Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2017; 5(8F):3416-3422 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2017.v05i08.078

Original Research Article

The effect of phenytoin monotherapy on serum 25-hydroxyvitamin D and bone health markers -A prospective study

Aastha Bansal[1], Kiran Dahiya[2], Surekha Dabla[3], Veena Singh Ghalaut[4], Abhishek Dubey[5], Richa Goel[6], Narander kumar singla[7]

[1]Senior Resident, Department of Biochemistry, Maulana Azad Medical College, New Delhi- 110002
[2]Professor, Department of Biochemistry, Pt. B. D. Sharma PGIMS, Rohtak, Haryana-124001
[3]Professor, Department of Medicine, Pt. B. D. Sharma PGIMS, Rohtak, Haryana- 124001
[4]Professor & Head, Department of Biochemistry, Pt. B. D. Sharma PGIMS, Rohtak, Haryana- 124001
[5]Senior Resident, Department of Biochemistry, Maulana Azad Medical College, New Delhi- 110002
[6]Senior Resident, Department of Biochemistry, M.M. Institute of Medical Sciences & Research, Mullana, Ambala, Haryana,
[7]Senior Consultant, Department of Medicine, Shri Action Balaji Medical Institute, Delhi, 110063

***Corresponding author**
Dr. Aastha Bansal
Email: aastha.singla2013@gmail.com

Abstract: Phenytoin monotherapy in patients with epilepsy affects calcium metabolism and bone turnover markers leading to hypovitaminosis D, hypocalcemia, reduced bone mineral density and its imminent consequences. This study was planned to assess how early these changes may arise and to find out their correlation with phenytoin levels. In this prospective study, bone mineral density (BMD), 25-hydroxy vitamin D, urinary hydroxyproline were estimated at baseline, 2 and 6 months after phenytoin monotherapy and serum phenytoin levels were measured at 2 and 6 months of therapy. At 6 months, BMD showed a decrease (T-score -1.22±1.049 to -1.412±1.055, p value <0.001) while vitamin D levels started decreasing as early as 2 months after therapy and decreased further after 6 months (32.93±6.38 ng/mL to 31.46±5.99 ng/mL at 2 months and then to 29.96±5.94 ng/mL, p value <0.05 and <0.001 respectively). Urine hydroxyproline levels were 16.65±2.22 mg/day at diagnosis and increased to 16.97±2.25 mg/day after 2 months and to 18.544±2.83 mg/day after 6 months (pvalue <0.001 at 6 months). Mean serum phenytoin levels at 2 months were 15.74 ± 9.49 mg/L and while at 6 months these were observed to be 15.92 ± 5.54 mg/L. Urine hydroxyproline levels correlated positively with phenytoin levels (r-0.447, p value <0.05). Bone health derangement starts at around 2 months while at six months of phenytoin therapy, there is significant decline in bone health as indicated by status of markers like BMD and urine hydroxyproline.
Keywords: Phenytoin monotherapy, Bone mineral density, 25 hydroxy vitamin D, urine hydroxyproline, Bone formation and resorption marker

INTRODUCTION

Epilepsy is a common neurological disorder, defined as two or more unprovoked seizures characterized by sudden, recurrent excessive electrical activity in brain. Incidence of epilepsy is 0.3-0.5% in different population throughout the world while prevalence has been estimated as 5-10 people per 1000 population [1]. However in India, incidence has been estimated as 38-49.3 per 100,000 populations per year from two community based studies and prevalence stands at around 5 per thousand populations [2]. Epilepsy itself, in addition to antiepileptic drugs (AEDs) already has an increased propensity for increasing fracture risk by various mechanisms. Patients with epilepsy have 2-6 times greater risk of fracture than general population due to poor bone health [3].

Chronic therapy with AEDs is quite well known to lead to reduction in bone mineral density and abnormalities in calcium metabolism causing

hypocalcemia, hypophosphatemia, elevated levels of serum alkaline phosphatase and parathyroid hormone, reduced levels of biologically active vitamin D metabolites and are generally associated with rickets and osteomalacia. These effects are more commonly seen in patients on therapy with hepatic enzyme inducers like phenytoin, carbamazepine, primidone and phenobarbitone which are thought to induce enzymes leading to inactivation or breakdown of vitamin D. However, the exact mechanism of poor bone health with antiepileptic drugs is still not clear [4]. Studies on treatment pattern revealed that 40% of pediatric neurologists and 28% of neurologists treating adult patients screen patients on AEDs for bone disease [5].

Phenytoin is the most commonly prescribed drug in our setup due to its cost-effectiveness, efficacy and easy availability [6, 7]. It has a moderately large volume of distribution and is approximately 90% bound to plasma proteins and is prone to competitive displacement. Due to its saturable pharmacokinetics, it has a narrow therapeutic index [8]. Treatment with phenytoin monotherapy in patients with epilepsy affects calcium metabolism and bone turnover leading to hypocalcemia, reduced bone mineral density and its imminent consequences [4].

About 10% of body hydroxyproline is excreted in urine while 90% is catabolized in liver to urea and carbon dioxide. Hydroxyproline is found mainly in collagen and accounts for 13% of total aminoacids. However, hydroxyproline is not specific for collagen as it is present in other proteins as well and also, ingestion of meat or gelatin can also increase hydroxyproline excretion [9]. Urinary hydroxyproline level as a marker of resorption is known in patients suffering from osteoporosis and breast cancer [10]. It is not being done in routine now a day, but it has potential to be an easy, non-invasive marker which can reflect the bone activity [10, 11].

The physiology of each individual body is unique and serum levels of phenytoin are bound to affect its actions. Very few studies are there in literature to correlate the serum levels of phenytoin with vitamin D and other bone markers.

Therefore, this study was planned to find out effect and correlation of serum phenytoin levels, if any, with vitamin D and bone turnover in patients with epilepsy.

MATERIAL AND METHODS

The present study included 25 newly diagnosed patients with epilepsy. The diagnosis was made according to the International League against Epilepsy classification 2010 and was based on thorough history taking and neurological examination along with electroencephalography and neuroimaging (computerised tomography or magnetic resonance imaging). Patients suffering from any other chronic disease (renal, hepatic, endocrinal, malignancy), on any drugs or supplements that may alter vitamin D and calcium levels were excluded from the study. Pregnant or lactating females and patients with other risk factors of hypovitaminosis like gastric or bowel resection, malabsorption were excluded. Phenytoin therapy was initiated in a standard dose of 300 mg/day. All the patients were ambulatory and had an age appropriate activity and adequate diet as assessed by history.

Blood sample was collected from all patients at the time of diagnosis and after 2 and 6 months of phenytoin therapy for measuring serum 25-hydroxyvitamin D (25-(OH) D), serum phenytoin levels (at 2 and 6 months). All the patients underwent dual energy X-ray absorptiometry (DEXA) scan using Hologic Explorer QDR series (S/N 90797, Hologic Inc, Waltham, USA) at L1-L_4 at baseline, 2 and 6 months of therapy. Bone mineral density was measured and T-score (the difference in standard deviations between a given bone density and peak bone density in the normal reference population) and Z-score (age-adjusted T-score) were calculated [12]. Estimation of phenytoin levels was done by high performance liquid chromatography on Chromsystems, Germany based on principle of reverse phase adsorption chromatography [13]. Twenty four hour collected urine samples were analyzed for hydroxyproline colorimetrically using modified Neuman and Logan method.[14] Vitamin D was estimated by enzyme linked immunosorbent assay (ELISA) [15].

Patient's samples were grouped into I, II and III where group I was analysis at baseline, II was analysis at 2 months after therapy and III was analysis after 6 months of therapy.

The results were statistically analyzed using appropriate methods.

RESULTS

The mean age of the patients included in the study was 26.72±11.59 years (range 18-65years). The female/male ratio was 1:4, suggesting a male

preponderance in patients presenting with epilepsy to the outpatient department. Around 72% patients presented with partial seizures and one patient presented with neurocysticercosis. Majority of patients were students (44%) followed by farmers (28%), labourers (8%) and salesman and zamindar (4%) respectively. This suggests that outdoor activity and sun exposure was sufficient in these patients. Mean serum phenytoin levels in these patients at 2 months were 15.74 ± 9.49 mg/L (range-3.8-40.2 mg/L) while at 6 months were 15.92±5.54 mg/L (range-6.2-28.5 mg/L). This shows that majority of patients were in therapeutic range. At 2 months, 48% of the patients and at 6 months 68% of patients were having phenytoin levels within the therapeutic range (10-20 mg/L). Around 24% were in toxic range at 2 and 6 months and the rest 28% at 2 months and 8% at 6 months were in subtherapeutic range respectively.

Table-1: Comparison of bone mineral density and levels of serum phenytoin, serum vitamin D and urine hydroxyproline in various groups

Parameters		Group I	Group II	Group III	P value
Serum phenytoin (mg/L)	Mean±SD (Range)		15.74 ± 9.49 (3.8 - 40.2)	15.92 ± 5.54 (6.2 – 28.5)	
Vitamin D (ng/mL)	Mean±SD (Range)	32.93 ± 6.38 (22.3 – 45.2)	31.46 ± 5.99 (22.1 – 41.5)	29.96 ± 5.94 (20.0 – 40.0)	0.036* <0.001**
BMD (T-score)	Mean±SD (Range)	-1.22 ± 1.049 (-2.40 – 2.30)	-1.256 ± 1.065 (-3.00 – 2.20)	-1.412±1.055 (-3.00 – 1.90)	<0.001**
Urine hydroxyproline (mg/day)	Mean±SD (Range)	16.65 ± 2.22 (12.0 – 20.0)	16.97 ± 2.25 (13.4 – 21.0)	18.54± 2.83 (13.6 – 23.6)	<0.001**

*significant p value between group I and II
*significant p value between group I and III, Group II and III.

A significant decrease was observed in vitamin D levels amongst these groups **(Table 1).** It was observed that a significant difference appeared within 2 months of phenytoin therapy (p value 0.036), which further increased after 6 months of therapy (p value <0.001).

Urine hydroxyproline levels were increased as shown **(Table 1).** The change was found to be highly significant even after 2 months of therapy which further increased after 6 months. It was observed that the significant change in BMD levels appeared between a time intervals of 2 to 6 months after therapy. Mean difference between group I and II was 0.036 ± 0.045 but it was not statistically significant. Major difference appeared after 2 months of phenytoin therapy which was shown by a mean difference of 0.156 ± 0.031 between group I and III.

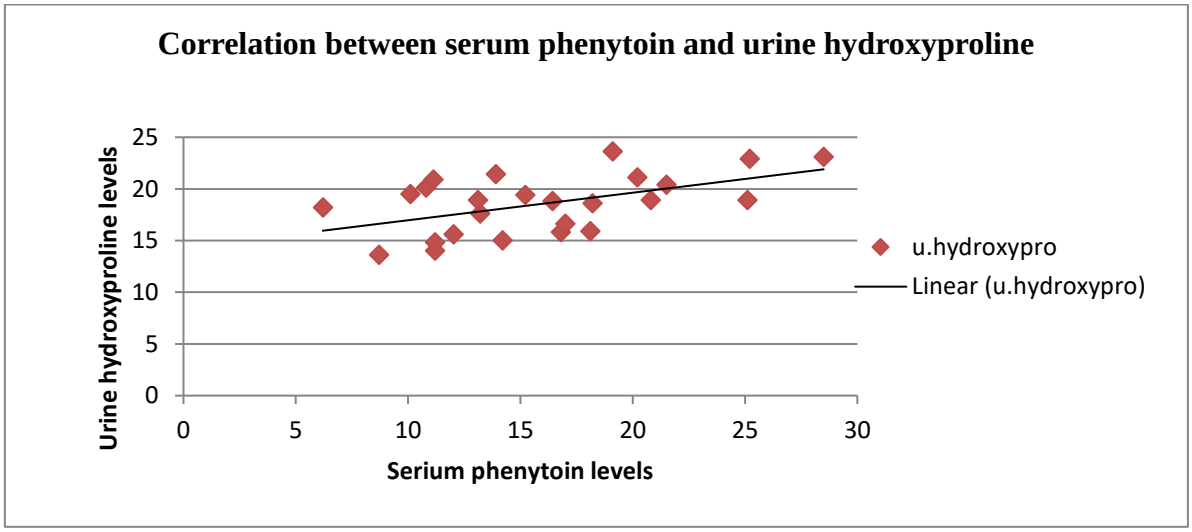

Fig-1: Correlation between serum phenytoin levels and urine hydroxyproline levels at 6 months of therapy

According to world health organization (WHO) criteria, at baseline, 80% of the patients were osteopenic and 20% were normal. After 2 months of phenytoin therapy one patient developed osteoporosis and 2 more patients became osteopenic while after 6 months of therapy one more patient became osteopenic and 2 more patients developed osteoporosis as compared to pretherapy status. There was no significant correlation between vitamin D levels and T-score for BMD as shown by Pearson's Correlation Coefficient test (r=0.004, p value-0.983). Also, no correlation was observed of phenytoin levels with vitamin D (r=0.119, p value- 0.573) and BMD (r=0.213, p value- 0.360). However, a positive correlation of phenytoin was observed with urine hydroxyproline levels at 6 months after therapy (r=0.447, p value <0.05) which was statistically significant (Table 1, Fig. 1).

DISCUSSION

The results of this study clearly indicate that patients on phenytoin monotherapy are at a definite risk of osteomalacia and osteoporosis in later life. The parameters used in the study can help in predicting the future risk and can be used as novel screening markers in epilepsy patients. Both these markers i.e. DEXA scan for BMD and urine hydroxyproline are non-invasive, inexpensive and can be repeated without hassles and reflect metabolic activity and mineralization of the skeletal system.

In present study, out of 25 patients 20 were males and 5 females. This difference in incidence among sexes has also been documented in earlier studies [16]. The females have been reported to a marginally lower incidence of epilepsy and unprovoked seizures than males. This difference is usually attributed to male's greater exposure to risk factors.[17]The difference in presentation can also be due to social stigma involved especially in low socioeconomic groups resulting in comparatively less number of females attending the outpatient department for management. This difference may also be due to skewness of the sex ratio towards males in population of Haryana [18]. In the present study 48% patients were in therapeutic range and 24% were in toxic range after 2 months, while at 6 months 68% were in therapeutic range. This indicates that phenytoin level monitoring at a regular interval is an important tool to check the patient's compliance for dose titration. It can also helps us to study the dose related adverse effects at the earliest. Long term repeated exposure to high serum concentration of phenytoin may predispose patients to irreversible neurotoxicity and may also exarcerbate seizures [19].

Patients with epilepsy can, however, have an increased propensity for insufficient state of vitamin D as they tend to live a sedentary lifestyle and their outdoor activity is minimum due to the social stigma attached to epilepsy [20]. The differences can also be explained on the basis of variations in seasons, diet and sun exposure. Our study showed a significant decrease in vitamin D levels at 2 and further at 6 months. Similar findings were observed by Krishnamoorthy *et al*. who conducted a research to find out the effect of supplementation of vitamin D and calcium in patients with epilepsy (on phenytoin or valproic acid monotherapy) on biochemical parameters of bone health for a period of 3 months [21]. Other researches also have documented the similar changes in vitamin D levels in patients on phenytoin or any other enzyme inducing AEDs [22-25]. However some studies reported no significant reduction in vitamin D levels with use of AEDs [26, 27]. It has been stated that the decrease in vitamin D levels with use of certain drugs such as phenytoin, carbamazepine and phenobarbitone could be due to vitamin D inactivation by induction of hepatic microsomal enzymes which increases the catabolism of 25-OHD by 25-hydroxylase production [22]. Another proposed mechanism is activation of mixed oxidase system leading to conversion of active vitamin D to a less active and non-polar form, thereby, enhancing the loss of vitamin D and gastrointestinal system [28]. Phenytoin has a direct effect on calcium metabolism as well, thus leading to hypocalcemia which leads to secondary hyperparathyroism that further can lead to inactive form of vitamin D (24,25-$(OH)_2D$) [4]. Various drugs can also act through pregnane X receptor which is expressed in gastrointestinal tract, kidneys and liver cells and resemble vitamin D. Thus, instead of vitamin D pregnane X receptor binds to vitamin D responsive element (VDRE) at deoxyribonucleic acid (DNA) and acting as transcription factor regulates the gene expression. This leads to upregulation of 24-hydroxylases (CYP24) or an isoenzyme (CYP3A4 in liver and small intestine) and that further may lead to increased breakdown of $1,25(OH)_2D$ and 25-(OH)D [20, 29]. However no significant correlation of phenytoin levels could be found with vitamin D and BMD in the present study. It might be because of small sample size.

Studies on lifestyle of patients with epilepsy suggested that physical activity is limited in

them which may be due to the fear that it may provoke seizures or some mishappenings might occur. This decrease in mobility, outdoor activities, and weight bearing exercises increases the risk of osteoporosis.The decrease in mobility has been implicated as one of the risk factors for osteopenia and bone loss.[30] The low BMD levels indicate that there is a greater risk of osteoporotic fractures in later life. Many other studies have demonstrated decrease in BMD levels in adults taking AEDs [25,31,32].

In a study by Farhat *et al*., duration of administration of AEDs has been reported to correlate inversely with BMD and the decrease was found to be prominent during the early phase of treatment suggesting that the disease, itself, or some associated factors may also be responsible for the above finding. But no significant correlation of BMD was found with vitamin D levels in this study though decrease in vitamin D levels and BMD was observed with increased duration of treatment [23].

An increase in levels of urine hydroxyproline in the present study was observed at 6 months. Similar finding in urine hydroxyproline has been reported by in other studies [33, 34]. This effect may be explained on the basis of an imbalance between the bone formation and resorption phase which is inclined towards the osteoclastic phase. Another study by Pack et al. included cross linked N-telopeptide as a bone resorption marker in urine and found increased levels in patients treated with phenytoin but the change was not significant [35].

Thus, direct urinary assay of hydroxyproline to measure bone resorption may have clinical applications as part of screening programs to assess the risk of osteoporotic fractures. Urine hydroxyproline levels may prove to be a promising marker in these patients being non-invasive and simple procedure. Its levels were observed to correlate with the phenytoin levels in plasma at 6 months of therapy. No other conventional marker showed a correlation with phenytoin levels. So, urine hydroxyproline levels may prove to be a better marker for bone health in patients with epilepsy on phenytoin.

Thus, assessment of vitamin D, BMD and urine hydroxyproline in epilepsy patients indicates an altered bone health status even before the initiation of phenytoin monotherapy. The levels of these bone health markers further deteriorated at 2 and 6 months of therapy with phenytoin but a significant correlation of serum phenytoin levels could only be established with urine hydroxyproline levels.

CONCLUSION

Therefore, it is concluded that bone health is already compromised in epilepsy patients and it may be worsened by phenytoin therapy. These findings may call for selection of newer drugs for treatment which are less deleterious to bone health. Further the study also indicates towards use of BMD and urine hydroxyproline as economical, non-invasive and simple screening bone markers in epilepsy patients though further studies with larger sample groups are needed to support these findings.

REFERENCES

1. Lowenstein DH. Seizure and epilepsy. In: Lango DL, Kasper DL, Jameson JL, Fauci AS, Hauser SL, Loscalzo J, editors. Harrison Principle of Internal Medicine. 18thed. New York: McGraw Hill; 2011. p. 3251-8.
2. Ray BK, Bhattacharya S, Kundu TN, Saha SP, Das SK. Epidemiology of epilepsy-Indian perspective. J Indian Med Assoc 2002;100:322-6.
3. Souverein PC, Webb DJ, Weil JG, Van Staa TP, Egberts ACG. Use of antiepileptic drugs and risk of fractures. Case control study among patients with epilepsy. Neurology 2006;66:1318-24.
4. Valsamis HA, Arora SK, Labban B, McFarlane SI. Antiepileptic drugs and bone metabolism. Nutrition & Metabolism 2006;3:36.
5. Valmadrid C, Voorhees C, Litt B, Schneyer CR. Practice patterns of neurologists regarding bone and mineral effects of Antiepileptic drug therapy. Arch Neurol 2001;58:1369-74.
6. Krishnan A, Sahariah SU, Kapoor SK. Cost of epilepsy in patients attending a secondary level hospital in India. Epilepsia 2004;45:289-91.
7. Krishnan A, Ritvik, Chowdhury C. Cost of antiepileptic drugs in India. Neurol Asia 2007;12:42-3.
8. Richens A. Clinical pharmacokinetics of phenytoin. Clin Pharmacokinet 1979;4:153-69.
9. Risteli J, Winter WE, Kleerekoper M, Risteli L. Bone and mineral metabolism. In: Burtis CA, Ashwood ER, Bruns DE, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th edition. Philadelphia: WB Saunders; 2012. p. 307-28.
10. Jagtap VR, Ganu JV. Effect of antiresorptive therapy on urinary hydroxyproline in postmenopausal osteoporosis. Indian J Clin Biochem 2012;27:90–3.

11. Frenay M, Namer M, Boublil JL, Khater R, Viot M, Francois E, Milano G. Value of urinary hydroxyproline and bone isoenzyme of alkaline phosphatase in the early detection and follow-up of bone metastasis in breast cancer patients. Bulletin du cancer. 1988;75(6):533-9.
12. Watts NB. Fundamentals and pitfalls of bone densitometry using dual-energy X-ray absorptiometry (DXA). Osteoporos Int 2004;15:847-54.
13. Hortin GL, Goldberger BA. Chromatography and Extraction. In: Burtis CA, Ashwood ER, Bruns DE, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th edition. Philadelphia: WB Saunders:2012. P. 307-28.
14. Mitoma C, Smith TE, Davidson JD, Udenfriend S, Dalosta M, Sjoerdsma A. Improvements in methods for measuring hydroxyproline. J Lab Clin Med 1959;53:970-6.
15. Zerwekh JE. Blood biomarkers of vitamin D status. Am J Clin Nutr 2008;87:1087-91.
16. Sridharan R, Murthy BN. Prevalence and Pattern of Epilepsy in India. Epilepsia 1999;40:631-6.
17. McHugh JC, Delanty N. Epidemiology and classification of epilepsy: gender comparisons. Int Rev Neurobiol 2008;83:11-26
18. Dahiya K, Bansal P, Gahlaut VS, Dhankar R, Gahlaut PS. Therapeutic drug monitoring for antiepileptic drugs using HPLC: An experience at a tertiary care hospital in India. Neurol Asia 2010;15:233-7.
19. Reynolds EH, Trimble MR. Adverse neuropsychiatric effects of anticonvulsant drugs. Drugs 1985;29:570-81.
20. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D_3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D synthesis in human skin. J Clin Endocrinol Metab 1988;67:373-8.
21. Krishnamoorthy G, Nair R, Sundar U, Kini P, Shrivastava M. Early predisposition to osteomalacia in Indian adults on phenytoin or valproate monotherapy and effective prophylaxis by simultaneous supplementation with calcium and 25-hydroxy vitamin D at recommended daily allowance dosage: a prospective study. Neurol India 2010;58:213-9.
22. Feldkamp J, Becker A, Witte OW, Scharff D, Scherbaum WA. Long term anticonvulsant therapy leads to low bone mineral density: evidence for direct drug effects of phenytoin and carbamazepine on human osteoblast like cells. Exp Clin Endocrinol Diabetes 2000;108:37-43.
23. Farhat G, Yamout B, Mikati MA, Demirjian S, Sawaya R, El-Hajj Fuleihan G. Effect of antiepileptic drugs on bone density in ambulatory patients. Neurology 2002;58:1348-53.
24. Telci A, Cakatay U, Kurt BB, Kayali R, Sivas A, Akçay T, Gokyigit A. Changes in bone turnover and deoxypyridinoline levels in epileptic patients. Clin Chem Lab Med 2000;38:47-50.
25. Valimaki MJ, Tiihonen M, Laitinen K, Tahtela R, Karkkainen M, Lamberg-Allardt C, Makela P, Tunninen R. Bone mineral density measured by dual-energy X-ray absorptiometry and novel markers of bone formation and resorption in patients on antiepileptic drugs. J Bone Miner Res 1994;9:631–7.
26. Tsukahara H, Kimura K, Todoroki Y, Ohshima Y, Hiraoka M, Shigematsu Y, Tsukahara Y, Miura M, Mayumi M. Bone mineral status in ambulatory pediatric patients on long-term anti-epileptic drug therapy. Pediatr Int 2002;44:247-53.
27. Verrotti A, Greco R, Latini G, Morgese G, Chiarelli F. Increased bone turnover in prepubertal, pubertal, and postpubertal patients receiving carbamazepine. Epilepsia 2002;43:1488-92.
28. Wahl TO, Gobuty AH, Lukert BP. Long term anticonvulsant therapy and intestinal calcium absorption. Pharm Ther 1981;30:505-12.
29. Grober U, Klisters K. Influence of drugs on vitamin D and calcium metabolism. Dermatoendocrinol 2012;4:158–66.
30. Holick MF, Krane SM . Introduction to bone and mineral metabolism. In: Harrison's principles of internal medicine. In: Eugene B, Fauci A, Kasper D, Hauser S, Longo D and Jameson JL, editor. Harrison's principles of internal medicine. 15th. New York, McGraw-Hill; 2001.p. 2192–94.
31. Sato Y, Kondo I, Ishida S, Motooka H, Takayama K, Tomita Y, Maeda H, Satoh K. Decreased bone mass and increased bone turnover with valproate therapy in adults with epilepsy. Neurology. 2001 Aug 14;57(3):445-9.
32. Kubota F, Kifune A, Shibata N, Akata T, Takeuchi K, Takahashi S, Ohsawa M, Takama F. Bone mineral density of epileptic patients on long-term antiepileptic drug therapy: a quantitative digital radiography study. Epilepsy research. 1999 Feb 28;33(2):93-7.
33. Bell RD, Pak CY, Zerwekh J, Barilla DE, Vasko M. Effect of phenytoin on bone and vitamin D metabolism. Ann Neurol 1979;5:374-8.

34. Liakakos D, Papadonlos S, Vlashos P, Boviatsi E, Varonos DD. Serum alkaline phosphatase and urinary hydroxyproline values in children receiving phenobarbital with and without vitamin D. J Pediatr 1975; 87: 291-296.

35. Pack AM, Morrell MJ, Marcus R, Holloway L, Flaster E, Doñe S, Randall A, Seale C, Shane E. Bone mass and turnover in women with epilepsy on antiepileptic drug monotherapy. Annals of neurology. 2005 Feb 1;57(2):252-7.