UNCONTROLLED SEIZURES AND BONE HEALTH AMONG ADULT EPILEPSY PATIENTS

BY

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Uncontrolled Seizures and Bone Health among Adult Epilepsy Patients

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Abstract

PURPOSE: Uncontrolled seizures negatively impact the quality of life of epilepsy patients, and bone health represents one of the more serious adverse outcomes of epilepsy and its treatment. The objectives of this study were to determine the association between seizure status and bone metabolism and to determine the association between seizure status and the history of fractures.

METHODS: A retrospective case-controlled study was conducted. Patient data was collected at the Comprehensive Epilepsy Center at the University of Kansas Medical Center (CEC KUMC). Adult patients with a positive diagnosis of epilepsy (age range 21-50) treated with at least one antiepileptic drug for more than six months were included in the study sample. Patients with a diagnosis of psychogenic nonepileptic seizures, obesity, abnormal liver transaminases, comorbidities and concomitant medications that alter bone metabolism were excluded from participation. The patients' alkaliphosphatase (ALP) level was used as a biomarker for bone metabolism. The case was defined as ALP levels higher than the median ALP levels among the epilepsy patients at the CEC KUMC or positive history of fracture. Logistic regression was used to assess the association and to account for potential confounders.

RESULTS: Among 2,607 patients, 161 patients were eligible for this study: 85 cases and 76 controls were identified. Patients with uncontrolled seizures demonstrated 1.964 times higher odds of ALP elevation relative to the odds of ALP elevation among epilepsy patients with well-controlled seizures (95% CI: 1.049-3.680, p=0.0341). Therapeutic category and proportion of enzyme-inducing AEDs were independent risk factors that altered ALP levels. The number of comorbidity, the number of concomitant medications, and the length of epilepsy were considered potential confounders. No association was found between seizure status and prevalence of fractures.

CONCLUSIONS: Uncontrolled seizure status is a significant risk factor for alteration of bone metabolism when liver transaminases are normal. Further investigation is necessary to determine the influence of vitamin D intake on ALP elevation and uncontrolled seizures.

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Introduction

Refractory seizures negatively impact the quality of life of epilepsy patients. A refractory seizure is defined as "having at least 1 seizure per month and having not responded positively to at least 2 antiepileptic drugs (AEDs)".[1] Approximately 30% of epilepsy patients have experienced refractory seizures.[2-5] One report shows that almost 10% of patients with epilepsy never become seizure-free with a trial of a third AED or multiple AEDs.[6] Despite the availability of multiple AEDs, optimizing seizure treatment remains difficult.

Bone health in patients with epilepsy is a major concern. The fracture risk is two times to six times greater among patients with epilepsy compared to the general population.[7] Although fractures may occur as a consequence of seizures, adverse effects of AED treatment and changes in brain function due to seizures negatively impact bone health.[8,9] Numerous studies demonstrate that all AEDs alter various biomarkers of bone damage.[7,8,10-37] Some AEDs induce isoenzymes, resulting in rapid metabolism and less bioavailability of endogenous and exogenous vitamin D and sex hormones.[38] These hormones are essential in maintaining bone quality and in remodeling.[39,40] Moreover, changes in brain function interfere with the regulation of the hypothalamus-pituitary-endocrine gland axis and its feedback system.[41] Therefore, patients with epilepsy have several risk factors for fractures.

Fractures significantly reduce quality of life and limit physical activities.[42] Moreover, expenditures for fracture treatment are costly.[43] Current costs for fracture treatment secondary to osteoporosis are reported to be $19 billion in the United States and are predicted to increase to $25.3 billion by 2025.[43] Thus, appropriate fracture prevention and risk reduction among patients with epilepsy are critical to improving quality of life.

Published studies demonstrate an association between AED use or duration of AED treatment and negative bone-health outcomes. However, none of the studies has determined the association between seizure severity and bone health. The aim of this study was to determine if patients with uncontrolled seizures show higher alkaline phosphatase (ALP) levels, the biomarker for bone formation, compared to

epilepsy patients whose seizures were well controlled. Also, we conducted an investigation to determine if uncontrolled seizures increased the risk for fractures.

Methods

Database

All data were collected from the Comprehensive Epilepsy Center at the University of Kansas Medical Center, and 2,607 charts were reviewed. Monthly seizure frequency, gender, race (Caucasian, African American, and others), history of fractures, diagnosis date of epilepsy based on the International Classification of Diseases, Ninth Revision code or ICD9 code (345.XX), last office visit date, age, smoking status, vitamin D and calcium (Ca) intake, length of epilepsy, utilization of Vagus Nerve Stimulator (VNS), the number of AEDs and their daily doses, the number of concomitant medications and comorbidities, weight, and height were obtained for the study. Seizure frequency was captured during the patients' most recent clinic visit. Uncontrolled seizure status is defined as when a patient experiences at least one seizure per month despite trials of two or more AEDs.[1] Seizure-free is defined as "a patient has to be seizure free for at least 1 year, or 3 times the longest inter-seizure interval (whichever is longer)."[44] In this study, seizure frequency was used as a marker of seizure status, regardless of the number of current or previous AEDs. The reason for this was that patients did not remember the number of previous AED(s) correctly or that previous AEDs were not listed in a referral letter. If the daily dosage of AED was more than the recommended dose listed in the Anatomical Therapeutic Chemical classification system and the Defined Daily Dose (ATC/DDD) provided by World Health Organization,[48] the dose was classified as high. The compliance rate of AEDs was calculated as follows: 28 days minus a number of AED missing days, then divided by 28 days. A compliance rate of 80% or more was considered as good medication compliance. Body mass index (BMI) was calculated as body weight in kilograms divided by height in meters squared. Laboratory data collected were ALP, alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum calcium concentration, and vitamin D levels. Alkaline phosphatase is a frequently tested isoenzyme and usually is measured with other liver function tests, ALT, AST, and

gamma glutamyl transpeptidase. Approximately 50% of total ALP is derived from the liver and another 50% from the bone.[45] Bone ALP is produced by the surface of osteoblasts, making ALP elevation an indicator of bone formation, when other liver function tests are within a normal range.[39,46,47]

Patient selection

Figure 1 showed the algorithm of patient selection. Of the 2,607 patients, those who met the following conditions were included in this sample: a positive diagnosis of epilepsy (generalized [345.0x, 345.1x, 345.2x, or 345.3x], partial [345.4x, 345.5x, or 345.7x]; or unclassified [345.8x or 345.9x]); aged 21-50 at the last visit; treated with at least one AED for more than six months; and with liver function tests (ALP, ALT, and AST) within a year from the last clinic visit. Additionally, it was necessary for the study that female patients experienced a monthly menstrual cycle. A patient having what we refer to as an uncontrolled seizure status was a patient who had one or more seizures per month. Likewise, seizure free was defined as not having a seizure at least one year after the previous clinic visit.

Excluded from the study were patients with a diagnosis of psychogenic nonepileptic seizures (nonepileptic seizure or pseudo seizure); patients with obesity (defined as a BMI of 30 or greater); patients whose ALT and AST levels exceed 1.5 times above the upper normal range; patients with comorbidities that alter ALP levels, vitamin D levels, and bone metabolism (e.g., menopause, liver disease, viral hepatitis, renal disease, chronic kidney disease, pancreatic diseases, primary hyperparathyroidism, hyperthyroidism, inflammatory conditions, rheumatoid arthritis, Paget's disease, osteoporosis, alcoholism); patients treated with medications that alter bone metabolism except AEDs (e.g., glucocorticoid, immunosuppressants, gonadotropin-releasing hormone analogues; and patients with chronic use of heparin, warfarin, chemotherapy agents, medroxyprogesterone acetate, diuretics, metoclopramide, methotrexate, and antiretroviral therapy for HIV). Also excluded were patients who did not have laboratory data within one year of the last visit as well as patients whose seizure frequency was not recorded in their chart or whose monthly seizure frequency was less than 1.

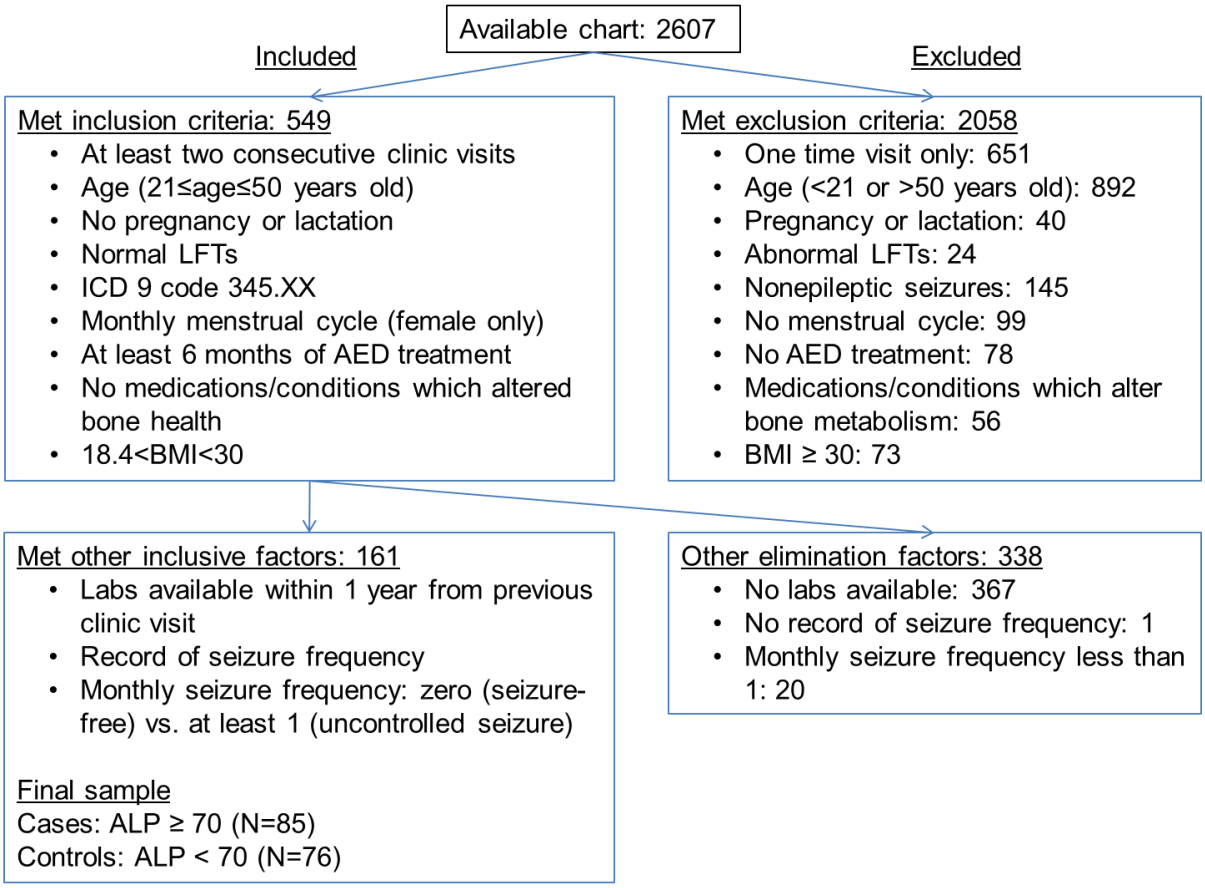

Figure 1: The algorithm of patient selection for the primary outcome.

Case-control study design

To assess the association between ALP elevation and seizure frequency among adult epilepsy patients, a retrospective case-control analysis was conducted using the data described in the previous section. Two categorical variables were examined as outcomes: ALP elevation and the history of fractures. The ALP elevation was defined as ALP levels higher than the median ALP levels among the epilepsy patients at the Comprehensive Epilepsy Center at the University of Kansas Medical Center. If a patient ever experienced fractures in lifetime and was listed on the charts, it was counted as a positive history of fracture.

Statistical Analysis

Statistical analysis was performed using SAS version 9.3 (Cary, NC, United States). Differences in categorical variables, such as diagnosis (generalized epilepsy, partial epilepsy, or unclassified); gender; race (White, Black, or other); BMI (underweight, normal, or overweight); smoking status (smoker or nonsmoker); seizure status (controlled, defined as seizure free for more than one year, vs. uncontrolled, defined as having a seizure at least once a month); length of epilepsy (0-1 year, 1-10 years, 11-20 years, 21-30 years, and more than 30 years); vitamin D intake (none, multivitamin, or vitamin D supplement defined as taking at least 400 IU daily); calcium intake (none, multivitamin, Ca supplement, defined as taking at least 500 mg daily); serum Ca levels (cutoff point: median value); ALP categorical (cutoff point: median value); history of fracture; compliance rate of AEDs (cutoff point: 80%); use of VNS; proportion of enzyme-inducing AEDs (EIAEDs: none, 0%-50%, more than 50%); proportion of high-dose of AEDs (high-dose defined as utilization of AED dose higher than the one listed in the ATC/DDD [48]: none, 0%-50%, more than 50%) were tested by Chi Square test or Fisher exact test where appropriate. Differences in continuous variables, (e.g., age, serum Ca levels, ALP, number of comorbidity, number of concomitant medication, and number of treatment AEDs) were tested by independent two sample *t* test, Student's *t* test or Wilcoxon Rank Sum test where appropriate. *P* values of less than 0.05 were considered statistically significant.

The primary endpoint of this study was to detect the difference in ALP elevation between controlled and uncontrolled seizure patients. The secondary outcome was to detect the association between uncontrolled seizures and the history of fractures. To assess the association between ALP elevation/the history of fracture and the variables, unadjusted odds ratios (OR) with 95% confidence intervals (CI) were calculated in logistic regression. The *p* values of less than 0.05 were considered as associated with ALP elevation/the history of fracture. In the logistic regression, if the coefficient value of uncontrolled seizure (β_1) was altered by another variable by more than 10% compared with the β_1 without the variable in the model, then the variable was considered a potential confounder. The Cochran-Mantel-

Haenszel statistics were used to analyze how degrees of variables affect the value of the association (e.g., absence or present, dose differences).

Furthermore, logistic regression was used for the calculation of crude and adjusted OR for ALP elevation (categorical) and the history of fracture. The logistic regression model was as follows, according to the hypothesis of previous reports regarding bone health among epilepsy patients[7,8,10-37]:

$$\textit{Logit (p) of ALP elevation} = \textit{intercept} + \beta_1 x_{\textit{refractory seizure}} + \beta_2 x_{\textit{age}} + \beta_3 x_{\textit{gender}} + \beta_4 x_{\textit{number of comorbidity}} + \beta_5 x_{\textit{number of concomitant medication}} + \beta_6 x_{\textit{length of epilepsy}} + \beta_7 x_{\textit{vitamin D intake}} + \beta_8 x_{\textit{Ca intake}} + \beta_9 x_{\textit{serum Ca level}} + \beta_{10} x_{\textit{number of AED}} + \beta_{11} x_{\textit{proportion of enzyme inducing AED}} + \beta_{12} x_{\textit{therapeutic category.}}$$

Also: $$\textit{Logit (p) of history of fracture} = \textit{intercept} + \beta_1 x_{\textit{refractory seizure}} + \beta_2 x_{\textit{age}} + \beta_3 x_{\textit{gender}} + \beta_4 x_{\textit{number of comorbidity}} + \beta_5 x_{\textit{number of concomitant medication}} + \beta_6 x_{\textit{length of epilepsy}} + \beta_7 x_{\textit{vitamin D intake}} + \beta_8 x_{\textit{Ca intake}} + \beta_9 x_{\textit{serum Ca level}} + \beta_{10} x_{\textit{number of AED}} + \beta_{11} x_{\textit{proportion of enzyme inducing AED.}} + + \beta_{12} x_{\textit{therapeutic category}} + \beta_{13} x_{\textit{alp elevation.}}$$

Age and gender were included as covariates. The final multivariate model included variables when they were retained in a backward analysis with a p value of 0.10.

Boluk and colleagues demonstrated that ALP levels among healthy adults and adult epilepsy patients were 95±36 IU/L and 111±32 IU/L, respectively.[49] Based on their data, a group sample size was calculated, and 73 patients in each group (total sample size of 146) provided 80% power to detect a difference of ALP between two groups.

Results

A. Uncontrolled seizures and ALP elevation

The study cohort was a total of 161 patients: 85 cases and 76 controls (Figure 1). The demographic characteristics were summarized in Table 1. No statistically significant differences were found between the cases and controls in age, diagnosis, race, BMI, length of epilepsy, vitamin D intake, Ca intake, serum Ca levels (continuous and categorical), number of concomitant medications, medication compliance rate, number of treatment AEDs, proportion of high-dose AEDs, and history of fractures. A

statistically significant difference was found in seizure status, gender, therapeutic category (monotherapy vs. polytherapy), VNS use, and proportion of EIAEDs.

Table 1. Characteristics of Cases and Control Subjects

	Cases: ALP ≥ 70 (N=85)	Controls: ALP < 70 (N=76)	P value
Seizure status, % (N)			0.0341
Seizure free	41.2 (35)	57.9 (44)	
Uncontrolled	58.8 (50)	42.1 (32)	
Age (median), years	33.0	32.0	0.4872
Gender, % (N)			0.0009
Male	68.2 (58)	37.6 (32)	
Female	31.8 (27)	62.4 (44)	
Diagnosis, % (N)			0.2887
Partial	77.7 (14)	84.2 (64)	
Generalized	16.5 (14)	14.5 (11)	
Unclassified	5.9 (5)	1.3 (1)	
Race, % (N)			0.6876
White	84.7 (72)	80.4 (61)	
Black	14.1 (12)	17.1 (13)	
Others	1.2 (1)	2.6 (2)	
BMI, % (N)			0.2295
Underweight	37.6 (32)	28.9 (22)	
Normal	31.8 (27)	44.7 (34)	
Overweight	30.6 (26)	26.3 (20)	

Smoking, % (N)			0.4500
Nonsmoker	75.3 (64)	80.3 (61)	
Smoker	24.7 (21)	19.7 (15)	
Medication compliance, % (N)			0.4500
≥ 80%	92.9 (79)	93.4 (71)	
< 80%	7.1 (6)	6.6 (5)	
Length of epilepsy, year			0.2535
1-10 years	17.6 (15)	30.3 (23)	
11-20 years	29.4 (25)	28.9 (22)	
21-30 years	23.5 (20)	19.7 (15)	
> 30 years	29.4 (25)	21.0 (16)	
Vitamin D intake			0.7049
Vitamin D supplement	24.7 (21)	19.7 (15)	
Multivitamin	12.9 (11)	15.8 (12)	
None	62.3 (53)	64.5 (49)	
Ca intake			0.2208
Ca supplement	27.1 (23)	15.8 (12)	
Multivitamin	11.8 (10)	14.5 (11)	
None	61.2 (52)	69.7 (53)	
Serum Ca level (median)	9.2	9.2	0.7859
Number of comorbidity			0.1658
None	42.4 (36)	48.7 (37)	
1-5 (inclusive)	48.2 (41)	44.7 (34)	
> 5	9.4 (8)	6.6 (5)	

Number of concomitant meds			0.6509
None	30	33	
1-5 (inclusive)	49	42	
> 5	6	1	
Number of AEDs, % (N)			0.1447
1	32.9 (28)	50.0 (38)	
2	30.6 (26)	30.3 (23)	
3	24.7 (21)	14.5 (11)	
4	5.9 (5)	2.6 (2)	
5	5.9 (5)	2.6 (2)	
Therapeutic category			0.0280
Monotherapy	32.9 (28)	50 (38)	
Polytherapy	67.1 (57)	50 (38)	
VNS			0.0142
Yes	16.5 (14)	5.3 (4)	
No	83.5 (71)	94.7 (71)	
Proportion of EIAEDs			0.0053
None	35.3 (30)	60.5 (46)	
~50%	42.3 (36)	23.7 (18)	
> 50%	22.4 (19)	15.8 (12)	
Proportion of high-dose AEDs			0.3052
None~50%	29.4 (25)	40.8 (31)	
> 50%	27.1 (23)	21.0 (16)	
> 50%	43.5 (37)	38.2 (29)	

BMI: Underweight (<18.5), normal weight (18.5–24.9), overweight (25–29.9)

Table 2 shows the association between ALP elevation and various factors. Based on the crude ORs, patients with uncontrolled seizures demonstrated 1.964 times higher odds of ALP elevation relative to the odds of ALP elevation among epilepsy patients with well-controlled seizures (95% CI: 1.049-3.680, p=0.0341). Gender, therapeutic category (monotherapy vs. polytherapy), and proportion of EIAEDs also were significantly associated with ALP elevation. The number of comorbidity, the length of epilepsy, and the number of AEDs were considered potential confounders that significantly altered seizure status and OR of ALP elevation. A univariate analysis in a logistic regression model demonstrated that only seizure status, gender, therapeutic category, and proportion of EIAEDs showed a statistically significant association with ALP elevation.

Previous reports showed that EIAEDs alter vitamin D metabolism and that vitamin D can alter the absorption of Ca from the intestine. Thus, the influences of vitamin D intake, Ca intake, serum Ca levels, and proportion of EIAEDs on seizure status and ALP elevation also were examined. No significant interactions were seen between vitamin D metabolism, Ca intake, and proportion of EIAEDs on the effect of ALP.

Table 2. Univariate and multivariate logistic regression analysis for ALP elevation

Risk factor	Univariate				Multivariate			
	OR	95%CI		P value	OR	95%CI		P value
Seizure frequency	1.964	1.049	3.679	0.0350	1.732	0.811	3.700	0.1562
Race	0.736	0.362	1.497	0.3973	0.736	0.331	1.636	0.4525
Diagnosis	1.586	0.826	3.046	0.1658	1.493	0.707	3.151	0.2931
Gender	0.339	0.178	0.645	0.0010	0.342	0.162	0.723	0.0050
BMI	0.931	0.628	1.380	0.7216	0.977	0.614	1.555	0.9229
Smoking	1.334	0.631	2.824	0.4508	1.952	0.793	4.801	0.1453
Compliance	1.078	0.315	3.687	0.9043	0.981	0.223	4.312	0.9794

Number of comorbidity	1.564	0.892	2.745	0.1188	1.140	0.428	3.036	0.7930
Number of concomitant medications	1.263	0.769	2.074	0.3568	1.105	0.472	2.590	0.8180
Length of epilepsy	1.313	0.988	1.745	0.0602	0.951	0.663	1.364	0.7858
Vitamin D intake	0.902	0.620	1.311	0.5887	0.927	0.265	3.240	0.9054
Calcium intake	0.743	0.507	1.091	0.1299	0.848	0.448	1.603	0.6112
Therapeutic category	1.483	1.087	2.022	0.0129	1.325	0.900	1.950	0.1533
Proportion of enzyme inducing AEDs	1.408	1.065	1.861	0.0163	1.566	1.134	2.163	0.0064
Proportion of high-dose AEDs	1.250	0.873	1.788	0.2227	1.096	0.693	1.734	0.6957

Table 3 shows a summary of the results of the final regression model. Variables in the regression model were selected based on prior evidence, clinically relevant factors, and the covariates that showed statistical significance upon univariate logistic regression analyses. The univariate logistic regression analysis was used to determine the final model. The variables that provided *p* values of less than 0.1 were selected in the final equation. The final model was as follows, and uncontrolled seizures, and higher proportion of EIAEDs were significant factors that elevated serum ALP levels:

$$\textit{Logit (p) of ALP elevation} = -0.2157 + 0.8465x_{\textit{uncontrolled seizure status}} + (-1.0610)x_{\textit{gender}} + 0.5239x_{\textit{proportion of enzyme inducing AED}}$$

Table 3. Logistic regression of factors associated with ALP elevation

Parameter	Estimate (β)	SE	P value	OR	95% CI
Intercept	-0.2157	0.3340	0.5183		

Uncontrolled seizures	0.8465	0.3445	0.0140	2.331	1.187-4.580
Gender	-1.0610	0.3436	0.0020	0.346	0.176-0.679
Proportion of EIAEDs	0.5239	0.0.2227	0.0187	1.689	1.091-2.613

B. Uncontrolled seizures and history of fracture

The study cohort was a total of 161 patients: nine had at least one fracture in their lifetime and 152 did not have fractures. The demographic characteristics were summarized in Table 4. The number of cases did not provide adequate power for the analyses of interest. No statistically significant differences were found between the cases and controls in all variables except with the serum Ca level (p=0.0127). The global null hypothesis test for β=0 was not rejected by the Likelihood Ratio test when conducting univariate and multivariate analysis with logistic regression. Serum Ca levels and the proportion of EIAEDs were the only variables that satisfied the regression model (p=0.0065 and 0.0488, respectively). The crude OR of serum Ca levels and the proportion of AED for the history of fractures were 0.101 (95% CI: 0.012-0.825, p=0.0134) and 2.360 (95% CI: 0.985-5.655, p=0.0542).

Table 4. Characteristics of cases and control subjects (history of fracture)

	Cases: positive history of fracture (N=9)	Controls: negative history of fracture (N=152)	P value
Seizure status, % (N)			0.4959
Seizure free	33.3 (3)	50.0 (76)	
Uncontrolled	66.7 (6)	50.0 (76)	
Age (median), years	36.0	32.0	0.1478
Gender, % (N)			0.5092
Male	44.4 (4)	56.6 (86)	
Female	55.6 (5)	43.4 (66)	

Diagnosis, % (N)			0.4088
	Partial	77.8 (7)	80.9 (123)	
	Generalized	11.1 (1)	15.8 (24)	
	Unclassified	11.1 (1)	3.3 (5)	
Race, % (N)			0.2884
	White	66.7 (6)	83.5 (127)	
	Black	33.3 (3)	14.5 (22)	
	Others	0 (0)	2.0 (3)	
BMI, % (N)			0.6309
	Underweight	44.4 (4)	32.9 (50)	
	Normal	22.2 (2)	38.8 (59)	
	Overweight	33.3 (3)	28.3 (43)	
Smoking, % (N)			0.4196
	Nonsmoker	66.6 (6)	78.3 (119)	
	Smoker	33.3 (3)	21.7 (33)	
Medication compliance, % (N)			1.0000
	≥ 80%	100 (9)	92.8 (141)	
	< 80%	0 (0)	7.2 (11)	
Length of epilepsy, year			0.6262
	1-10 years	11.1 (1)	24.3 (37)	
	11-20 years	44.4 (4)	29.3 (43)	
	21-30 years	11.1 (1)	22.4 (34)	
	> 30 years	33.3 (3)	25.0 (38)	
Vitamin D intake			0.5360

Vitamin D supplement	22.2 (2)	22.4 (34)	
Multivitamin	0 (0)	15.1 (23)	
None	77.8 (7)	62.5 (95)	
Ca intake			0.7691
Ca supplement	22.2 (2)	21.7 (33)	
Multivitamin	0 (0)	13.8 (21)	
None	77.8 (7)	64.5 (98)	
Serum Ca level (median)	8.8	9.2	0.0127
Number of comorbidity			0.5426
None:	22.2 (2)	40.1 (61)	
1-5 (inclusive)	77.8 (7)	55.3 (84)	
> 5	0 (0)	4.6 (7)	
Number of concomitant meds			1.0000
None	44.4 (4)	45.4 (69)	
1-5 (inclusive)	55.6 (5)	46.0 (70)	
> 5	0 (0)	8.5 (13)	
Number of AEDs, % (N)			0.1855
1	33.3 (3)	41.4 (63)	
2	22.2 (2)	30.9 (47)	
3	22.2 (2)	19.7 (30)	
4	22.2 (2)	3.3 (5)	
5	0 (0)	4.6 (7)	
Number of AEDs, % (N)			0.7382
Monotherapy	3 (33.3)	63 (41.5)	

	Polytherapy	6 (66.7)	89 (58.5)	
VNS				0.2675
	Yes	22.2 (2)	10.6 (16)	
	No	77.8 (7)	89.4 (135)	
Proportion of EIAEDs				0.1056
	None	22.2 (2)	48.7 (74)	
	0-50%	33.3 (3)	33.5 (51)	
	> 50%	44.4 (4)	17.8 (27)	
Proportion of high-dose AEDs				0.3080
	None	11.1 (1)	36.2 (55)	
	0-50%	33.3 (3)	23.7 (36)	
	> 50%	55.6 (5)	40.1 (61)	
ALP (continuous, median)		81.0	70.0	0.0811
ALP (categorical)				0.5017
	ALP<70	33.3 (3)	48.0 (73)	
	ALP≥70	66.7 (6)	52.0 (79)	

Discussion

Our study population showed an association between uncontrolled seizures and ALP elevation, indicating that frequent seizures alter bone metabolism. However, other factors, such as therapeutic category, proportion of EIAEDs, and a combination of those factors with uncontrolled seizure status may result in ALP elevation. To the best of our knowledge, this is the first study to determine the relationship between seizure frequency and bone metabolism among ambulatory epilepsy patients treated with one or more AED. Published studies showed that AED treatment results in an elevation of ALP or bone ALP when compared to epilepsy patients with health population.[10-17,19-27,50] Our study identified another risk

factor--uncontrolled seizure condition--that altered the bone metabolism among epilepsy patients. However, in our study, uncontrolled seizures did not increase the risk of fracture. The small number of patients (n=9) with fractures may explain the negative finding in our study.

One possible mechanism for why uncontrolled seizures can be a significant risk factor of alteration of bone metabolism is that frequent seizures alter excretion of sex hormones, such as estrogens and androgens. Prior studies indicated that seizures interfere with the regulation of hormone release via the hypothalamus-pituitary-endocrine organ axis, resulting in hypogonadism, sexual dysfunction, infertility, and osteoporosis.[41, 51-53] Indeed, sex hormones are essential in maintaining adequate bone density.[39,54,55] Androgens are known to be crucial in bone formation, because androgens increase osteoblast activity.[55,56] The long-term testosterone therapy increased bone mineral density (BMD) by 26% ($p<0.0001$) compared to the BMD before therapy.[56] Estrogen enhances osteoblast activities but decreases the lifespan of osteoclasts.[39,54,57-60] In fact, the Women's Health Initiative's randomized controlled trial showed that estrogen replacement therapy decreased the risk of hip fracture (hazard ratio: 0.66, 95% CI: 0.45-0.98) compared to individuals who did not undergo estrogen replacement therapy.[61] Additionally, men and women with epilepsy tend to experience a reduction of gonadotropin and sex hormones as well as a higher serum concentration of sex hormone-binding globulin (SHBG). [41, 83-85] This illustrates that epilepsy patients with uncontrolled seizures may induce less biologically available free-sex hormones (i.e., unbound sex hormones to SHBG). Additionally, estrogen is known to enhance the conversion from 25-hydroxy vitamin D_3, the intermediate metabolite of active vitamin D_3, to 1α,25-$(OH)_2D_3$, the active vitamin D_3, and also known to inhibit the conversion from 25-hydroxy vitamin D_3 to 24,25-dihydrovitamin D_3, inactive metabolite of vitamin D.[62] Therefore, an uncontrolled seizure status may induce an inadequate production of sex hormones, a low concentration of biologically available sex hormone, and active vitamin D production, which subsequently altered bone metabolism.

Our study showed that the crude OR of EIAED used for ALP elevation was 1.408 (95% CI: 1.065-1.861, p=0.0163). That is an indication that poor bone health is positively associated with a

proportion of EIAED use. Carbamazepine (CBZ), phenytoin (PHT), phenobarbital (PB), and primidone (PRM) are known as EIAEDs.[63,64] Although our data did not show significant differences in serum Ca levels between ALP elevated and non-ALP elevated groups, a published study showed that EIAEDs increased vitamin D metabolism, which leads to lower serum calcium levels and to an enhancement of parathyroid hormone release.[46] Additionally, some EIAEDs altered bone resorption markers (e.g., cross-linked C-telopeptide, cross-linked N-telopeptides of type I collagen levels), and bone formation markers (i.e., elevation of ALP or bone-specific ALP, osteocalcin, C-terminal propeptide of Type I procollagen, N-terminal propeptide of Type III procollagen, and N-terminal propeptide of Type I procollagen).[27,28,35,50,65-74]

Another significant aspect in EIAED use is recognizing the roles of nuclear receptors in CYP450 regulation. The nuclear receptors are essential in understanding how isoenzymes are related to bone metabolism. Recently, the roles of nuclear receptors in CYP450 regulation have been widely known.[38,75,76] CBZ and PHT bind to specific nuclear receptors, such as the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR), which induce CYP1, CYP2, and CYP3 isoenzymes, resulting in enhancement of steroid metabolism.[38,75,76] We discussed that uncontrolled seizures may reduce the quantity of the biologically available sex hormone. Adding the fact that estrogens and androgens increase the survival of osteocyte but reduce the lifespan of osteoclasts,[39,54,57-60] the use of EIAEDs accelerates the loss of sex hormones by enhanced metabolism of these hormones.

Nuclear receptors also are responsible in vitamin D metabolism for maintaining healthy bones. In the CYP family, the CYP24 isoenzyme converts 25-hydroxy vitamin D_3 to 24,25-dihydrovitamin D_3 and 1α, 24,25-trihydrovitamin D_3, the inactive metabolites of vitamin D_3.[76] Valproic acid binds to the vitamin D receptor and induces the CYP24 isoenzyme.[77] This enzyme induction due to valproic acid results in an increase of inactive forms of vitamin D_3 and of bone loss.[77]

In our study, no differences were found in the degree of vitamin D and Ca intake and the serum Ca levels between patients with or without ALP elevation. This indicates that our study sample did not have

hypocalcemia and that vitamin D and Ca supplementation did not make significant differences in serum Ca levels between the two groups. However, interestingly, when controlling for vitamin D intake (patients who took a vitamin D supplement of at least 400 IU/day or more, N=36), patients with uncontrolled seizures showed higher OR for ALP elevation compared to seizure-free patients (OR= 5.0000, 95% CI: 1.1949-20.9218, p=0.0233). But patients who took multivitamins or no vitamin D supplement did not show significant differences in ALP elevation (OR= 2.4500, 95% CI: 0.4561-13.1605, p= 0.2921, N=23; OR= 1.3745, 95% CI: 0.6304-2.9973, p= 0.4233, N=102, respectively). No association was found between vitamin D and Ca intake, the proportion of EIAED use, and serum vitamin D levels ($p>0.05$). We expected that higher vitamin D intake might show lower ALP levels, but the result was the opposite of the expectation.

Several reasons may explain this result, and these might be related to the limitations of this study. One possible reason is that patients using a vitamin D supplement might not have been on the supplement for enough time to receive the benefit from it in this cross-sectional study design. Because we did not capture the duration of the supplements during the chart review, we did not examine the influence of the duration of the supplementation on seizure status and ALP elevation. Some of the clinical studies to investigate the efficacy of vitamin D supplements spent at least 60 days evaluating the efficacy.[74,78-80] Additionally, the amount of vitamin D in this study might not be enough to correct bone abnormalities. In fact, the range of vitamin D administration was between 2,000 IU and 120,000 IU in the clinical trials with epilepsy patients treated with AEDs.[74,78-80] Moreover, we did not capture the compliance rate of the vitamin D and Ca supplement during our chart review. Thus, it is unclear if all patients who reported positive vitamin D supplementation truly took the supplement. Another reason might be that vitamin D intake might not correlate to serum vitamin D levels. We did capture serum vitamin D levels during data collection. However, only nine of 161 epilepsy patients had serum vitamin levels on the charts. This made it impossible to investigate the influence of uncontrolled seizures and vitamin D intake on serum vitamin D levels.

Other limitations of this study were hormone levels (estrogens, androgens, and vitamin D) and other biomarkers that determine bone health status, such as bone-specific ALP and BMD data from DEXA scan, both of which were not available. Additionally, the duration of AED therapy and vitamin D and Ca supplementation were not available to confirm bone damage. Additionally, the ALP level was measured within a year from the last clinic visit, which might not directly reflect the adverse reactions from the AEDs with which patients were treated or from the seizure status at the latest clinic visit.

In summary, an uncontrolled seizure condition is negatively associated with bone health. The mechanism of how uncontrolled seizures affect bone health is unclear, but an abnormal excretion of sex hormones and vitamin D metabolism may influence the structure of the bones. Further investigation is necessary to confirm this hypothesis. Additionally, healthcare providers should pay attention to bone health, especially when seizures are uncontrollable even with adequate AED treatment. Routine analysis of serum ALP is convenient and can be significant to detect the alteration of bone metabolism.

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