Comparison between the effects of older versus newer generations of antiepileptic drugs on bone metabolism in adult Iraqi patients: an observational study [version 1; peer review: awaiting peer review]

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Abstract

Background: Metabolic bone disorder is a significant endocrine system disorder that encompasses any disorder that alters the natural skeleton's mineralization process. Epilepsy is a prevalent central nervous system disorder that can cause biochemical abnormalities involving bone metabolism in the epileptic patients. The present study aimed to investigate the effects of chronic use of older compared to the newer generations of antiepileptic drugs on bone metabolism-related biomarkers.

Methods: The study included fifty-one epileptic outpatients who attended the Consultation Clinic of Baghdad Teaching Hospital at the Medical City Complex from October/2021 to December/2021. The selected patients were on antiepileptic drugs for more than 2 years, hence were grouped according to their antiepileptic therapy into: Group-1: 24 epileptic patients on old antiepileptic drugs (Carbamazepine or Valproate), Group-2: 27 epileptic patients on new antiepileptic drugs (Levetiracetam), compared with Group-3: 28 healthy control subjects. Serum was obtained from their blood specimens to measure: calcium and inorganic phosphate by colorimetric assays, parathyroid hormone, and level of bone alkaline phosphatase activity.

Results: Data analysis revealed that the median value of serum parathyroid hormone levels was significantly elevated in the epileptic patients' groups compared to the healthy control group. However, group-2 (new generation antiepileptic drugs) presented higher values. Whereas serum calcium and inorganic phosphate levels showed non-significant variation for all the studied groups. Furthermore, serum bone alkaline phosphatase activity exhibited significantly higher values in the patients compared to the healthy subjects group, with more significant elevation among those on old generation
antiepileptic drugs (Carbamazepine or Valproate).

**Conclusion:** Epileptic individuals who had been on AEDs for more than two years had increased parathyroid hormone levels, which were boosted by the newer antiepileptic drug Levetiracetam. Furthermore, BAP serum levels were considerably greater in epileptic patients than in healthy control participants, with larger values generated by older antiepileptic medications.

**Keywords**
Antiepileptic drugs, Bone disorders, Calcium, Inorganic phosphate, Parathyroid hormone, Bone alkaline phosphatase.

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Introduction
Metabolic bone disorder (MBD) is a significant endocrine system disorder that encompasses any disorder that alters the natural skeleton’s mineralization process. The illness is caused by abnormalities in the temple or bone mass, vitamin D concentration, also minerals involved in bone metabolisms, such as calcium and phosphorus. Osteoporosis, osteomalacia (including tumor-induced osteomalacia), primary hyperparathyroidism, fluorosis, fibrous dysplasia, Paget’s disease, and osteogenesis imperfect are the most common manifestations of MBD. Calcium is the most prevalent mineral in the body; it combines with phosphorus in the bones and teeth to make calcium phosphate. Osteomalacia is a disturbance of "bone softens" in adults due to the prolonged decrease of vitamin D that results in disorder in osteoid mineralization. Osteoporosis is one type of skeletal disorder that is described as rejecting osteoid strength due to soft osteoid microarchitecture and elevated sensitivity to break. Epilepsy is a prevalent CNS disorder that can cause considerable morbidity and mortality, and it is a disabling general chronic illness of neurological disorder. The biochemical abnormalities involving bone metabolism in the generality of epileptic patients on therapy with antiepileptic drugs (AEDs). These anomalies include low blood calcium concentration, low phosphate level, vitamin D breakdown, and enhanced parathyroid hormone (PTH) level. AEDs are also destructive of the intracellular response to PTH, resulting in increased bone rebuilding.

The goal of this research is to investigate the effects of chronic use of older (Carbamazepine or Valproate) compared with newer (Levetiracetam) antiepileptic drugs on serum biomarkers related to bone metabolism, including calcium, inorganic phosphate, parathyroid hormone, and bone alkaline phosphatase activity levels among Iraqi adult epileptic patients.

Methods
Study design
This is an observational, cross-sectional study that was designed to involve adult Iraqi epileptic patients on different antiepileptic regimens; including those on the old antiepileptic drugs (OED) (Carbamazepine or valproate), compared to those on the new antiepileptic drugs (NED) (Levetiracetam) for more than two years period.

Sample size
G*Power version 3.1.9.7 software was used to calculate the sample size. The minimal total sample size was determined to be 70 patients with 90% power at a 95% confidence interval, a two-tailed alpha of 0.05, and an effect size of 0.80 (f). A total of 79 participants, 51 in the patient group and 28 in the control group, were included in the study.

Eligibility criteria
Adult patients aged 18 years old and above from both sexes (male, female), and on antiepileptic drugs (AEDs) more than two years.

The study included fifty-one epileptic outpatients attending the Consultant Clinic of Baghdad Teaching Hospital at the Medical City – Complex from October 2021 to December 2021. First, the selected patients were treated with antiepileptic drugs for more than two years. Then the patients were grouped according to their antiepileptic therapy as follows:

Group-1: 24 (18 Male and six Female) epileptic patients on old generation antiepileptic drugs (Carbamazepine or valproate), with ages ranging between 20 and 50 years old.

Group-2: 27 (seven Male and 20 Female) epileptic patients on new generation antiepileptic drugs (levetiracetam), with ages ranging between 20 and 50 years old.

Additionally, a group of apparently healthy subjects with age & sex matching that of the patients’ groups was included as a control group:

Group-3: 28 (13 male and 15 female) healthy control subjects with ages ranging between 20 and 50 years old.

A venous blood specimen (8 ml) was withdrawn from each participant (control or patient) to obtain serum. Serum was divided into aliquots and kept frozen (about - 20°C) for later measurement of calcium (Ca\(^{2+}\)), inorganic phosphate (PO\(_{4}\)\(^{-3}\)) by colorimetric assays, while parathyroid hormone (PTH) and bone alkaline phosphatase (BAP) activity were assayed by using specific ELISA Kits.

Exclusion criteria
Any patient with cardiovascular disease, diabetic or hypertensive patients, patients with autoimmune disease, pregnant & lactating women, patients with kidney & liver diseases, those on steroid or antibiotics (rifampicin), patients with primary bone diseases, history of previous fracture, and patients have malignant disease were excluded.
Bias
Selection bias may arise during the process of identifying the research sample. This is especially true in case-control and retrospective cohort studies, when exposure and outcome have already happened before people are chosen for study participation. However, selection bias is less likely in this Prospective research since the result is unknown at the time of participation. The ideal research population is well-defined, easily available, dependable, and at high risk of developing the desired result.

Materials and instruments
The chemicals used in this study were of highest available purity.

Ethical consideration
The study was conducted with the approval of the Human Research Ethics Committee of the Ministry of Health of Iraq with approval number 5033 at 10/4/2021. Informed consent forms were obtained from each participant before beginning the research.

Data analysis
IBM SPSS for Windows, version 25 (IBM SPSS Statistics, RRID:SCR 016479) was used to perform statistical analyses. A one-way ANOVA testing was carried to compare the three investigated groups (group-1, group-2, and group-3) and assess the degree of significance using a quantitative analysis test. The non-normally distributed variables were reported using non-parametric measures, and significant differences between the three groups were determined using the Kruskal-Wallis H test. The difference between the two groups was examined using the Student’s t-Test (independent and Mann-Whitney). Correlation coefficient (r) utilizing Spearman’s test for examining the correlations between variables; qualitative associations were tested using the Chi-square test; P-values less than 0.05 were statistically significant. We did a missing values analysis in SPSS (under the “analyze” tab) to examine whether the values were Missing Completely at Random (MCAR) or if there was a correlation among missing data. If no correlations are found, missing data may be dealt with either pairwise or listwise deletion. However, if the missing value analysis finds a particular tendency, the imputation was performed.

Results
There was a significant elevation of serum PTH levels among the epileptic patients’ groups compared to the healthy control group. However, in group-2, epileptic patients on new antiepileptic drugs presented higher values than those on the old drugs, as shown in Table 1.

| Table 1. Serum parathyroid hormone (PTH) levels among study groups. |
|-----------------|--------|----------------|----------------|-----------------|-----------------|
| Number Median Interquartile range Mean rank P-value |
| PTH (pg/mL) |
| Group-1 24 | 1840.3500 | 592.53 | 38.46 | 0.000* |
| Group-2 27 | 2244.3100 | 314.10 | 60.70 |
| Control 28 | 1277.3750 | 429.40 | 21.36 |

*Values are significantly different (α=0.05), Group-1=Patients on old generation drugs, Group-2=Patients on new generation drugs, PTH: Parathyroid Hormone.

| Table 2. Serum calcium and inorganic phosphate levels among the study groups. |
|-----------------|--------|----------------|----------------|-----------------|-----------------|
| Number Median Interquartile range Mean rank P-value |
| Ca+2 (mg/dL) |
| Group-1 24 | 9.5000 | 0.67 | 40.92 | 0.907 |
| Group-2 27 | 9.5000 | 1.20 | 38.43 |
| Control 28 | 9.5000 | 0.80 | 40.73 |

| PO43- (mg/dL) |
| Group-1 24 | 3.0500 | 0.77 | 36.52 | 0.539 |
| Group-2 27 | 3.0000 | 1.20 | 39.43 |
| Control 28 | 3.1000 | 0.83 | 43.54 |

Group-1=Patients on old generation drugs, Group-2=Patients on new-generation drugs, Ca+2: Calcium, PO43-: Inorganic Phosphate.
Serum calcium and inorganic phosphate levels were presented with non-significant variation among the studied groups, as seen in Table 2.

The serum activity of bone alkaline phosphatase was estimated for each specimen in the study groups (group-1, group-2, and control), as demonstrated in Table 3. The epileptic patients' groups were significantly elevated compared to the healthy control subjects.

Discussion

To this day, there is mounting evidence that epilepsy and its treatments might impair bone minerals and cause metabolic disorders. 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 non-enzyme-inducing antiepileptic drugs NEIAED also cause bone mineral impairment. The serum level of calcium, phosphorus and 25(OH) Vitamin D was significantly decreased and iPTH and ALP levels were increased in patients taking anti-epileptic drugs for > 5 years. There was a significant negative correlation between serum calcium, phosphorus and vitamin D levels and significant positive correlation with ALP and iPTH levels with duration anti-epileptic therapy. The findings of Buket Tüğan Yıldız et al. (2021) study where bone turnover markers were used to identify the effects of antiepileptics on bone metabolism, showed there is a balance of bone remodeling throughout life, and these events are mediated by osteoblasts producing bone matrix and osteoclasts that degrade it, the results of that study also showed that epilepsy patients’ serum levels of BAP were lower than healthy control persons. Because these are indications of enhanced bone formation, the low levels in AED users show that anti-epileptic drugs have a direct impact on bone mineralization. This study reported that the serum bone alkaline phosphatase activity of the studied groups (group-1, group-2 and control). Significant differences between the epileptic patients (group-1, group-2); as well as with the healthy control subjects. Calcium plays a dynamic role in metabolic processes.

Reported by Hale Maral Kir et al. (2012) found significantly higher PTH levels between two groups compared to control groups with carbamazepine groups.

The present study reports significant differences in parathyroid hormone levels between the epileptic patients' groups and the healthy control group. However, group-2 (new antiepileptic drugs) presented higher values, as shown in Table 2.
At the same time, serum parathyroid hormone level is negatively correlated at a significant level with bone alkaline phosphatase (r=-0.380*, p-value=0.034), as shown in Table 4.

Because active vitamin D, also known as 1,25-dihydroxy vitamin D, improves calcium absorption in the gastrointestinal tract, increased catabolism of active vitamin D to inactive vitamin D metabolites will result in a decrease in calcium absorption in the gastrointestinal tract, hypocalcemia, and an enhance in PTH levels. In response to a fall in serum calcium levels, PTH increases renal calcium reabsorption and stimulates 1-hydroxylase in the kidney, and mobilizing skeletal calcium. Furthermore, persistently high levels of PTH enhance bone turnover, favoring bone resorption over bone production. PTH is a hormone that controls osteoclast activity, renal calcium reabsorption, and intestinal calcium absorption to regulate serum calcium levels.

Serum calcium and inorganic phosphate levels showed no significant variation for all the studied groups (Table 2). Inorganic phosphate in serum is correlated positively at a significant level with serum calcium (r=0.438*, p-value=0.016) in Table 4. None of the studied parameters significantly correlated with serum calcium levels among patients on new antiepileptic drugs (Table 5). However, serum inorganic phosphate levels strongly correlate with bone alkaline phosphatase activity in those on new antiepileptic drugs (r=-0.396*, p-value=0.021), as shown in Table 5.

Many research examining the effects of antiepileptic drugs on body minerals and electrolytes concluded that the most common side effects were Ca++, P, and Vitamin D. One of these studies, conducted by Ashrafi MR et al. (2005), evaluated the effects of antiepileptic drugs on bone minerals, particularly PTH, in patients using antiepileptic drugs with controls. According to the Shah QA et al. (2001) study, untreated epileptics have unaltered Ca++ levels, consistent with other investigations. On the other hand, many studies have shown that a low level of Ca++ is responsible for the onset of convulsions. Therefore, the significantly greater levels of Ca++ in people with epilepsy treated with valproate and carbamazepine could be a signal for better seizure control with AED medication. Compared to controls, epileptic patients treated with valproate and carbamazepine have a considerable increase in Ca++ levels.

The epilepsy patients experienced significant changes in bone metabolism after three months of taking carbamazepine compared to when they first started taking it. Conversely, it was not observed that levetiracetam significantly reduced vitamin D and calcium levels three months after taking it compared to when they began taking the drug. However, Meier C et al. (2011) found that people with epilepsy treated with antiepileptic enzyme-inducing medicines such as carbamazepine had a significant drop in bone mineral density and a higher risk of bone fracture. Such findings with a recent study by Beerhorst K et al. (2013) they reported that provide identification at a glance of the critical association between carbamazepine and bone metabolic disturbances in patients with epilepsy, that explored the effect of antiepileptic drugs on minerals in the body and concluded that the most common effects of these drugs were on calcium and vitamin D. It’s worth noting that vitamin D is required for calcium absorption and aids in preventing additional bone loss. The hepatic cytochrome P450 enzyme, directed mainly by carbamazepine, increases serum levels of inactive forms of vitamin D.

Moreover, carbamazepine accelerates bone metabolic activity. It has been confirmed that the danger of bone fracture related to AEDs is widely known. As for levetiracetam in this regard, there are limited studies that revealed no unhealthy effects. Calcium, inorganic phosphate, magnesium, and parathyroid hormone levels were within normal limits. However, when compared to group carbamazepine, serum calcium levels in the control group were significantly lowers. Our study reports that the serum bone alkaline phosphatase activity was estimated for each specimen in the study groups (group-1, group-2, and control) demonstrated (Table 3). Both epileptic patients (group-1 and group-2) had significant differences compared to the healthy control subjects. BAP is a particular marker of osteoblast bone-forming activity generated by osteoblasts and is thought to be involved in the calcification of the bone matrix. Different results have been reported in studies related to BAP. This result is dissimilar to the finding of Kir et al. (2012) study showed evaluated an epileptic patient group given carbamazepine and showed no significant difference from the control group concerning BAP levels. In the current study, serum BAP levels were lower in epilepsy patients than in the control group. These results demonstrate that AEDs impair bone mineralization by directly affecting bone turnover. Valproate has been shown to cause a significant increase in serum ALP levels in some investigations. However, the tiny sample size of such studies limits them. The ALP levels in patients receiving valproate were within the normal reference range, confirming a previous result, indicating that valproate reduces epilepsy-induced ALP levels. This was reinforced by the finding that patients on valproate-assisted multitherapy had lower ALP levels than those on non-valproate-assisted multitherapy.

A high ALP level was linked to a significantly reduced vitamin D level in the patients. ALP measures in the lab usually comprise a mix of isoenzymes, mainly from the liver, bone, and intestines. A high ALP level is likely to be associated with
low serum vitamin D levels and impaired bone metabolism if other liver function tests are normal. Likewise, lower calcium levels appear to be linked to greater ALP and low vitamin D levels. Hale Maral Kir et al. (2012) study found a significant difference was not found between control groups and carbamazepine groups in terms of mean serum BAP. The results of a study by Buket Tuan Yldz et al. (2021) showed that epilepsy patients’ serum levels of BAP were lower than healthy control persons. Because these are indications of enhanced bone formation, the low levels in AED users show that anti-epileptic drugs have a direct impact on bone mineralization. The frequency of patients with abnormal biochemical parameters and the laboratory results of the analyzed groups. Patients had reduced serum calcium, 25(OH) D, and OPG and increased ALP compared to control volunteers. In ambulatory adults with epilepsy and on chronic treatment with AEDs, there is an increased risk of bone loss (osteopenia/osteoporosis) and fracture, hypocalcemia (52%) and 25(OH) D deficiency/insufficiency, increased bone turnover as indicated by lower serum levels of OPG and higher levels of sRANKL and sRANKL/OPG ratio (markers of bone remodeling), and metabolic bone compromise with prolongation of the duration of treatment with AEDs due to disturbed mineral metabolism associated with compromise of bone remodeling with accelerated bone turnover, biochemical abnormalities of bone-related mineral metabolism included hypocalcemia (52%) and hypovitaminosis D (93.33%). 25(OH) D levels were positively correlated with calcium levels.

This study reported that the serum bone alkaline phosphatase activity the of the studied groups (group-1, group-2 and control). Significant differences between the epileptic patients (group-1, group-2); as well as with the healthy control subjects. The other common laboratory abnormalities described in relation to AED are elevated levels of ALP and hyperparathyroidism. The hepatic induction of cytochrome P450 enzymes by enzyme inducing AEDs (e.g., phenobarbital, phenytoin, and CBZ) increases the metabolism and clearance of vitamin D with secondary hypocalcaemia and hyperparathyroidism. The mechanism of disturbed mineral metabolism due to VPA is not completely understood. VPA may cause renal tubular dysfunction, which could indirectly influence bone and mineral metabolism with increased urinary loss of calcium and phosphorus. In support, VPA has been associated with reversible Fanconi syndrome. Verrotti et al. reported that VPA doses do not seem to change bone turnover in adult epileptic patients. This could be explained by vitamin D-independent pathways such as impaired calcium absorption, resistance to parathyroid hormone and inhibition of calcitonin secretion, direct effects of AEDs on bone cells (9), hyperparathyroidism and calcitonin deficiency. Singla et al. (2017) study compared serum Ca+2, P, parathyroid hormone (PTH), vitamin D, ALP levels, and DEXA scores of 25 AED users and 25 healthy control subjects. Serum Ca+2 and protein levels were significantly decreased, and serum PTH and ALP levels were significantly increased in AED users. The serum level of calcium, phosphorus and 25(OH) Vitamin D was significantly decreased and iPTH and ALP levels were increased in patients taking anti-epileptic drugs for > 5 years. There was a significant negative correlation between serum calcium, phosphorus and vitamin D levels and significant positive correlation with ALP and iPTH levels with duration anti-epileptic therapy.

No significant difference the effects of VPA on serum calcium level between the VPA group and the control group due to the serum calcium level is affected by hormones such as PTH and 25(OH)D3, thus, the change of serum calcium level is not as sensitive as PTH and 25(OH)D3. A previous research demonstrated that patients with osteoporosis have normal serum calcium level before treatment. No significant difference regarding the effects of VPA on serum PTH level between the VPA group and the control group, the serum BAP level in the adult VPA group was significantly higher than that in the control group. Pack AM et al. (2004) study among users of AEDs, showed significant hypocalcaemia, hyperparathyroidism, and increased levels of serum alkaline phosphatase when compared to their age, gender, and socioeconomic status matched controls. Hypocalcaemia affects between 3% and 30% of persons with epilepsy receiving AEDs. Several theories on the mechanism of AED associated bone disease have been proposed, but no single one explains all the reported findings. The most common explanation is that AEDs such as carbamazepine that induce hepatic cytochrome P450 enzymes cause increased conversion of Vitamin D to inactive metabolites in the liver microsomes. The decreased biologically active Vitamin D leads to decreased absorption of calcium in the gut, resulting in hypocalcaemia and an increase in circulating PTH.

The study’s implications center on the enduring need for patients to be followed up on chronic treatments for parameters related to bone diseases, as well as the proclivity to use new drugs in terms of their effect on bone metabolism based on the results of a study of their effect on calcium, phosphate bone alkaline phosphatase, and PTH levels.

Limitations

The number of patients in our study was limited, thus we propose expanding the number of patients in future research. Bone turnover indicators may also be studied between patients who utilize older and newer generations. Because a history of prior fracture had been an exclusion criteria, we were unable to analyze the prevalence of no traumatic fractures in people with epilepsy. As a result, further research is needed to estimate the risk of no traumatic fractures while using AEDs.
Conclusion
The epileptic adults on more than two years AEDs were presented with higher parathyroid hormone levels, with greater values by group-2 (new antiepileptic drugs - levetiracetam). In addition, BAP serum levels were significantly elevated in epileptic patients (group-1, group-2) compared to the healthy control subjects, with higher values produced by the old antiepileptic drugs. Meanwhile, serum inorganic phosphate levels expressed a significant negative correlation with Bone alkaline phosphatase activity on new antiepileptic drugs.

Data availability
Underlying data

This project contains the following underlying data:

Data.xlsx: (Demographic data, anthropometric measurements, and clinical parameters).

Extended data

This project contains the following underlying data:

Diagnostic Kits and Their Suppliers.pdf: Diagnostic Kits and Their Suppliers

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

References


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