 ± 0.7	**< 0.001***	9.21 ± 0.69	10.9 ± 0.75	**< 0.001***	1
Serum phosphorus (mg/dl)	5.5 ± 0.46	5.3 ± 0.49	0.13	5.36 ± 0.46	5.43 ± 0.46	0.36	0.4
Alkaline phosphatase (iu/l)	86.7 ± 19.7	87.9 ± 16	0.75	85.6 ± 17.3	82.5 ± 19.4	0.34	0.69
Parathyroid hormone (pg/ml)	30.2 ± 8.9	28.8 ± 8.5	0.37	31.8 ± 9.2	27.9 ± 7.3	0.26	0.24

Data are represented as mean ± SD or number (%)

Data are analyzed using one way ANOVA test followed by Tukey's test for multiple comparisons; *P < 0.05 is considered significant. P > 0.05 is considered non-signifcant

Vit D: Vitamin D; P1: Male patient Vs Male control; P2: Female patient Vs Female control; P3: Male patients Vs Female patients

Regarding gender, there was a statistically significantly lower serum vitamin D and calcium in male and female patients than in controls (P < 0.001). Moreover, there were significantly higher serum concentrations of sclerostin in male and female patients groups than control groups (P < 0.001). However, no significant difference between patient groups, either male or female, and the control group regarding age, weight, height, BMI, serum phosphorus, alkaline phosphatase, or parathyroid hormone was observed (Table 4).

The results revealed that the duration of the disease has a statistically significant negative correlation with serum vitamin D levels (r = —0.82 and P = < 0.001) and calcium (r = -0.7 and P < 0.001) and a significant positive correlation with sclerostin concentrations (r = 0.29, P = 0.04) and alkaline phosphatase (r = 0.4 and P = 0.009). Moreover, it has a negative correlation with serum phosphorus (r = -0.12 and P = 0.08) and a positive correlation with parathyroid hormone (r = 0.07and P = 0.34) but without significant difference.

In addition, BMI correlates significantly positively with serum vitamin D (r = 0.33 and P = 0.03). However, it positively correlates with serum phosphorus, and serum sclerostin, and negatively correlates with serum calcium, alkaline phosphatase, and parathyroid hormone, but without significant difference (Table 5).

Furthermore, sclerostin has a significant negative correlation with vitamin D (r = −0.35 and P = 0.02), alkaline phosphatase (r = −0.5 and P = < 0.001), and parathyroid hormone (r = −0.31 and P = 0.04), while it has a negative correlation with serum phosphorus but without significant correlation. Moreover, it positively correlates with age, and serum calcium but without significant correlation (Table 6).

Table 5 Correlation between BMI and duration of disease with the biochemical parameters

Biochemical parameters	BMI		Duration of disease	
	r	P	r	P
Vit D	0.33	0.03*	− 0.82	**< 0.001***
Serum sclerostin	0.01	0.89	**0.29**	**0.04**
Serum calcium	− 0.03	0.67	− **0.7**	**> 0.001**
Serum phosphorus	0.01	0.82	− 0.12	0.08
Alkaline phosphatase	− 0.05	0.44	0.4	**0.009***
Parathyroid hormone	− 0.04	0.54	0.07	0.34

r: Pearson coefficient; *P < 0.05 is considered significant; P > 0.05 is considered non-significant

Table 6 Correlation between Sclerostin with Age and serum laboratory data of patients

	Sclerostin	
	r	P
Age	0.31	0.04
Serum VIT D	− 0.35	**0.02***
Serum calcium	0.07	0.31
Serum phosphorus	− 0.08	0.28
Alkaline phosphatase	− 0.5	**< 0.001***
Parathyroid hormone	− 0.31	**0.04***

r: Pearson coefficient; *P < 0.05 is considered significant; P > 0.05 is considered non-significant

Table 7 Multiple linear regression analysis for bone turnover according to Sclerostin

Variables	B	SE	P
Duration of treatment	5.2	3.5	**0.009***
Age	0.95	0.067	0.09
BMI	5.7	3.8	**0.03***
Serum VIT D	− 0.27	0.93	0.76
Serum calcium	8.2	6.7	0.21
Serum phosphorus	− 13.5	12.8	0.29
Alkaline phosphatase	− 0.15	0.32	0.63
Parathyroid hormone	− 0.61	0.65	0.34

BMI: Body mass index; B: Estimated unstandardized regression coefficient; SE: Standard error

*P < 0.05 is considered significant

P > 0.05 is considered non-significant

Multiple linear regression analyses were conducted to predict the contributing factors of sclerostin. Only duration of treatment and BMI (P = 0.009 & P = 0.03) respectively, were significant predictors of high levels of sclerostin. Meanwhile, the other factors failed to contribute significantly (Table 7).

Discussion

Epilepsy and bone health relationship has been explored for more than three decades [17–19]. It is known that the risk of fracture in adults with epilepsy is higher than in the general population. This risk increases with age [20, 21].

Although vitamin D deficiency is reported in epileptic patients, there is no evidence-based for routine screening for vitamin D deficiency and bone health in children with epilepsy. We hypothesized that there is a significant relationship between the use of ASMs in epileptic children and serum vitamin D levels and bone health. Thus, we investigated the influence of ASMs on vitamin D status and sclerostin as one of the bone turnover biomarkers.

The prevalence of vitamin D deficiency was 53.3% in our epileptic children. This rate aligns with findings from previous studies in in Egypt such as the one conducted by Elmazny et al. [22] in newly diagnosed pediatric patients with idiopathic generalized epilepsy. In this study, 40% of patients reported having vitamin D deficiency and 38% had vitamin D insufficiency. Moreover, this is in line with Wang et al. [23]; Dong et al., [24] results. However, when we considered both vitamin D deficiency and insufficiency together as hypovitaminosis D, our results indicated that approximately two-thirds of children with epilepsy in Egypt suffer from vitamin D deficiency despite the tropical climate.

Our study showed a significant difference between epileptic children and normal subjects according to the laboratory markers serum level. Patients on poly ASMs therapy had a significantly low level of serum calcium, serum phosphorus, and vitamin D and a significantly high level of serum alkaline phosphatase and PTH than the control group. This aligns with Oner et al. [25]; Hamed et al. [26]; Mintzer [27] studies.

Our epileptic children treated with mono ASM therapy, have a significantly lower value of serum vitamin D, serum calcium, and serum phosphorus when compared to the control group. However, serum alkaline phosphatase and PTH showed no significant difference between the patient and control groups.

In different treatment types, our patients who were treated with carbamazepine (CBZ) had a significantly lower vitamin D level than those on other drug types. A study by Borusiak et al. [28] indicated that ASMs particularly CBZ, are frequently associated with vitamin D deficiency. CBZ, as an enzyme-inducer ASMs, was associated with lower vitamin D levels in epileptic patients [29, 30]. In the same line, evidence from a previous study conducted by Aksoy et al. [31] demonstrated that conventional ASMs such as CBZ had significant effects on bone mineral density (BMD) and vitamin D serum level.

In a meta-analysis conducted by Junges et al. [32] reported that the prevalence of vitamin D deficiency was found to be 33% in epileptic children treated with enzyme-inducer ASMs, compared to 24% for those on non-enzyme inducers.

The ASMs, that induce CYP450, reduce plasma level of vitamin D by increasing its hepatic metabolism [20]. Epileptic patients who were treated with enzyme-inducer drugs need a higher supplement of vitamin D than those receiving non-enzyme inducers [33].

CBZ activates the pregnane X receptor (PXR), whose DNA-binding domain shares 60% homology with the vitamin D receptor (VDR). The PXR has been shown to mediate the induction of cytochrome P450 enzymes involved in drug metabolism. Emerging evidence indicates that the activation of PXR can increase the expression of CYP24, a VDR target gene, both in cultured cells and in vivo in mice. CYP 24 is an enzyme that directs the side chain oxidation and cleavage of 25(OH)2 D3 and 1, 25 (OH)2D3 to carboxylic acid end products (calcitroic acid), thereby reducing cellular concentrations of active vitamin D [34].

Although the number of ASMs used by the child negatively influences their serum vitamin D level [35], in our study, children on mono ASM therapy or poly ASM therapy had a similar risk for vitamin D deficiency. Similarly, many risk factors contribute to vitamin D deficiency. However, epileptic children with poly ASM were at high risk for vitamin D deficiency, elevation of alkaline

phosphatase and parathyroid hormone as compared to children with mono ASM therapy. This is consistent with the opinion of Nettekoven et al. [36].

As commonly recognized, environmental, behavioral, and demographic factors primarily influence vitamin D status. In this retrospective study, age, gender, and BMI were identified as the key determinants. There is an apparent negative association between serum vitamin D levels and patients' age, which aligns with results from previous reports [37–39].

However, there are different results from several studies. Siddiqee et al. [40] and McGillivray et al. [41] reported that vitamin D levels presented a considerable drop in the younger age group. Nevertheless, the ages of the subjects participating in these studies ranged from newborn to 5 years old. However, age is one of the most important determinants of serum vitamin D levels [20, 42].

The effect of gender on serum vitamin D is still controversial. Some reports [40, 43]) have reached different conclusions regarding serum vitamin D levels, with some indicating significantly higher levels in males than females, while other studies find no correlation for both sexes [35, 44, 45]. The effect of sex differences was not observed in our retrospective study. The median serum level of vitamin D in healthy children was approximately 1.5 times higher than that in epileptic children within the same sex group. Interestingly, these differences were noted in serum calcium levels.

Our study proved that a high body mass index and a longer duration of using ASMs were independent risk factors for low serum vitamin D levels. This is in line with another study conducted on Korean pediatric patients which reported a lower level of vitamin D in pediatric group who received ASM for at least two years than those on ASMs for less time. Sixty-five per cent of epileptic patients with vitamin D deficiency were diagnosed with either osteoporosis or osteopenia. Consequently, they concluded that the duration of the ASMs is a major factor in the development of vitamin D deficiency, which leads to epilepsy-related bone disease [46].

In addition, serum calcium concentrations in our control group were in age-specific normal ranges, whereas children under ASMs therapy were found to be significantly below this range, and serum calcium was significantly negative correlated with the duration of treatment. This agrees with Krishnamoorthy et al. [47], who conducted a study on 114 epileptic children received different ASMs who initially had a normal serum level of calcium and vitamin D, they reported a decline in calcium and vitamin D levels after six months of ASM therapy on maintenance doses and a further decrease after 90 days.

Contrarily, Mintzer et al. [27] and Öner et al. [25] reported no decrease in the serum calcium concentrations of children treated with ASMs, while Sato et al. [48] showed increased serum concentrations of ionized calcium in adults treated with valproate. These contrasting results may be due to differences in methodology and/or study populations.

Bone metabolism can be assessed by bone turnover markers which not only reflect the bone quality or fracture risk but also helps understand the pathophysiology of metabolic bone disease and secondary osteoporosis and guides in early prevention and monitoring the response to treatment [49].

The Wnt signaling pathway assists osteoblast differentiation from mesenchymal cells and promotes bone growth and quality. This pathway is regulated by endogenous factors, among which sclerostin is significant. Primarily produced by osteocytes, sclerostin acts as a key player in hindering osteoblast differentiation, thereby inhibiting bone formation [50].

For the first time, this study demonstrated that the Wnt inhibitors are modulated in epileptic children treated with ASMs. Thus, there were higher sclerostin levels in children with epilepsy receiving ASMs when compared with age-matched healthy controls. However, the group receiving lamotrigine as a monotherapy had a more intense elevation in sclerostin serum levels in pediatric epileptic patients. Poly ASMs caused significantly higher serum sclerostin levels than mono ASMs, indicating higher bone resorption.

In the context of experimental data, Parveen et al. [51] reported that higher serum sclerostin were noted in women (20–40 years of age) on monotherapy with ASMs for at least a year compared to age-matched healthy controls. The effect on bone health is via modifying the Wnt signaling mechanisms.

Circulating and skeletal sclerostin negatively relate with histomorphometric and circulating parameters of bone formation [52]. In our study, sclerostin levels were higher after ASM and negatively correlated with alkaline phosphatase, PTH, and vitamin D; this suggests an effect on bone formation through inhibition of the Wnt signaling pathway. Bellido et al. [53] and Keller and Kneissel [54] had reported that osteocytes are crucial target cells for the PTH. Moreover, PTH promotes new bone formation by reducing the expression of sclerostin.

Coppola et al. [55] in a study including children, adolescents, and young adults, found abnormal bone mass density (BMD) in 58% of the patients. Furthermore, 25% of them had osteoporosis, and the remaining 75% had osteopenia. They also indicated that the duration of treatment and the number of ASMs were correlated with abnormal BMD to a greater extent. Moreover, Tekgul

et al. [56] and Tosun et al. [57] reported that polytherapy for epilepsy is related to decreased BMD in children. In parallel, a study by Yaghini et al. [58] indicated that both cytochrome p450- inducing and non-inducing ASMs had caused a reduction in BMD, but there was no significant decrease in BMD in untreated epileptic children.

An animal study conducted by Simko et al. [59] revealed that treatment with LTG decreases the whole-body BMD, specifically in the femurs. This reduction consequently impaired the mechanical strength of the bones in orchidectomized rats.

Lamotrigine induces UDP-glucuronosyltransferase (UGT) enzymes, an isoenzyme of the UGT1 and UGT2 families, that play a vital role in the metabolism of xenobiotics, steroidal hormones, thyroid hormones, fat-soluble vitamins, bilirubin, and biliary acids. This mechanism could explain the effects of LTG on bone health [60].

The literature points to the fact that pediatric patients are at greater risk for metabolic bone disease than adults. It was reported that people who began taking ASMs when they were under the age of 18, had significantly lower bone mass and a higher risk of fractures than patients who started their therapies as adults. The importance of bone growth and bone formation, especially during childhood, is because anything that interferes with the bone formation process causes bone loss in the bones and the risk of bone loss increases in later ages [44].

Simm et al. [61] studied the effect of ASMs on the bone's health in pediatric patients (aged between 5 and 18), including twins or siblings. Compared with controls, the patients who received ASMs displayed a higher frequency of fractures and a clinically significant reduction in trabecular volumetric BMD at the tibia. Appropriate selection of subjects based on age, height, and bone development helps eliminate other factors affecting bone.

According to our results, sclerostin levels are significantly associated with age. This is in context with Mödder et al. [62]; Amrein et al. [63]. Elevation of sclerostin level with age suggests age-related impairment in bone formation, as sclerostin is a potential candidate as a biomarker for bone formation [64]. Because age is a predictor of sclerostin levels, we re-estimated this relationship after adjusting for age. Surprisingly, no significant association was found after controlling this confounder. Therefore, our study suggests that the effects of ASMs on Wnt inhibitors and 25(OH)D may be independent of each other.

Additionally, multiple regression analysis confirmed our results and revealed that duration of treatment and BMI were the strongest predictors of bone turnover by elevated sclerostin serum levels.

It has been found that longer duration of epilepsy is associated with a progressive reduction in bone mineral content in 20–65% of the patients as compared to controls [65]. Furthermore, during epileptogenesis, the canonical Wnt pathway has been implicated affecting neuronal remodeling and the hippocampal dentate gyrus neurogenesis [66, 67]. Thus, the risk of fractures increases with the cumulative duration of exposure to the ASMs and the dysregulation in the Wnt pathway [68].

BMI was positively correlated with serum sclerostin levels [69]. A higher level of sclerostin level was found in subjects with metabolic syndrome, and its level increases significantly with the increasing number of metabolic syndrome components. Additionally, there is a significant positive correlation between sclerostin serum level and waist circumference [70]. Currently, no available clear explanation. One hypothesis could be based on the observation that sclerostin production is decrease in physically active person than less active person. During physical activity, mechanical stress is applied to the skeleton, sensed by the osteocytes, which lead to reduction in sclerostin expression [71]. Another hypothesis is the possible role of sclerostin in adipogenesis [52, 72]. Kim et al. [73] showed that mice overexpressing the SOST gene had excess adipose tissue.

Therefore, it is likely that ASMs might alter bone health via vitamin D-independent effects. However, the exact mechanisms of these pathological changes have not been clearly defined. One theory postulate that AMSs directly affects bone formation by increasing sclerostin secretion, leading to a potent inhibition of bone formation and mineralization [74]. Other possible mechanisms are decreased serum vitamin D level and secondary reduced intestinal calcium transport. Considering these findings collectively, our results support the hypothesis that ASMs induced changes in vitamin D and bone metabolism can be expected to promote bone remodeling over time.

This is a unique study because, to our knowledge, no previous study has evaluated the effect of ASMs on Wnt signaling in child epileptic patients. Some limitations need to be considered when examining the results of this study. Physical activity levels were not assessed in the present study. Both acute and chronic conditions are known to affect bone metabolism, which is associated with increased PTH concentrations. Therefore, the effect of physical activity level on bone metabolism cannot be ignored. Our results need more confirmation by studies with larger samples in different populations.

Conclusion

Pediatric patients seem to be more susceptible to the negative effects of ASMs on bone forming processes. ASMs generally lower vitamin D concentration and usually the decreases are more expressed in patients on

polytherapy, along with alterations in both markers of bone formation and resorption, assuming an accelerated skeletal turnover.

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Author contributions
Sarhan AM, Mahmoud W, Aldarah MJ, and Hashim NA carried out this work. Sarhan AM designed the study and did the statistical analysis. Mahmoud W, Aldarah MJ, and Hashim NA collected the patients, gathered clinical data and wrote the manuscript. All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors read and approved the final version to be published.

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Availability of data and materials
All raw data will be available on the editor request.

Declarations

Ethics approval and consent to participate
The study was approved by the Institutional Ethics Committee of the Faculty of Medicine, Zagazig University. Written informed consent was obtained from all study participants after explaining the details and benefits as well as risks to them. Surrogate consent from the patient's legal guardian or designated health proxy was permitted in cases where the patient did not have decision-making capacity.

Consent for publication
Not applicable**.**

Competing interests
The authors declared that they have no conflicts of interest with respect to the authorship and/or publication of this article.

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