American-Eurasian Journal of Toxicological Sciences 7 (4): 256-260, 2015
ISSN 2079-2050

DOI: 10.5829/idosi.aejts.2015.7.4.9693

# Serum Vitamin D and Carboxy-Terminal Telopeptide Type I Collagen Levels: As Markers for Bone Health Affection in Patients Treated with Different Antiepileptic Drugs

*[1]Moetazza M. ALshafei, [1]Hala M. Abdelkarim, [2]Eitedal M. Daoud and [1]Hassan Z.Hassuna*

[1]Department of Nutrition and Food Science, National Research Center, Giza, Egypt
[2]Complementary Medicine Department, National Research Center, Giza, Egypt

**Abstract:** Epilepsy is a common neurological disorder affecting all age groups. It is one of the world's most prevalent non-communicable diseases. Increased evidence suggesting that long term usage of anti epileptic drugs can have adverse effects on bone mineralization and bone molding. Aiming to study these effects and to give guide lines to support bone health through early intervention. From Neurology Out-Patient Clinic kaser Elaini University Hospital, 60 Patients were enrolled, 40 patients on antiepileptic drugs for at least two years and 20 controls matched with age and sex,epileptic but before starting treatment both chosen under specific criteria. Patients were divided into four groups, three groups with monotherapy treated with either Phynetoin, Valporic acid or Carbamazipine and fourth group treated with bothValporic acid and Carbamazipine. Estimation of serum Carboxy-Terminal Telopeptide of Type I- Collagen(ICTP) bone resorption marker, serum 25(OH) vitamin D, calcium, magnesium and phosphorus were done. Results showed that all patients on AED had significant low levels of 25(OH) vit D ($p<0.001$), with significant elevation of ICTP ($P<0.05$) versus controls. In group treated with Phynetoin highly significant elevation of (ICTP) marker and decrease of both serum 25 (OH) vitamin D ($P<0$, 0001) and serum calcium ($P<0.05$) versus control. Double drug group showed significant decrease of serum 25(OH) vitamin D ($P<0.0001$) and decrease in Phosphorus ($P<0.05$) versus controls. Serum magnesium showed no significant differences between studied groups. We concluded that Anti- epileptic drugs appears to be an aggravating factor on bone mineralization, so therapeutically it can be worth wile to supplement calcium and vitamin D even before initiation of antiepileptic therapy. ICTP marker can be used to evaluate change in bone resorption before and during AED therapy.

**Key words:** Antiepileptic Drugs · Vitamin D · Carboxy-Teminal Telopeptide Type-1-Collagen Bone Resorption Marker · Bone Minerals

## INTRODUCTION

Epilepsy is a neurologic disorder associated with many established co morbidities, one of which is a reduced bone health, it is world's most prevalent non-communicable diseases [1, 2]. In fact, it is well known that changes in the normal functioning of the bone tissue, mostly insidious and asymptomatic, occur more frequently in patients with epilepsy than the general population [3, 4]. In patients with epilepsy there is evidence of biochemical abnormalities indicating a disturbed bone metabolism, a decreased bone density and 2-6 times increased risk of fractures compared to the general population [5, 6]. Osteoporosis is characterized by low bone mineral density (BMD) and loss of the structural and biomechanical properties that are required to maintain bone Homeostasis. Physicians know about the association of osteoporosis with aging, postmenopausal status and secondary causes as drugs that cause such side effect. Drug-induced osteoporosis is a significant health problem and contribute to significant bone loss and fractures. All anti-epileptic drugs (AEDs), both enzyme inducers (phenytoin (PHY), Phenobarbital (PB), carbamazepine (CBZ)) and enzyme non inducers, such as valproate (VPA), are associated with accelerated bone loss and subsequent increased risk of osteoporotic fracture [7, 8].

**Corresponding Author:** Moetazza Mostafa Alshafei. Department of Nutrition and Food Science, National Research Center, postal code: 12622, Giza, Egypt. E-mail: Moetazza1_alshafei@yahoo.com.

Unfortunately, the adverse effects of AEDs on bone metabolism, especially phenytoin, are also seen in young patients [9]. Earlier studies in children [10] have shown decreased urinary excretion of calcium in patients on PHY, VPA and CBZ. Ninety percent of the subjects studied longitudinally developed either vitamin D deficiency or insufficiency at the end of 6 months of therapy.

Several theories exist concerning the mechanisms of AED-induced bone loss. The cytochrome P450 enzyme-inducing AEDs, such as phenytoin, phenobarbital and carbamazepine, accelerate inactivation of vitamin D which decreases calcium uptake and drives secondary hyperparathyroidism which exacerbate bone loss [11]. Animal studies suggest a direct inhibitory effect of phenytoin on osteoblast (The bone forming cells) proliferation and decreases in carboxylatedo-steocalcin, leading to poor bone mineralization [12]. It is unclear how non-enzyme-inducing AEDs reduce BMD and increase fracture, drug like VPA has been associated with fractures due to the development of hypophosphatemia secondary to Fanconi syndrome [13].

There are other mechanisms of AED induced bone loss include the drug decreases intestinal calcium absorption [14] decrease level of vitamin D3 necessary for maintenance of aromatase activity necessary in osteoblast bone formation function [15]. Another causes are the decreased vitamin K status (PHY), Onodera *et al.* [16] hepatic enzyme inhibition (VPA), Guo *et al.* [17] and by induction of the 25-hydroxyvitamin D-24-hydroxylase by the steroid hormone xenobiotic receptor [18]. AED therapy with multiple drugs is shown to be associated with high risk of bone mineral metabolism affection than monotherapy [19]. Bone resorption is currently evaluated by collagen degradation products, an assay of the breakdown of mature type I collagen carboxy-terminal telopeptide of type I collagen (ICTP), was developed [20] and has been shown to be a sensitive marker of bone resorption in various kinds of bone disorders [21-23]. As type I collagen is the most common protein in the skeleton, assays of the turnover of this protein should be good markers of bone turnover. The synthesis of type I collagen can be measured by PICP (Bone formation marker) [24]. Evidence-based strategies for prevention, screening, monitoring and treating bone loss and osteoporosis associated with AEDs are limited. Routine evaluation of 25-hydroxy vitamin D before treatment and every 6-12 months of AED therapy is recommended to ensure adequate vitamin D levels [25]. Patients on non-enzyme-inducing AEDs generally require 1000-1200 IU/day of vitamin D while those on enzyme-inducing AEDs need 2000-4000 IU/ day to maintain adequate vitamin D levels [26, 27]. Patients should also receive adequate calcium supplementation. When safe to do so, patients with epilepsy should be encouraged to participate in regular weight-bearing exercises as tolerated [28]. In a survey of 624 neurologists to assess their practice, only 28% were aware that AEDs are associated with reduced bone mass and only 9% of pediatric neurologists and 7% of those treating adults prescribed prophylactic calcium and vitamin D supplements for their epilepsy patients. Systematic control of the state of bones in all patients on long-term treatment with AEDs is nowadays recommended without qualification. Until further evidence from additional randomized trials is available, recommendations are that patients on long-term AEDs should be screened for bone health by DEXA (Dual energy x-ray analysis) [29].

## MATERIALS AND METHODS

Patients enrolled in the study were 60 collected from Neurology Out-Patient's Clinic kaser Elaini University Hospital after taking their consent, 40 patients on antiepileptic drugs for at least 2 years. 20 controls recently diagnosed as epileptics and before starting treatment matched with age and sex, Patient s and controls were chosen to have the following criteria: range of age (14-22) years mean in patient 18.4±2.4 and 19.4±2.5 in control, age coincides with maximum bone density period (12-30 years), sex ratio was 1:1 male to female in both groups, no regular supplementation of calcium or vitamin D for two years and all cases diagnosed as Grand mal epilepsy. Patients were divided into four groups, group I treated with phynetoin (PHY), group II on valporic acid (VAP), group III on carbamazipine (CBZ) and group I V treated with both valporic acid and carbamazipine (V+C).Blood was collected, separation of serum by centrifugation and stored under -20 °C tell analysis. Estimation of serum 25(HO) vitamin D and serum carboxy-terminal telopeptide of type I collagen( ICTP) marker for bone resorption both estimated by Elisa kit (Human Cross-linked Carboxy-terminal telopeptide of type I collagen, CTX-I ELISA Kit) [30]. Serum calcium Ca, phosphorus Ph and magnesium Mg estimated by calorimetric method [31-33]. All results were expressed as mean± SEM, Student test was calculated $P \leq 0.05$ considered significant difference.

## RESULTS

All the values are expressed as mean (±SEM). The age of the study population were (18.4±2.4) in patient and (19.4±2.5) in control. Biochemical results showed that

Table 1: Serum values of (OH) vitamin D, ICTP marker, serum calcium, phosphorus and magnesium indifferent AIDs treated Patients and untreated control groups

| | Control | PHY | VAP | CBZ | V+P |
|---|---|---|---|---|---|
| (OH) Vit D nmol/l | 86 ±19.5 | 17.3±3.4*** | 64.4± 8□ | 46.8± 15□ □ | 27.6± 5*** |
| ICTP ng/ml | 2.92±0.5 | 6± 1** | 3.2±0.7 | 3.7±1 | 3.4 ±1.3 |
| Ca ml/dl | 9.5± 0.7 | 8.3± 0.8** | 9.5± 0.5 | 9.6± 0.7 | 9.5± 0.6 |
| Ph ml/dl | 4.5± 0.7 | 4.1± 0.5 | 4.5± 0.4 | 4.3± 0.7 | 4.3± 0.3□ |
| Mg ml/dl | 3.8± 0.2 | 3.9± 0.18 | 3.8± 0.08 | 3.7± 0.2 | 3.8± 0.06 |

P≤0.0001***, P≤0.001□ □, P≤0.05 □ (ng/ml = nanogram/ml ; nmol/l = nanomol/l)

patients under different drug treatment had significant low levels of 25 (OH) vitamin D compared to control (38.7± 20.9 versus 86 ±19.5 nmol/l) p≤0.0001 and significant elevation of C-Teminal Telopeptide of Type-1-Collagen (ICTP) 4.1 ±1.6 versus 2.92±0.5ng/ml in controls (P<0.05). Group treated with Phynetoin (PHY) showed highest significant elevation of bone resorption marker ICTP 6± 1 versus 2.92±0.5 ng/ml in controls (P≤0.0001), insignificant elevation in other groups (VAP), (CBZ) and both drugs (V+C) (3.2±0.7,3.7±1.1,3.4 ±1.3 ng/ml) respectively versus 2.92±0.5 in control. Serum 25(OH) vitamin D showed highly significant decrease in phenytoin and double drug groups (17.3±3.4, 27.6± 5) versus 86 ±19.5 nmol/l in controls (P≤0.0001), CBZ (46.8± 15 nmol/l, P≤0.001) while in VAP. (64.4± 8 nmol /l, P≤0.05). Serum calcium showed significant decrease in phenytoin group (p≤0.01) but not in other groups serum Ca was (8.3± 0.8, 9.5± 0.5, 9.6± 0.7,9.5±0.60 versus 9.5±0.7ml/dl)in PHY, VAP, CBZ and double drug groups respectively. Significant decrease in Phosphorus in double drug group (p≤0.05), but not in other groups. Serum Ph was (4.1± 0.5, 4.5± 0.4, 4.3± 0.3, 4.3± 0.7)versus 4.5± 0.7ml/dl) in Phy, Vap, V+C and CBZ respectively. Serum Mg showed no difference versus control in all groups (3.9± 0.18, 3.8± 0.08, 3.8± 0.06, 3.8 0.2 versus 3.8± 0.2ml/dl) in Phy,VAP,V+CandCBZ respectively. Table 1.

## DISCUSSION

In this study patients under AED showed marked 25 (OH) vitamin D deficiency with levels in PHY, CBZ, V+P (17.3±3.4, 46.8± 15, 27.6± 5) nmol/l respectively while in VAP group was with better level in VAP group 64.4± 8 nmol/l but still in the sufficient area. Optimal status of vitamin D is level > 50 nmol/l, vitamin D sufficiency is between 52.5-72.5 n mol/l while deficiency is < 50 nmol/l [34].

PHY group showed the highest decrease in vitamin D level. The three first groups are under treatment of enzyme inducers AED which cause destruction of vitamin D this was observed in study done by Valsamis [11] and Meier and Kraenzlin [26] colleagues. While VAP is a non enzyme inducer so VAP group showed better vitamin D level, studies showed that VAP does not affect bone resorption marker. This was reported in many studies [35]. Physiological concentrations of vitamin D3 are necessary for maintenance of aromatase activity necessary in osteoblasts function and so affect bone formation [15]. In the present group of subjects, enzyme inducing AED (PHY, PB and CBZ) and enzyme inhibiting AED (VPA) affected the 25 (OH) D metabolisms and caused decrease in its level alike. This leads us to speculate that bone mineral metabolism is affected on those with AED at sub-therapeutic plasma concentrations of the drug level as mentioned by Zare *et al.* [35]. The cytochrome P450 enzyme-inducing AEDs, such as phenytoin and carbamazepine, accelerate inactivation of vitamin D which decreases calcium uptake drives secondary hyperparathyroidism and accelerates bone loss [11].

All AEDs, both enzyme inducers (phenytoin, carbamazepine) and enzyme non-inducers, such as valproate, are associated with accelerated bone loss and subsequent increased risk of osteoporotic fracture [7, 8, 17]. A meta-analysis found AED therapy to be associated with increased risk of fracture, with the relative risk (RR) of 2.2 (95% CI 1.9–2.5) [36].

The fracture risk is dependent on the duration and cumulative dose of AEDs. A recent retrospective study evaluated nearly 16,000 patients over 50 years of age using AEDs for epilepsy and non-epilepsy indications. Use of carbamazepine and phenytoin were associated with a significant increase in non traumatic fractures, while valproate was not [37]. Animal study showed that valporic acid decreased bone mineral density in rats in dose depending manner [38].

Evidence-based strategies for prevention, screening, monitoring and treating bone loss and osteoporosis associated with AEDs are poor. Routine evaluation of 25-hydroxy vitamin D before treatment and every 6-12 months of AED therapy is important to ensure adequate vitamin D levels and early supplement of both vitamin D and calcium [34].

Low vitamin D status in patients with epilepsy has various health problems. Low serum 25(OH) D concentrations are associated with a higher risk for hip fracture and seizure related injuries [37] Significant elevation of ICTP showed in all patients in this study versus control emphasizing the fact that AED can cause bone health affection and bone resorption with highest elevation in PHY group this goes with many studies showing that phynetoin is very deterious drug causing bone resorption [34].

Serum calcium of cases showed no difference between control and drug groups except for group treated with PHY showing significant decrease than controls. This was mentioned in literature as AEDs affects intestinal absorption of Ca and cause vitamin D deficiency or insufficiency which affects ninety percent of the subjects studied longitudinally at the end of 6 months of therapy [10].

Mg showed no difference among the groups with studies confirming the observation. In our study, Mg was unaltered in all groups of epileptics and this is consistent with many studies [40, 41]. In contrast, some studies reported low Mg levels in epileptics [42].

In conclusion AED appear to have a deleterious effect on bone health and as treatment is long term one it is Mandatory to predict, investigate, support and treat bone affection as early as start of treatment. Although the study was with limited number of cases due to the specific enrolling criteria still it is important to recommend dietary follow up for all cases under such drugs. ICTP can be used as sensitive diagnostic test for bone affection.

## REFERENCES


1. Scott, R.A., S.D.L. Hatoo and J.W.A. Sander, 2001. The treatment of epilepsy in developing countries: where do we go from here□ Bull World Health Organ, 79: 344-351
2. Baroncelli, G.I., S. Bertelloni, F. Sodini and G. Saggese, 2005. Osteoporosis in children and adolescents: etiology and management. Paediatr. Drugs, 7: 295-323.
3. Petty, S.J., T.J. O'Brienand, J.D. Wark, 2007. Anti-epileptic medication and bone health. Osteoporos. Int., 8: 129-142.
4. Samaniego, E. and MR.D. Sheth, 2007. Bone consequences of epilepsy and antiepileptic medications. Semin. Pediatr. Neurol., 14: 196-200.
5. Henderson, R.C., R.K. Lark, M.J. Gurka, G. Worley, E.B. Fung, M. Conaway, V.A. Stallings and R.D. Stevenson, 2002. Bone density and metabolism in children and adolescents with moderate to severe cerebral palsy. Pediatrics, 110(1 Pt 1): e5.
6. Beerhorst, K., F.C. Huvers and W.O. Renier, 2005. Severe early onset osteopenia and osteoporosis caused by antiepileptic drugs. J. Med., 63: 222-226
7. Souverein, P., D. Webb, J. Weil, T. Van Staaand, A. Egberts, 2006. Use of antiepileptic drugs and risk of fractures: case-control study among patients with epilepsy. Neurology, 66: 1318-1324
8. Shen, C., F. Chen, Y. Zhang, Y. Guoand M. Ding, 2014. Association between use of antiepileptic drugs and fracture risk: a systematic review and meta-analysis. Bone, 64: 246-253
9. Pack, A.M., M.J. Morrell, A. Randall, D.J. McMahonand, E. Shane, 2008. Bone health in young women with epilepsy after one year of antiepileptic drug monotherapy. Neurology, 70(18): 1586-1593
10. Altay, E.E., A. Serdaroðlu, L. Tümer, K. Gücüyener and A. Hasanoðlu, 2000. Evaluation of bone mineral metabolism in children receiving carbamazepine and valproic acid. J. Pediatr. Endocrinol. Metab., 13(7): 933-939.
11. Valsamis, H., S. Arora, B. Labban and S. McFarlane, 2006. Antiepileptic drugs and bone metabolism. Nutr.Metab. (Lond), 3: 36.
12. Pack, K., A.B. Gidal and B. Vazquez, 2004. Bone disease associated with antiepileptic drugs. Cleve. Clin. J. Med., 71(2): S42-S48.
13. Dhillon, N. and W. Hogler, 2011. Fractures and Fanconi syndrome due to prolonged sodium valproate use. Neuropediatrics, 42: 119-121
14. Koch, H.U., D. Kraft, D. Von Herrath and K. Schaefer, 1972. Influence of diphenyl hydantoin and phenobarbital on intestinal calcium transport in the rat. Epilepsia, 13: 829-8
15. Yanase, T., S. Suzuki, K. Goto, M. Nomura, T. Okabe, R. Takayanagi and H. Nawata, 2003. Aromatase in bone: roles of Vitamin D3 and androgens. J Steroid Biochem. Mol. Biol., 86: 393-397
16. Onodera, K, A. Takahashi, S. Sakurada and Y. Okano, 2002. Effects of phenytoin and/or vitamin K2 (menatetrenone) on bone mineral density in the tibia of growing rats. Life Sci., 70: 1533-1542

17. Guo, C., G.M. Ronen and S.A. Atkinson, 2001. Long-term valporate and lamotrigine treatment may be a marker for reduced growth and bone mass in children with epilepsy. Epilepsia, 42: 1141-1147
18. Zhou, C., M. Assem, J.C. Tay, P.B. Watkins, B. Blumberg, E.G. Schuetz and E. Thummel, 2006. Steroid and xenobiotic receptor and vitamin D receptor crosstalk mediates CYP24 expression and drug-induced osteomalacia. J. Clin. Invest., 116(6): 1703-1712
19. Pack, A. and M. Morrell, 2001. Adverse effects of antiepileptic drugs on bone structure: epidemiology, mechanisms and therapeutic implications. CNS drugs, 15(8): 633–642
20. Risteli, J., I. Elomaa, S. Niemi, A. Novamo and L. Risteli, 1993. Radioimmunoassay for the pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen: a new serum marker of bone collagen degradation. Clin. Chem., 39: 635-40.
21. Elomaa, I., P. Virkkunen, L. Risteli and J. Risteli, 1992. Serum concentration of the cross-linked carboxy-terminal telopeptide of type I collagen(ICTP) is a useful prognostic indicator in multiple myeloma.Br. J. Cancer, 66: 337-41
22. Hakala, M., L. Risteli, J. Manelius, P. Nieminen and J. Risteli, 1993. Increased type I collagen degradation correlates with disease severity in rheumatoid arthritis. Ann. Rheum. Dis., 52: 866-9.
23. Kylmala, T., T. Tammela, L. Risteli, J. Risteli, T. Taube and E. Lomaa, 1993. Evaluationof the effect of oral Clodronate on skeletal metastases with type I collagen metabolites.A controlled trial of the Finnish Prostate Cancer Group. Eur. J. Cancer, 29A: 821-5.
24. Melkko, J., S. Niemi, L. Risteli and J. Risteli, 1990. Radioimmunoassay of the carboxy-terminal propeptide of human type I procollagen. Clin. Chem., 36: 1328-32.
25. Sheth, R.D., N. Binkley and B.P. Hermann, 2008. Progressive bone deficit in epilepsy. Neurology, 70(3): 170-176.
26. Meier, C. and M. Kraenzlin, 2011. Antiepileptics and bone health. Ther. Adv. Musculoskelet. Dis, 3: 235-243
27. Menon, B. and C.V. Harinarayan, 2010. The effect of anti epileptic drug therapy on serum 25-hydroxyvitamin D and parameters of calcium and bone metabolism-A longitudinal study. Seizure, 19(3): 153-158
28. Drezner, M., 2004. Treatment of anticonvulsant drug-induced bone disease. Epilepsy Behav., 5(2): S41-S47
29. Valmadrid, C., C. Voorhees, B. Littand C.R. Schneyer, 2001. Practice patterns of neurologists regarding bone and mineral effects of antiepileptic drug therapy. Arch Neurol., 58: 1369-1374.
30. Kobayashi, N., K. Ueda, M. Tsutsumi, Y. Tabata, K. Shimada., 1993. Enzyme immunoassay for plasma 25-hydroxyvitamin D3 employing immune affinity chromatography as a pretreatment method.J.Steroid Biochem. Mol. Biol., 44(1): 93-100
31. Biggs, H.G. and W.R. Moorehead, 1974. Estimation of serum calcium clin. chem., 20: 1458-1460
32. El-Merzabani, M.M., A.A. AlAaser and N.L. Zkhary, 1977 estimation of serum phosphorus. J. clin. chem. clin. Biochem., 15: 715-718
33. Bohuon, C., 1962. Estimation of serum magnesium. clin. chem. Acta, 7: 811-817
34. Lips, P., 2007. Relative value of 25(OH) D and 1,25(OH)D2 measurements. J. Bone Miner Res., 22: 1668-1671.
35. Zare, M., M.R. Ghaye Ghazvini, M. Dashti, M.R. Najafiand A.M.A. Naeini, 2013. Bone turnover markers in epileptic patients under chronic valproate therapy. J Res Med. Sci., 18: 338-40.
36. Vestergaard, P., 2005. Epilepsy, osteoporosis and fracture risk-a meta-analysis. Acta Neurol. Scand., 112: 277-286
37. Jette, N., L. Lix, C. Metge, H. Prior, J. McChesney and W. Leslie, 2011. Association of antiepileptic drugs with nontraumatic fractures: a population-based analysis. Arch. Neurol., 68: 107-112
38. Takahashi, A. and K. Onodera., 2005. Procceding valporic acid induced eoseopenia and its prevention with alfacalcidol and alendronate. J. hard tissue biology, 14(2): 275-6
39. Pearson, J.A., E. Burkhart, W.B. Pifalo, T. Palaggo-Toy and K. Krohn, 2005. A lifestyle modification intervention for the treatment osteoporosis. Am. J. Health Promot., 20(1): 28-33
40. Verrotti, A., S.A. Gostinelli, G. Coppola, P. Parisi and F.A. Chiarelli, 2010. 12-month longitudinal study of calcium metabolism and bone turnover during valporate monotherapy. J. Neurol. Sci., 290: 131-4.
41. ALshafei, M., S. Kassem and M. Abdel kader, 2013. Effect of Long Term Treatment with Antiepileptic Drugs on Oxidant Status, Zinc and Magnesium in Epileptic Patients, World Applied Sciences Journal, 28(3): 316-323
42. Ozcelik, A., N. Kurt., E. Kutsal and N. Sevinc, 2011. Effects of epilepsy and antiepileptic drugs on nitric, oxide, lipid peroxidation and xanthine oxidase system in children with idiopathic epilepsy. Seizure, 20(2): 138-42.