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Investigation of the effect of anti-epileptic drugs on bone metabolism using osteoprotegerin and bone-specific alkaline phosphatase: The direct effects of antiepileptic drugs on bone metabolism

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Ethics Committee Approval
Approval for the study was granted by the Kahramanmaraş Sütçü Imam University Ethics Committee (Number: 2018-03/06).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Anti-epileptic drugs are long-term medications; thus, side-effects are frequently seen. An important but insufficiently known side-effect is the emergence of metabolic bone diseases. The mechanism of this entity is not clearly known, but it is usually seen with the use of cytochrome P450 enzyme-inducing anti-epileptics**.** However, recent studies demonstrated that non-enzyme-inducing molecules also cause bone mineral impairment. The aim of this study was to shed light on the pathogenesis of anti-epileptic metabolic bone disease using bone turnover markers.

Methods: This comparative, prospective case-control study included 37 patients followed-up in our outpatient clinic and 39 healthy control subjects. All the patients were female, aged over 18 years and in the premenopausal period, and had received the same anti-epileptic treatment for at least 3 months. Male patients, females who were <18 years old, pregnant, in the postmenopausal period, those with osteoporosis, gastrointestinal malabsorption, physical impairment that may prevent normal ambulation, endocrine and metabolic disease, musculoskeletal and joint disease or a history of medication use were excluded from the study. A healthy control group was formed of age-matched premenopausal women, with no disorders causing gastrointestinal malabsorption, no physical impairment preventing normal ambulation, no endocrine or metabolic disorder, or history of medication use that may affect bone turnover. The levels of serum calcium, alkaline phosphatase, 25-hydroxyvitamin D, osteoprotegerin and bone-specific alkaline phosphatase were assessed, and the results were recorded.

Results: Evaluation was made of 37 female epilepsy patients with a mean age of 30.8 (8.1) years and a healthy control group of 39 age- and body mass index-matched females (P=0.69, P=0.85, respectively). The mean duration of AED use was 6.1 (5.5) years. The calcium (P=0.09), phosphate (P=0.906) and alkaline phosphatase (P=0.22) levels were similar in both groups. The levels of 25-hydroxyvitamin D (P=0.049), osteoprotegerin (P=0.025), and bone- specific alkaline phosphatase (P=0.037) were significantly lower in the epilepsy group.

Conclusion: Our study showed that serum levels of osteoprotegerin and bone-specific alkaline phosphatase, which are markers of increased bone formation, were lower in epilepsy patients. Probably many factors cause the bone mineral disorder seen in epilepsy patients. Antiepileptic use is one of them. These results suggest that antiepileptics may not only affect enzyme induction but also bone turnover. Neurologists should be aware of this issue and monitor patients regularly with respect to bone mineralization to enable early treatment when necessary.

Keywords: Antiepileptic drugs, Metabolic bone disease, Osteoporosis, Osteoprotegerin, Bone-specific alkaline phosphatase

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Introduction

Epilepsy, one of the most common diseases at any age [1], is estimated to affect approximately 50 million people worldwide [2,3]. Most epilepsy patients use anti-epileptic drugs (AED). However, AEDs have many side effects which can cause patients to discontinue use, resulting in treatment failure. One of these is metabolic bone disease, which may range from bone mineral reduction to pathological fractures [4, 5]. However, the mechanism of this side effect is still unclear. Some authors advocated that enzyme-inducing anti-epileptic drugs (EIAED) cause secondary hypocalcemia and hyperparathyroidism by increasing vitamin D metabolism [6-8] and recent studies have shown that non-enzyme-inducing anti-epileptic drugs (NEIAED) can also lead to bone mineral deficiency [9, 10]. These recent outcomes suggest that factors other than enzyme induction play a role in the emergence of this side-effect, raising the question of whether anti-epileptic drugs have a direct impact on bone turnover.

The aim of this study was to investigate the effects of AEDs on bone mineralization using bone turnover markers and determine at which stage this side-effect occurs. To the best of our knowledge, this is the first study to have used bone-specific alkaline phosphatase (BALP) and osteoprotegerin (OPG) concurrently as two important bone turnover markers.

Materials and methods

This comparative, prospective case–control study included female patients aged >18 years who were being followed up in our outpatient clinic and had received the same anti-epileptic treatment for at least 3 months.

Male patients, and females aged <18 years, or who were pregnant or in the postmenopausal period were excluded from the study. Other exclusion criteria were the presence of osteoporosis, gastrointestinal malabsorption, physical impairment that may prevent normal ambulation, endocrine and metabolic disease (e.g., thyroid disorders, Cushing's Syndrome, hypogonadism, diabetes), musculoskeletal and joint disease (e.g., rheumatoid arthritis), or a history of medication use (e.g., corticosteroids, proton pump inhibitors).

A healthy control group was formed of age-matched premenopausal women, with no disorder causing gastrointestinal malabsorption, no physical impairment preventing normal ambulation, no endocrine or metabolic disorder, or history of medication use that may affect bone turnover.

A record was made for each patient of age, height, weight, body mass index (BMI), treatment duration, disease duration and type of antiepileptic medication prescribed. The levels of serum calcium (Ca), phosphate (P), alkaline phosphatase (ALP) and 25-hydroxyvitamin D [25(OH)D] were assessed and recorded. For the measurement of osteoprotegerin (OPG) and bone-specific alkaline phosphatase (BALP), fasting blood samples were withdrawn into anticoagulant-free tubes, and then centrifuged at 4000 rpm for 10 minutes after coagulation at room temperature. The serum BALP and OPG levels were determined using commercial ELISA kit procedures (201-12-1494; SunredBio, China, 201-12-1559; SunredBio, China).

This study was conducted in accordance with the Declaration of Helsinki. The ethical board approval was obtained from Kahramanmaraş Sütçü Imam University Ethics Committee (Number: 2018-03/06).

Sample size analysis

The sample size was determined using G*Power version 3.1 software. The minimum total sample size was calculated to be 68 (two groups, 34 per group) subjects with 90% power at a 95% confidence interval with a two-tailed alpha of <0.05 and a 0.80 effect size (f). A total of 76 participants, 37 in the patient group and 39 in the control group, were included in the study.

Statistical analysis

Data obtained in the study were analyzed statistically using SPSS for Windows v. 22 software (Statistical Package for the Social Sciences, IBM Corporation, Armonk, NY, USA). Continuous data were presented as mean (standard deviation (SD)) values and categorical variables were summarized as number (n) and percentage (%). The Kolmogorov Smirnov test was used for the evaluation of normal distribution. Comparisons between groups were made using Chi-square tests for categorical variables, Independent Samples Student's t-tests for normally distributed continuous variables and Mann-Whitney U-tests when the distribution was skewed. A *P*-value of <0.05 was considered statistically significant.

Results

Evaluation was made of 37 female epilepsy patients with a mean age of 30.8 (8.1) years and a healthy control group of 39 age and BMI-matched females (*P*=0.69, *P*=0.85, respectively). The mean duration of AED use was 6.1 (5.5) years. Monotherapy, most commonly levetiracetam, was found in 20 (54.05%) patients, and 17 (45.95%) patients were on multiple drug regimens. The Ca (*P*=0.09), P (*P*=0.906) and ALP (*P*=0.22) levels were similar in both groups. The levels of Vit D (*P*=0.049), OPG (*P*=0.025), and BALP (*P*=0.037) were significantly lower in the epilepsy group (Figures 1, 2, 3) (Table 1). In the correlation analysis of the study group data, OPG was positively correlated with vitamin D (rho = 0.479 / *P*=0.01) and BALP (rho = 0.571 / *P*=0.001), and negatively correlated with ALP (rho = -0.398 / *P*=0.036). The correlation analysis results are summarized in Table 2.

Table 1: Descriptive and analytical characteristics of the groups

	Epilepsy (n=37) Mean (SD) median (min-max)	Control (n=39) Mean (SD) median (min-max)	*P*-value
Age (years)	30.8 (8.1)	27.8 (5.9)	0.69
Duration of antiepileptic drug use (months)	6.1 (5.5)	-	
BMI (kg/m^2)	24.3 (3.6)	24.5 (5.5)	0.85
Ca^{++} (mg/dL)	9.1 (0.4)	9.2 (0.36)	0.09
Vit D (µg/L) *	6.9 (5)	8.0 (4.6)	0.049
ALP (U/L)	75.7 (26.7)	69.3 (18.1)	0.22
P (mg/dL)	3.4 (0.46)	3.4 (0.48)	0.906
Osteoprotegerin **	31 (13-751)	33.3 (12.7-725)	0.025
Bone-specific ALP **	28.5 (19.2-885.9)	49.4 (22.2-864.8)	0.037

BMI: body mass index, ALP: alkaline phosphatase, P: phosphorus, * Independent Samples t-test, ** Mann Whitney U-test, *P*<0.05, statistically significant difference

No correlation was found between the duration of drug use and Vit D level (rho=-0.229; *P*=0.173), OPG (rho= -0.311; *P*=0.108), and bsALP (rho= -0.125; *P*=0.512).

The levels of Ca (P=0.205), Vit D (P=0.95), ALP (P=0.36), OPG (P=0.46), and BALP (P=0.89) were similar in both the monotherapy and polytherapy groups.

Figure 1: Boxplot of osteoprotegerin according to the groups

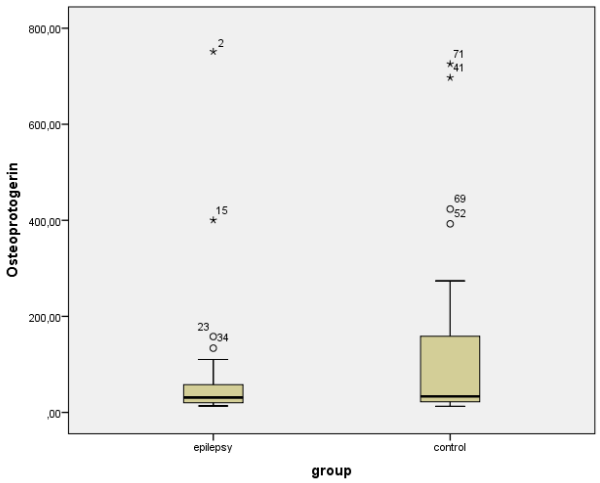

Figure 2: Boxplot of bone-specific alkaline phosphatase according to the groups

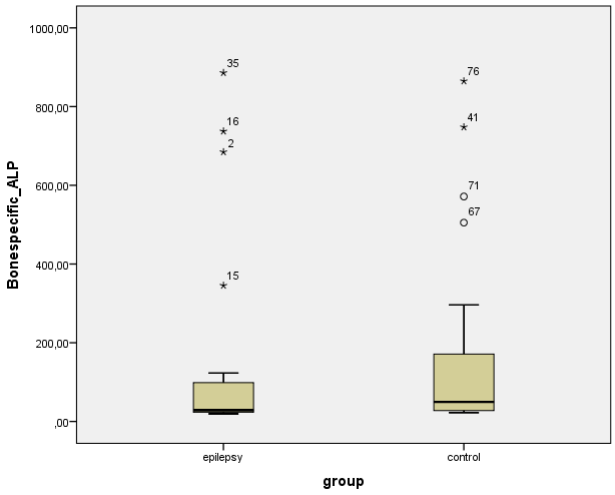

Figure 3: Boxplot of vitamin D according to the groups

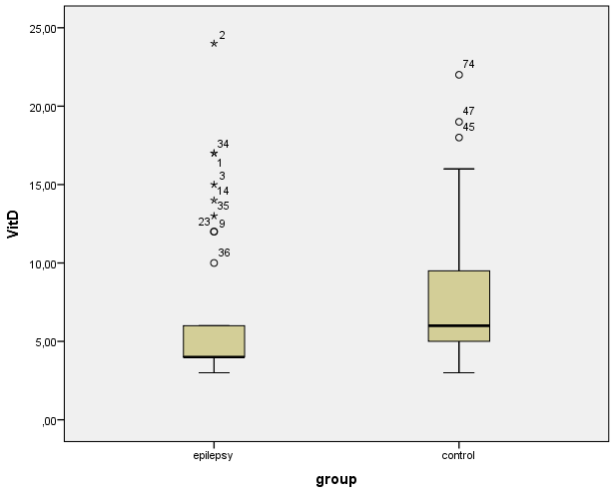

Table 2: Correlation analysis of the study group (epilepsy)

	Osteoprotegerin rho/*P*-value	Bone-specific ALP rho/*P*-value
Age (years)	0.233/0.232	-0.327/0.078
BMI (kg/m^2)	-0.194/0.323	-0.242/0.198
Duration (months)	-0.311/0.108	-0.125/0.512
Ca^{++}	0.178/0.366	0.324/0.081
VitD	0.479/0.01*	0.324/0.081
ALP	-0.398/0.036*	-0.155/0.414
P-value	0.092/0.641	0.228/0.226
Osteoprotegerin	-	0.571/0.001*

BMI: body mass index, ALP: alkaline phosphatase, P: phosphorus, * Spearman Correlation test, P<0.05: statistically significant difference

Discussion

The results of this study demonstrated that serum levels of OPG and BALP were lower in epilepsy patients compared to healthy control subjects. As both are markers of increased bone formation, the low levels in patients using AEDs suggests that anti-epileptics have a direct effect on bone mineralization.

AEDs are known to cause bone metabolism diseases, and although some studies have been carried out on this subject, no consensus has been reached and the pathogenesis is still a matter of debate.

Whereas earlier studies considered that the effects of AEDs on the cytochrome p450 enzyme system caused this side-effect, more recent studies have shown that NEIAED also cause bone mineral impairment.

Shen et al. [3] found that the use of both enzyme-inducing and non-enzyme inducing AEDs increased the risk of fractures, with a higher risk resulting from EIAED use.

Singla et al. [11] compared the levels of serum Ca, P, parathormone (PTH), vitamin D, ALP levels and DEXA scores of 25 AED users and 25 healthy control subjects. Serum Ca and protein levels were significantly decreased and serum PTH and ALP levels were significantly increased in AED users. In the comparisons between EIAED and NEIAED users, with the exception of ALP, no significant difference was found between the groups with respect to the changes in parameter levels. It was suggested that AEDs may affect the bone metabolism through some other mechanisms in addition to enzyme induction.

Although studies have been conducted using routine blood tests, Hamed et al. [8] used bone turnover makers as in the current study and found that epileptic patients had significantly lower serum Ca, 25OHD, OPG and higher Soluble Receptor Activator of Nuclear Factor-KappaB Ligand (RANKL) levels than the control subjects. It was concluded that low serum OPG and high RANKL levels indicated increased bone turnover. Although no correlation was found between serum parameters and the duration of treatment, a correlation was reported between the duration of treatment and bone mineral densities (BMD) measured using dual-energy X-ray absorptiometry (DEXA).

In an experimental animal model, Simko et al. [13] found a highly significant decrease in the OPG/RANKL* ratio in the phenytoin group. (*: Receptor Activator of Nuclear Factor-KappaB Ligand)

Although patients may have bone metabolism disorders due to anti-epileptics, these disorders may not be detected in the early stages on bone mineral densitometry or in routine blood tests such as Ca, P and ALP. In the current study, bone turnover markers were used to identify the effects of anti-epileptics on bone metabolism. There is known to be a balance of bone remodeling throughout life, and these events are mediated by osteoblasts producing bone matrix and osteoclasts that degrade it. OPG is a protein that inhibits osteoclastic bone resorption [14]. As in the study by Hamed et al. [8], OPG was used in the current study to investigate the effect of AEDs on bone turnover, and the results were consistent with those of Hamed et al. The OPG levels were significantly lower in the epilepsy group.

BALP, which is synthesized by osteoblasts and assumed to be involved in the calcification of the bone matrix, is considered a highly specific marker of the bone-forming activity of osteoblasts [15]. Different results have been reported in studies related to BALP.

Kir et al. [16] evaluated an epileptic patient group given carbamazepine and found no significant difference from the control group with respect to BALP levels. In the current study, serum BALP levels were lower in epilepsy patients than in the control group. These results demonstrate that AEDs impair bone mineralization by directly affecting bone turnover.

Limitations

Since the number of our patients was not high enough, we could not compare patients using AEDs as EIAED and NEIAED. In the future studies, by increasing the number of the patients, bone turnover markers can also be compared between patients who use EIAED and NEIAED.

Conclusion

Neurologists should be aware of this issue and monitor these patients regularly in terms of this complication. This situation is complicated by the presence of many factors which affect bone mineralization, a lack of studies on this subject, and the use of combined anti-epileptic treatments in some patients. There is a need for further studies of the pathogenesis of these side-effects to enable effective treatment planning.

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