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ORIGINAL ARTICLE

Effects of Long-Term Treatment with Different Types of Anti-Epileptic Drugs on Vitamin D2 and Osteoprotegrin Serum Levels in Iraqi Patients

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ABSTRACT

Epilepsy is a chronic neurological illness had been described as repeated seizures associated with abnormal neurological activities in the brain. Epilepsy affects nearly fifty million people worldwide, with 85 percent of them living in developing countries. Vitamin D2 (VD2) is ergocalciferol (made from ergosterol). Genetic disorders related to vitamin D metabolism are dysfunction of generally loss osteoid mineralization the leading cause to bone disorders including: rickets or osteomalacia. Osteoprotegerin (OPG) is an osteoclastogenesis inhibitory factor (OCIF), OPG has essential role in bone metabolism and bone mass level. The aim of the present study is to investigate the effects of long-term treatment with anti-epileptic drugs (old Vs new antiepileptics) on vitamin D2 and osteoprotegrin levels among Iraqi epileptic patients.

The study included fifty-one epileptic outpatients, whom attending the Consultant Clinic of Baghdad Teaching Hospital at the Medical City –Complex for the period from October/2021 to December/2021. The selected patients were on antiepileptic drugs for more than 2years, 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), to be compared with Group-3: 28 apparently healthy control subjects, with age and sex matching to that of patients.

Serum was obtained from their blood specimens to measure: serum VD2 and OPG concentrations among the study groups participants, using specific ELISA Kits.

Data analysis revealed that the mean vitamin D2 levels were not significantly different between group -1 (old antiepileptic drugs) and group-2 (new antiepileptic drugs), whereas, both of the patients groups expressed significantly lower values than the control group. However, serum osteoprotegrin levels were significantly elevated in group-1 compared to group-2. Furthermore, both of the epileptic patients groups had significantly higher values compared to the healthy subjects group.

Keywords: Osteomalacia, antiepileptic drugs, Vitamin D2, Osteoprotegrin.

INTRODUCTION

Osteomalacia is a vitamin D decrease caused by antiepileptic drugs that cause cytochrome p450 liver enzyme induction; other factors as well as; malnutrition and lack sun light exposure. In early stages, the process of osteomalacia in patient's used antiepileptic drugs is due to their ability of inducing the isoenzyme of cytochrome p450 system. The diffusion of osteomalacia in patients taking antiepileptic drugs , In addition to lower vitamin D levels, which are likely due to the direct and indirect effects of newer antiepileptic drugs (NEDs) such as levetiracetam, lamotrigine, oxcarbazepine, and gabapentin on vitamin D metabolism (cholecalciferol or ergocalciferol) (1). Vitamin D decrease is clinically expressed as rickets in children and osteomalacia in adults (2). Sometimes levels of enzymes and vitamins are used to diagnose diseases, as is the case in epilepsy, and to treat it similar to some cellular proteins (3). Osteoporosis is another bone disorder related to anti-epileptic drugs that show inhibition in bone mass, micro architectural discomfort, and increased skeletal failure and frailness, with subsequent decrease bone strength and high rates of death (4).

Generally antiepileptic drugs (AEDs) activity have been three mechanisms, as well as adjustment of calcium, sodium, or potassium dependent potential volts channel, enhance synaptic passage through Gamma Aminobutyric Acid (GABA), then decreased glutamate-induced catalyze (5). Antiepileptic medicines, particularly enzyme inducer antiepileptic drugs (EIAEDs), have a negative impact on vitamin D and calcium metabolism. These can stimulate the liver's cytochrome-P450 enzyme system, resulting in greater vitamin D inactivation and osteoid deficiency, an effect not seen with non-enzyme-inducing antiepileptic medications (NEIAEDs). Similarly, AEDs can directly affect bone cells, induce bone fracture result hypernatremia induced osteoporosis (6). Besides increasing the risk for osteoid fractures in patients with epilepsy (7). Especially, the pathophysiological reaction induced by valproate's ability to enhance the likelihood of CYP24 isoenzyme type CYP24 messenger RNA (mRNA) expression in the liver. The commonly known physiological properties of isoenzyme CYP24 is to enhance hydroxylation of 1, 25- di-hydroxyvitamin D, enhanced by the vitamin D3, as a physical negative feedback process. Such mechanism of activation by valproate drug leading to quickened vitamin D deactivation. However ,Valproate is unbelief underground enzyme-inducer antiepileptic drugs (EIAED), but rather an enzyme-inhibitor AED, and it is not prevalent to be linked to vitamin D and calcium concentrations, regardless of decrease vitamin D being generic during using valproate (8). On the other hand, osteoprotegerin (OPG) is an osteoclastogenesis inhibitory factor (OCIF), or known as a cytokine receptor of the tumour necrosis factor receptor (TNF) superfamily is encoded by the TNFRSF11B gene (TNFRSF11B). Osteoprotegerin is the decoy receptor which binds and opposes receptor activator of NF-κB Receptor Activator of Nuclear factor Kappa (RANK) ligand, which acts to activate osteoclasts and causes bone resorption process (9). Osteoprotegerin is considering as one type of glycoprotein, initially discovered as a bone resorption inhibitor (10), in addition to its ability to formation and preserve bone. It's generated in many tissues such as lung, bone, kidney, vasculature, heart, and placenta (11).

The current study is aimed to investigate effects of long-term treatment with anti-epileptic drugs with various mechanisms of action on serum vitamin D2 and osteoprotegrin levels among Iraqi patients with epilepsy.

MATERIALS AND METHODS

This is a cross sectional study, was designed to involve adults Iraqi epileptic patients on different antiepileptic regimens; including those on the old antiepileptic drugs (OED) (Carbamazepine or Valproate) compared to those on the new anti-epileptic drugs (NED) (Levetiracetam) for more than two years period.

The study included fifty-one epileptic outpatients, whom attending the Consultant Clinic of Baghdad Teaching Hospital at the Medical City –Complex for the period from October/2021 to December/2021. The study was conducted with approval of the Human Research Ethics Committee of The Ministry of Health of Iraq. Informed consent forms were obtained from each participant before beginning the research. The selected patients were selected to be treated by antiepileptic drugs for more than 2 years period (table-1). The patients were grouped according to their antiepileptic therapy as follows:

Group-1: 24 (18 Male & 6 Female) epileptic patients on old antiepileptic drugs (Valproate or Carbamazepine), with age ranged between 20 & 50 years old.
Group-2: 27 (7 Male & 20 Female) epileptic patients on new antiepileptic drugs (Levetiracetam), with age ranged between 20 & 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 & 15 Female) apparently healthy control subjects with age ranged between 20 & 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 - 20^0C), for later measurement of: Vitamin D2 (VD2) (12) and osteoprotegerin (OPG) concentrations (13) were assayed by using specific ELISA Kits.

Statistical analysis was performed using the (SPSS) Statistical Package for Social Sciences (version 25 for Windows). Quantity analysis were tested using one way ANOVA (Analysis of Variance) test to compare the three studied groups (group-1, group-2, group-3) and to determine the degree of significance between them. The non-normally distributed variables were presented by non-parametric measures as tested by Kruskal – Wallis H test for significant differences between the three groups. Student's t-Test (independent and Mann-Whitney) were applied for testing the difference between two groups. Correlation coefficient (r) using Spearman's test for testing the relationships between variables; while qualitative relationships were evaluated using Chi-square test, the P-value (<0.05) were considered statistically significant.

Table 1: Type of Antiepileptic Drugs Used by Study Groups

			Type drug			
			Carbamazepine	Valproate	Levetiracetam	P-value
Sex	M	Count	9	9	7	0.005 *
		% within Sex	23.7%	23.7%	18.4%	
		% within Type drug	69.2%	81.8%	25.9%	
	F	Count	4	2	20	
		% within Sex	9.8%	4.9%	48.8%	
		% within Type drug	30.8%	18.2%	74.1%	

*Association between (sex and type drug), M: Male, F: Female

RESULTS

Vitamin D2 Levels Among Study Groups:

There were no significant differences between group-1 (old antiepileptic drugs) and group-2 (new antiepileptic drugs) considering their serum vitamin D2 concentrations, whereas the patients groups were presented with significantly decreased values compared to the control group, shown in (table-2).

Table 2: Serum Vitamin D2 (VD2) Levels Among Study Groups

		Number	Median	Interquartile Range	Mean Rank	P-value
VD2 (ng/mL)	Group-1	24	43.0450	7.87	30.54	0.000*
	Group-2	27	42.7600	6.32	29.19	
	Control	28	58.3900	14.15	58.54	

* = values are significantly different (α =0.05), VD2: Vitamin D2

Osteoprotegrin (OPG) Levels Among Study Groups:

Significantly higher serum osteoprotegrin levels were detected in group-1 compared to group-2. Furthermore, both of the epileptic patients groups (group-1 and group-2) had significantly higher levels than the control group, as illustrated in (table-3).

Table 3: Serum Osteoprotegrin (OPG) Levels Among Study Groups

		Number	Median	Interquartile Range	Mean Rank	P-value
OPG (pg/mL)	Group-1	24	3.1900	0.69	57.23	0.000*
	Group-2	27	3.0300	0.39	48.67	
	Control	28	1.9500	0.39	16.88	

* = values are significantly different (α =0.05), OPG: Osteoprotegrin

Table 4: Spearman's Correlation of Group-1 (Patients on the old antiepileptic drugs) for Serum Ca^{+2}

Variable	R	P-Value
Vit.D2	0.066	0.379
OPG	0.419*	0.021
BAP	0.064	0.383

VD2: Vitamin D2, OPG: Osteoprotegrin, BAP: Bone Alkaline Phosphatase

DISCUSSION

According to WHO data from 2018, epilepsy deaths in Iraq accounted for 246 deaths, or 0.14 percent of all deaths, with an age adjusted mortality rate of 0.78 per 100,000 people, placing Iraq 158th in the world (14). Epilepsy is among the most frequent major brain disorders, with more than 70 million population affected globally by 2022 (15).

A study by **Hamed et al. (2011)** reported that epileptic patients had significantly lower serum Ca^{+2}, 25OHD, OPG, and higher Soluble Receptor Activator of Nuclear Factor-KappaB Ligand (RANKL) levels than healthy control subjects (16). Low serum OPG and high RANKL levels were found to indicate that bone turnover was increasing. Although no link was discovered between serum parameters and treatment time, there was a link between treatment length and BMD as evaluated by dual-energy X-ray absorptiometry (DEXA) (17).

Vitamin D2 concentrations of group-1(old antiepileptic drugs) were presented with no significant differences from that of group-2 (new antiepileptic drugs); however these values were significantly lower than the vitamin D2 level of the control group; (table-2). Several theories have been suggested to clarify the mechanisms of antiepileptic drugs for inducing bone diseases but none of the theories alone can explain all findings related to bone disorders (18). Antiepileptic medications, such as carbamazepine, are thought to enhance the translation of vitamin D to inactive forms, resulting in a rise in parathyroid hormone (PTH) and accelerated bone turnover. (19). Previous research that showed increased bone turnover or bone loss despite vitamin D deprivation had not taken this mechanism into account (20). Instead of decreasing serum 25-OH vitamin D, direct effects of these medications on bone cells, intestinal calcium transport, and resistance to parathyroid hormone have been hypothesized as alternate mechanisms (21). This contradicts the findings of **Albaghdadi et al. (2016)**, who discovered the importance of monitoring epileptic patients for bone structure and osteoporosis development during their childhood and concluded that these medications caused low bone mineral density and hypovitaminosis D in epileptic adults (22).

Increased inactive forms of Vitamin D contribute to the induction of the hepatic microsomal enzyme cytochrome -P450, which is mostly influenced by AEDs such carbamazepine, phenytoin, phenobarbital, and primidone (23). Our findings, however, showed that the medicines used by both groups of patients had no significant differences in their effects on vitamin D2 metabolism and, as a result, serum levels. The current study examined the effect of Valproate (Depakin®) or Carbamazepine

(Tegretol®) medication on changes in both biochemical Vitamin D and BMD markers in 51 epileptic patients with the **Rahimdel et al. (2016)** study (24). The effects of antiepileptic drugs on calcium absorption and vitamin D metabolism have an impact on calcium homeostasis. When quitting antiepileptic medicines such valproate, phenytoin, and carbamazepine was linked to high calcium levels in an Indian study, and when seeking for calcium and vitamin D supplementation in idiopathic hypoparathyroidism, the effects of AEDs on calcium should be considered (25, 26). **Verrotti et al. (2002)** (27) revealed that bone turnover may rise despite normal vitamin D levels as a consequence of a study involving the administration of carbamazepine to epilepsy patients. Another study found anomalies in bone metabolism in patients treated with AEDs who were not deficient in vitamin D(20). Many studies had linked carbamazepine and phenytoin use to a reduction in 25(OH) D levels(28).

In the current investigations, patients using carbamazepine and those on levetiracetam (both of which have a non-inducing effect on the cytochrome p450 system) had lowered serum vitamin D levels to reach levels below that of the healthy subjects (control), demonstrating that there was no substantial difference between these antiepileptic drugs.

OPG is a non-signaling decoy receptor that binds to OPGL and prevents RANK activation. This system could be a goldmine of therapeutic targets and solutions for disorders including low bone mass, excessive bone resorption, and fracture vulnerability (29). The serum osteoprotegrin levels were not different between group-1 and group-2 compared to the healthy control group. Furthermore, both of them group-1 and group-2 patients had significantly higher levels than control group (table-3), indicating that old drugs applied in this study (Valproate & Carbamazepine) could be related to increased serum osteoprotegrin levels upon long term use, as well as, to those on new drugs (Levetiracetam). Opposite finding by **Buket Tuan Yldz et al. (2021)** study serum osteoprotegrin levels were significantly lower in the epilepsy group when they used cytochrome P450 enzyme-inducing anti-epileptics. The levels of 25-hydroxyvitamin D were significantly lower in epilepsy patients, as were serum levels of osteoprotegerin and bone-specific alkaline phosphatase, both of which are markers of increased bone formation (17). Furthermore, osteoprotegrin show a significant positive correlation (r= 0.419*, p- value=0.021) with serum calcium, by those on old antiepileptic drugs (table-4). Our finding of such increase of osteoprotegrin levels in epileptic patients, despite the type of antiepileptic drug used reflecting a preventive mechanism against bone loss (30). The findings of a study by **Buket Tuan Yldz et al. (2021),** which reported that epilepsy patients' serum levels of OPG and BAP, were lower than healthy control people. The low levels in patients using AEDs indicated that anti-epileptics have a direct influence on bone mineralization, as both are markers of enhanced bone growth (17). **Simko et al. (2016)** discovered a highly significant drop in the OPG/RANKL* ratio (*: Receptor Activator of Nuclear Factor Kappa B Ligand) in the phenytoin group, which is a powerful CYP-450 enzyme inducer (31), in their investigation. The current study used OPG to explore the effect of AEDs on bone turnover, which contradicts the findings of **Hamed et al. (2011)**, who found that the epilepsy group's OPG levels were much lower (16).

CONCLUSION

Long-term treatment with different types of anti-epileptic drugs in Iraqi patients; can be summarized as follows:-

1 There were no significant differences between (old antiepileptic drugs) and (new antiepileptic drugs) considering serum vitamin D2 concentrations, whereas the control group had significantly higher values compared to both of the patients groups.

2 Significantly higher serum osteoprotegrin levels in epileptic patients on old antiepileptic drugs) compared to those on new antiepileptic drugs. Furthermore, both of the epileptic patients groups had significantly higher levels of serum osteoprotegrin than control group.

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