How to Cite:
Raju, G. J. K. P., Rajendran, K., Dwajani, S., Adarsh, E., & Paveena, A. S. (2022). Prognosticators of bone health in pediatrics with epilepsy using anti-epileptic drugs: A prospective interventional study. *International Journal of Health Sciences*, *6*(S10), 918–929. https://doi.org/10.53730/ijhs.v6nS10.13735

Prognosticators of bone health in pediatrics with epilepsy using anti-epileptic drugs: A prospective interventional study

G. J. K. Prakash Raju
Ph.D. scholar, Department of Pharmacology, Saveetha Medical College and Hospital, Chennai, Tamilnadu, India
Corresponding author email: jaya.krishna89@gmail.com

Dr. Kannan Rajendran
Professor, Department of Medicine, Saveetha Medical College and Hospital, Chennai, Tamilnadu, India

Dr. Dwajani S.
Associate Professor, Department of Pharmacology, Senior Research Associate, Central Research laboratory, Rajarajeswari Medical College and Hospital, Bengaluru, Karnataka, India

Dr. Adarsh E.
Professor, Department of Pediatrics, Rajarajeswari Medical College and Hospital, Bengaluru, Karnataka, India

A. S. Paveena
Department of studies in statistics, University of Mysore, Manasagangotri, Mysore

Abstract---Introduction: Epilepsy is a common and long-lasting neurological disorder in children who require long-term treatment with Antiepileptic drugs (AED). Such long-term AED use may have negative effects on bone causing bone loss and osteoporosis, necessitating frequent monitoring. Data on the effect of AED on bone health in children is scarce compared to adults and hence this study was undertaken. Aim: To compare and quantify the effects of sodium valproate (SV), carbamazepine (CBZ), and levetiracetam (LEV) on bone health in children using specific bone biomarkers such as vitamin D, calcium, phosphorus, ALP, osteocalcin (OCN), and beta serum cross laps (CTx). Materials and methods: A prospective interventional study was carried out in Rajarajeswari Medical College and Hospital, Bangalore, between October 2019 to March 2022. A total of 79 confirmed cases of epilepsy within the age group of 1-18 years, receiving treatment with sodium valproate (SV), carbamazepine (CBZ),

and levetiracetam (LEV) monotherapy for a period of minimum one month were enrolled. Serum samples of calcium (Ca), phosphorus (PO_4), alkaline phosphatase (ALP), Vitamin D were analyzed at the time of initiation of study and these levels were considered as baseline levels. Bone biomarkers such as beta serum cross-laps (CTx), and osteocalcin (OCN) levels were measured at the time of initiation of study which was considered as a baseline, and then at third month and sixth month follow-up visit. The Electrochemiluminescence method was used to analyze the levels of bone biomarkers. Results: Total 79 subjects were included in the study. The mean age group in the study was 7.8 ± 5.1years. 19 (23.75%) subjects had focal seizures. Among 79, 37 (46.25%) were on SV, 15 (18.75%) were on CBZ , and 28 (35.0%) on LEV as monotherapy. Bone-specific biomarkers, beta CTx and OCN did not show any significant difference across the timeline (baseline v/s 3rd month v/s 6th month) as well as between the drugs (sodium valproate v/s carbamazepine v/s levetiracetam). A significant difference was noted in osteocalcin levels between the drugs only at 6th month of treatment duration (p-value=0.032). Conclusion: This study revealed that there was no significant difference in the bone biomarker levels between the antiepileptic drugs at each point of time and also no significant difference was noted across the timeline for each drug. Hence, we suggest that long-duration studies with frequent follow-ups and monitoring of bone biomarkers are necessary to analyze the exact time period of bone loss if any.

Keywords---bone biomarkers, bone health, carbamazepine, levetiracetam, monotherapy, pediatrics with epilepsy, sodium valproate.

Introduction

Epilepsy is a noncommunicable chronic brain disorder that affects individuals of all ages. Epilepsy affects around 50 million people worldwide, having a prevalence of 4-10/1000 in developed nations (1). Nearly 80% of patients with epilepsy (PWE) are living in low- and middle-income countries. Prevalence is considered to be higher in children and in the elderly (2). For adequate and long-term seizure control, anti-epileptics like carbamazepine or sodium valproate serve as first-line treatment (3). In addition to known side effects such as alopecia, hair curling, weight gain, diplopia, ataxia, dizziness, weakness, and increased bleeding tendency (4), t*here is substantial evidence that these medications may have the potential to impair bone and mineral metabolism, which has been linked to bone diseases like osteopenia, and osteoporosis, thus increasing the risk of fracture* (5,6,7). Anti-epileptic medications are known to cause deleterious effects on bone mineral status in both pediatrics and adults. *(8). The use of Anti-epileptic drugs (AED) on a long-term basis has been associated with lower bone mineral density (BMD), as well as changes in bone turnover (9). When compared to the general population, the risk of fracture is said to be increased 2-6 times, with pathological conditions associated with bone metabolic disorders accounting for more than 15%*

of fractures (10). Dual X-ray absorptiometry (DEXA) is the most frequently used method for quantifying BMD and is considered as the gold standard test for diagnostic purposes. Detection of bone loss using DEXA is considered after 2-3years of therapy with drug treatment. Early changes in bone remodeling is not picked up by DEXA. Hence specific bone biomarkers like beta serum cross laps and osteocalcin can be considered for predicting bone loss at the early stage (11).

Beta cross laps (*β* CTx) is a type I collagen C-terminal telopeptide that is the major component (about 90%) of a protein bone substrate. Its estimation serves as a specific marker for such breakdown of mature bone collagen type I (12). It is critical for patients who have received long-term epilepsy treatment to know the possible adverse effects, which include bone loss. To minimize the risk of bone loss from long-term anti-epileptic drugs (AEDs), frequent analysis and monitoring of certain bone biomarkers *β* CTx would be beneficial during AED treatment. One bone remodeling cycle takes at least three weeks to three months. As a result, changes in serum markers, if any, can only be seen after a month of treatment, and the drug's effect on bone can only be seen after one remodeling cycle is completed. *Hence, the goal of this study was to assess the effect of sodium valproate (SV), carbamazepine (CBZ), and levetiracetam (LEV) monotherapy on bone health using bone-specific biomarkers osteocalcin (OCN) and beta serum cross laps (β CT x) in children with epilepsy. There is no data or limited data available on the effect of AEDs on bone health among children with epilepsy when compared with adults. Hence this study was carried out to determine the effect of* bone health in children receiving sodium valproate (SV), carbamazepine (CBZ), and levetiracetam (LEV).

Aim

To compare and quantify the effects of sodium valproate (SV), carbamazepine (CBZ), and levetiracetam (LEV) on bone health in children with epilepsy using specific bone biomarkers osteocalcin (OCN), and beta serum cross laps (*β* CTx).

Objectives
Primary objective

To Compare the effects of bone biomarkers osteocalcin (OCN), and beta serum cross laps (*β* CTx) at each visit (baseline v/s 3rd month v/s 6th month in patients receiving Sodium Valproate, Carbamazepine, and Levetiracetam monotherapy.

Secondary objective

To determine the association of bone biomarkers with AEDs (SV/CBZ/LEV).

Materials and Methodology

This was a prospective interventional study, which was carried out after receiving approval from the institutional ethics committee (RRMCH-IEC/68/2018-19).The study was carried out in the pediatric department of Rajarajeswari Medical College and Hospital, Bangalore, between October 2019 to March 2022. Children with epilepsy, ≤18 years of age receiving sodium valproate, carbamazepine, and

levetiracetam monotherapy, who were on treatment for at least 1 month were enrolled in the study after obtaining parental or legal guardian consent. Children with motor function disorders, febrile seizures, diseases affecting their bone and mineral metabolisms, Paget's disease, liver and kidney disease, thyroid disease, Malabsorption, vitamin D supplements, and those on alternate or complimentary treatment for epilepsy were excluded from the study as the bone marker levels among these patients may introduce bias in the study (the decrease in bone biomarker level may not be due to AED but because of the presence of above-mentioned diseases and hence the actual effect of AED on bone health cannot be studied).

Sample size was calculated using the Yamane equation, $n=N/1+Ne^2$ (13). Here, the N=average number of children with epilepsy attending the pediatric outpatient department in a month was 10/month, hence it was 120/year, N=120, e= absolute precision=5%, $n=120/1+120(0.05)^2=92.30$. This was approximated to 100. 21 children were excluded because of incomplete, or no prescription details and few patients were put on different medications finally, 79 children were included in the study.

Data on demographic characteristics like age and gender was collected using interview method. Socioeconomic status was calculated as per the Kuppuswamy scale (14). Disease details like type of seizures, duration of disease, Diagnosis of epilepsy, and the type of seizures were confirmed by a pediatric neurologist. Treatment details like (AED used, duration of treatment, dose, dosage, family history, and alternate or complimentary drug used for epilepsy) were collected. All the patients receiving AEDs were prescribed by a pediatric neurologist and were calculated as per age and body weight. Hence all patients were within the standard dose range as mentioned. AEDs considered for the study and their normal doses were Sodium valproate: 15-30mg/kg/day; Levetiracetam: 10-30 mg/kg/day; Carbamazepine: 15-30mg/kg/day (15).

Hematological parameters such as hemoglobin and calcium [$Ca^{+2,}$ normal range-9.0-11.0 mg/dl] (16), phosphorous [PO_4, normal range:3.5-6.6 mg/dl)] (16), alkaline phosphatase [ALP, normal range:100-560 U/L](16), and vitamin D levels [normal range:2-50ng/ml](16) were measured from the blood samples collected from all children enrolled in the study. These were analyzed only once, i.e., on the day of enrollment, and it was considered as a baseline level for enrollment. Only those patients with normal lab values were subjected to further measurement of bone biomarkers such as bone formation marker osteocalcin [normal range:5-225 ng/ml] (17) and bone resorption marker serum cross laps or C-terminal telopeptide of type I collagen [beta CTx, normal range:538-462 ng/ml] (18). A study by Yang et al (19) has concluded osteocalcin as most promising marker and serum cross laps as the convenient method for assessing bone health among children and hence these two bone biomarkers were considered for this study. Serum osteocalcin and Beta CTx levels were measured on day 1 which was considered as baseline and then at 3rd month and 6th month of AED treatment. The serum samples for Beta CTx and osteocalcin were stored at -80 ^{0} Celsius until analysis. Bone biomarkers analysis was carried out using Electrochemiluminescence [Elecsys Cobas e411 by Roche] technique at Central Research Lab, Rajarajeshwari Medical College, and Hospital, Bangalore.

Statistical analysis

Descriptive statistics like frequency and percentages were used to present qualitative data, mean and standard deviation was used to present quantitative data like age. The data were expressed as mean + SE and analyzed by two way repeated measures analysis of variance. When it was found significant multiple comparison test was carried out using Bonferroni 't' test for drugs, months and the interaction. Within-group and between-group comparisons also were carried out. A probability of 0.05 and less was considered as statistically significant. SigmaPlot 14.5 version (Systat Software Inc., San Jose, USA) was used for statistical analysis and graph plotting.

Results

The mean age of the PWE was 7.8 ± 5.1 and the age ranged between 1-18 years. 54 (68.4%) were males and 25 (31.6%) were females. 24 (30.4%) belonged to 11-15 years age group which was higher (Table 1). 51 (65%) were from urban area and 30 (38.75%) belonged to the upper middle class which was calculated as per the Kuppuswamy scale (Table 1).

Table 1
Distribution of demographic characteristics among the patients

Variable	Number (%)
Gender	
Male	54 (68.4)
Female	25 (31.6)
Age in years	
1-5	34 (43.0)
6-10	15 (19.0)
11-15	24 (30.4)
16-18	6 (7.6)
Area	
Rural	28 (35.00)
Urban	51 (65.00)
Socio-economic status of the family	
Upper (I)	8 (10)
Upper middle (II)	30 (38.75)
lower middle (III)	27 (33.75)
Upper lower (IV)	13 (16.25)
Lower (v)	1 (1.25)

On analyzing the type of epilepsy 19 (23.75%) were diagnosed with focal seizures followed by generalized tonic-clonic seizures (GTCS) which was 15 (18.75%) .On assessing the AED treatment among 79 patients, 37 (46.25%) were on sodium valproate, 15 (18.75%) on carbamazepine, and 28 (35.0%) on levetiracetam who received these medications as monotherapy (Table 2). Doses of the drugs were well within the dose range which was calculated according to the age and body weight of the children which was prescribed by a pediatric neurologist. 15

(18.75%) had a family history of seizures among the near and dear ones. None of the patients received any alternate or complementary medications for the treatment of epilepsy. Biochemical investigations like Vit D, Calcium, Po4, and ALP were analyzed before AED treatment which was considered as baseline parameters where all children were within normal limits.

Table 2
Type of Epilepsy and AED utilization pattern

Types of epilepsy	Number of patients (%)
Focal seizure	19 (23.75)
GTCS	15 (18.75)
Myoclonic	8 (10.00)
Rolandic epilepsy	12 (15.00)
Others	19 (23.75)
Genetic	1 (1.25)
Idiopathic Epilepsy	1 (1.25)
Refractory Epilepsy	2 (2.50)
Complex partial	3 (3.75)
AED therapy	Number of patients (%)
Levetiracetam	28 (35.00)
Carbamazepine	15 (18.75)
Sodium Valproate	37 (46.25)

The mean values of serum osteocalcin of sodium valproate baseline, carbamazepine baseline, levetiracetam baseline, sodium valproate 3 months, carbamazepine 3 months, levetiracetam 3 months, sodium valproate 6 months, carbamazepine 6 months and levetiracetam 6 months are 19.965, 16.750, 26.239, 20.497, 17.460, 28.375, 25.677, 16.820 and 31.175 (ng/L) respectively. Two-way RM ANOVA showed significance among months (baseline, 3 months and 6 months) with $p < 0.001$. Within groups, the comparison showed significance among valproate baseline and valproate 6 months, valproate 3 months and valproate 6 months, and levetiracetam baseline and levetiracetam 6 months ($P < 0.001$, < 0.001 and 0.002 respectively). From baseline to 6 months sodium valproate showed an increase of 28.6 %, carbamazepine showed an increase of 0.4 % and levetiracetam showed an increase of 18.8 %. This shows that carbamazepine has no influence on osteocalcin, whereas sodium valproate and levetiracetam increases the level of osteocalcin. Two-way RM ANOVA showed significance among groups (sodium valproate, carbamazepine and levetiracetam) with ($P = 0.032$) (Table 3) (Figure:1). On comparing Between groups of SV vs LEV, SV vs CBZ, and CBZ vs LEV it showed significance only among carbamazepine and levetiracetam at 6 months ($P = 0.026$) . As carbamazepine has no influence on osteocalcin, the suspected drug is levetiracetam which increases the level of osteocalcin.

The mean values of serum beta CTx of valproate baseline, carbamazepine baseline, levetiracetam baseline, valproate 3 months, carbamazepine 3 months, levetiracetam 3 months, valproate 6 months, carbamazepine 6 months and levetiracetam 6 months are 219.6, 234.1, 267.6, 213.2, 231.1, 262.6, 207.9,

236.9 and 257.2 respectively(ng/L).Two-way RM ANOVA showed no significance among groups (valproate, carbamazepine and levetiracetam), months (baseline, 3 months and 6 months), and group X month interaction (P = 0.162, 0.266 and 0.689 respectively) This shows that the three antiepileptic drugs do not have any role on beta CTx.

Table 3
Comparison of antiepileptic drug treatments, sodium valproate, carbamazepine and levetiracetam on serum osteocalcin in children

S.No.	Drugs	Month	Mean + SE (ng/L)
1	Sodium valproate	Baseline	19.965 + 2.443
	Carbamazepine	Baseline	16.750 + 2.398
	Levetiracetam	Baseline	26.239 + 2.577
	Sodium valproate	3 months	20.497 + 2.599
	Carbamazepine	3 months	17.460 + 2.500
	Levetiracetam	3 months	28.375 + 2.584
	Sodium valproate	6 months	25.677 + 2.972
	Carbamazepine	6 months	16.820 + 2.093
	Levetiracetam	6 months	31.175 + 2.629
2	Significance among groups (Sodium valproate, carbamazepine and levetiracetam)		F = 3.589 P = 0.032
	Significance among months (baseline, 3 months and 6 months)		F = 8.444 P < 0.001
	Significance with groups X months (interaction)		F = 1.543 P = 0.193
3	Comparison within Sodium valproate (baseline and 3 months)		t = 0.430 P = 1.0
	Comparison within Sodium valproate (baseline and 6 months)		t = 4.621 P < 0.001
	Comparison within Sodium valproate (3 months and 6 months)		t = 4.191 P < 0.001
	Comparison within carbamazepine (baseline and 3 months)		t = 0.353 P = 1.0
	Comparison within carbamazepine (baseline and 6 months)		t = 0.377 P = 1.0
	Comparison within carbamazepine (3 months and 6 months)		t = 0.0434 P = 1.0
	Comparison within levetiracetam (baseline and 3 months)		t = 1.503 P = 0.405
	Comparison within levetiracetam (baseline and 6 months)		t = 3.474 P = 0.002
	Comparison within levetiracetam (3 months and 6 months)		t = 1.971 P = 0.152
4	Comparison between baseline (Sodium valproate and carbamazepine)		t = 0.712 P = 1.0
	Comparison between baseline (Sodium valproate and levetiracetam)		t = 1.741 P = 0.255
	Comparison between baseline (carbamazepine and levetiracetam)		t = 2.015

		P = 0.140
	Comparison between 3 months (sodium valproate and carbamazepine)	t = 0.673 P = 1.0
	Comparison between 3 months (valproate and levetiracetam)	t = 2.187 P = 0.094
	Comparison between 3 months (carbamazepine and levetiracetam	t = 2.318 P = 0.068
	Comparison between 6 months (sodium valproate and carbamazepine)	t = 1.656 P = 0.303
	Comparison between 6 months ((sodium valproate and levetiracetam)	t = 1.526 P = 0.391
	Comparison between 6 months (carbamazepine and levetiracetam)	t = 2.679 P = 0.026
n - Sodium valproate = 37; Carbamazepine = 14; Levetiracetam = 28. Two-way repeated measures analysis of variance with Bonferroni 't' test as multiple comparison.		

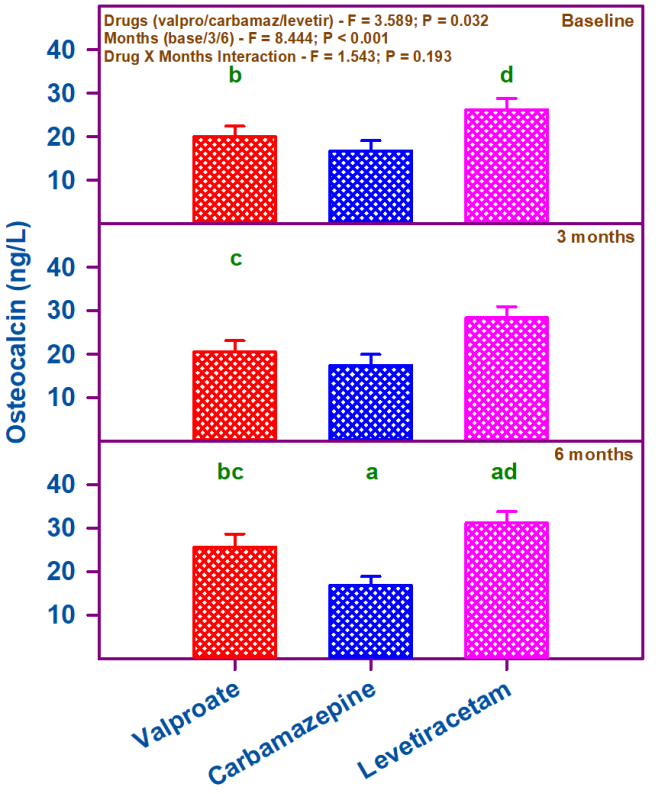

Figure 1. Comparison of baseline, 3 months and 6 months values of serum osteocalcin in antiepileptic drug treatment, valproate, carbamazepine and levetiracetam in children

Values are mean + SE. n - Sodium valproate = 37; Carbamazepine = 14; Levetiracetam = 28. The 'F' and 'P' values are by two-way RM ANOVA with Bonferroni 't' test for multiple comparison of between and within groups. Mutual alphabetical characters are statistically significant.

Discussion

Bone metabolism is a continuous process that alters between bone formation and bone resorption. Children's bone metabolism is more complex and distinct from that of adults, reflecting both growth and skeletal structure (8). Bone formation and resorption are closely associated with the remodeling observed in adults. Childhood is an important period for bone calcification. Children are prone to osteoporosis during high mineralization, which results in fractures (20). In recent years, there has been growing evidence that epilepsy and its treatment can have a deleterious impact on bone calcification and calcium metabolism (21). This study included 79 children aged between 1 to 18 years. Dwajani et al (22) conducted a study where similar age groups were included. The biochemical parameters such as calcium, phosphorus, and alkaline phosphatase were within normal levels. These results correspond with those of a 2002 study by Verrotti et al (23) which also found that the calcium and phosphorus levels were within normal limits.In this study, the difference between calcium, phosphorus, Vitamin D and ALP was not significant between the drugs which was similar to a study by Serin et al (24).

In this study, there was no noticeable difference in osteocalcin levels between the drugs at baseline and at third month, but there was a significant difference in osteocalcin levels between the drugs at the sixth-month visit (P-value = 0.032). Due to this difference in Osteocalcin levels at 6^{th} month compared to 3^{rd} month and baseline, osteocalcin might be considered as an early predictor for changes in bone health. In a study conducted by Monjardino et al (25) it was revealed that osteocalcin and Beta ctx showed moderate correlation with bone loss which is supportive to the findings of this study. In a study conducted by Takeshita et al. (26), serum osteocalcin levels were significantly higher among disabled children on antiepileptic treatment compared to the control group which was similar to the findings of this study. Erbayat Altay et al. (27) observed higher serum osteocalcin levels in children but had normal BMD despite receiving long-term CBZ therapy. Suljic and colleagues (28) concluded that children who received CBZ monotherapy for more than a year experienced a significant decrease in 25-OHD levels as well as significant increases in the bone-formation marker osteocalcin which was similar to this study. Tsukahara et al (29) observed that long-term treatment with SV or CBZ causes reduced bone turnover in children. Whereas Aksoy et al. (30), Caksen et al. (31), and Oner et al. (32) found that long-term valproic acid use had no effect on serum osteocalcin levels in children with epilepsy, Li Min et al. (33) found that there was no effect on serum osteocalcin levels in epileptic children, Shi etal.(34) found that children treated with levetiracetam showed no significant changes in bone metabolism, BMD, or thyroid hormone levels over a 12-month period which was contrary to this study.

In this study, Beta CTx showed no significant difference over a six-month treatment period with AED. Despite the fact that there had been very few studies on children with epilepsy using this CTx marker, Verrotti et al. (35) noticed a significant increase in bone turnover in epileptic post-pubertal males on 12-month Valproate monotherapy. Sato et al. (36) discovered an unusually high prevalence of BMD reduction in both sexes of epileptic adults, as well as high calcium and CTx concentrations that correlated negatively with BMD. Dwajani et al. (37) discovered that six months of levetiracetam monotherapy in adults for epilepsy had no adverse effects on bone health. Another study Koo et al. (38) revealed that LEV monotherapy for one year had no significant effect on bone strength or metabolism in adults.

Limitation

The study limitation is that the patients were followed up for a shorter duration of time. Long duration with frequent follow-ups studies are required to assess and analyze the effect of these drugs.

Conclusion

This research showed no significant difference in bone biomarker levels between antiepileptic drugs at each point of time. And, no significant difference was noted across the timeline for each drug. A significant difference in osteocalcin level was noted between the drugs at 6th month visit. Based on this osteocalcin can be considered as an early predictor for changes in bone health. Further studies with longer duration of follow-up are recommended in the future to study the effect of AED on bone health among children as bone loss begins only after long-term treatment with AED.

Conflict of interest: None

Acknowledgment

We, the authors, would like to express our gratitude to Dr. Vykunataraju for allowing us to collect patient data from his clinic.

References

1. Aksoy A, Sonmez FM, Deger O, Hosver I, Karaguzel G. The effects of antiepileptic drugs on the relationships between leptin levels and bone turnover in prepubertal children with epilepsy. J Pediatr Endocrinol Metab. 2011;24(9 –10):703 –8.
2. Arora E, Singh H, Gupta YK. Impact of antiepileptic drugs on bone health: Need for monitoring, treatment, and prevention strategies. J Family Med Prim Care. 2016 Apr-Jun;5(2):248-253.
3. Ashrafi M, Khoshhal F, Rabani A, Salajegheh N, Nasab AM, Shams S, et al. Study of Antiepileptic Drugs effects on bone metabolism. Iranian Journal of Pediatrics. 2005; 15(4):347-52.
4. *Barden HS, Mazess RB, Chesney RW, et al: Bone status of children receiving anticonvulsant therapy. Metab Bone Dis Relat Res 1982; 4:43–47.*

5. C. Meier, M.E. Kraenzlin. Antiepileptics and bone health. Adv Musculoskelet Dis, 3 (5) (2011): 235-243
6. Caksen H, Dulger H, Cesur Y, Odabas D, Tuncer O, Atas B. No effect of long-term valproate therapy on thyroid and parathyroid functions in children. Int J Neurosci. 2002;112(11):1371 –4.
7. Chaokromthong K, Sintao N. Sample Size Estimation using Yamane and Cochran and Krejcie and Morgan and Green Formulas and Cohen Statistical Power Analysis by G* Power and Comparisions. Apheit International Journal. 2021 Dec 24;10(2):76-86.
8. de Melo VC, Ferreira PR, Ricardi LO, Batista MC, França CN, Ferreira CE. Definition of reference ranges for β-isomerized carboxy-terminal telopeptide collagen type I for children and adolescents. Journal of Pediatric Endocrinology and Metabolism. 2018 Jun 1;31(6):637-40.
9. Dwajani S, et al. Effect of Sodium Valproate and Levetiracetam on Thyroid Hormones in Pediatric Patients with Epilepsy. Adv in Phar & Clin Tria 2020, 5(3): 000182
10. Dwajani S., GRK Sarma, Ganapathy Bantwal, Chanda Kulkarni. Effects Of Levetiracetam And Topiramate On Bone Health In Adults, Using Bone Specific Biomarkers. Med. Res. Chron., 2015, 2 (1), 61-66.
11. Erbayat Altay E, Serdaroglu A, Tumer L, Gucuyener K, Hasanoglu AJ. Evaluation of bone mineral metabolism in children receiving carbamazepine and valproic acid. J. Pedzziatr. Endocrinol. Metab. 2000; 13: 933–9.
12. Hamed SA, Moussa EMM, Youssef AH, Abd ElHameed MA, NasrEldin E. Bone status in patients with epilepsy: relationship to markers of bone remodeling. Front Neurol. 2014;5(August):142.
13. Hirokazu Tsukahara, Kouki Kimura, Yukiko Todoroki, Yusei Ohshima, Masahiro Hiraoka, Yosuke Shigematsu, Yasuyo Tsukahara, Masakazu Miura And Mitsufumi Mayumi. Bone mineral status in ambulatory pediatric patients on long-term anti-epileptic drug therapy. Pediatrics International (2002) 44, 247–253
14. Koo DL, Joo EY, Kim D, Hong SB. Effects of levetiracetam as a monotherapy on bone mineral density and biochemical markers of bone metabolism in patients with epilepsy. Epilepsy Res. 2013 Mar;104(1-2):134-9.
15. Kumar G, Dash P, Patnaik J, Pany G. Socioeconomic status scale-modified kuppuswamy scale for the year 2022. International Journal of Community Dentistry. 2022 Jun 10;10(1):1-6.
16. Min L, Chunyan W, Biaoxue R. Effects of valproic acid on skeletal metabolism in children with epilepsy: a systematic evaluation and meta-analysis based on 14 studies. BMC Pediatr. 2020 Mar 2;20(1):97.
17. Monjardino T, Silva P, Amaro J, Carvalho O, Guimaraes JT, Santos AC, Lucas R. Bone formation and resorption markers at 7 years of age: Relations with growth and bone mineralization. PloS one. 2019 Aug 22;14(8):e0219423
18. Morgan, Sarah L, and Ginnie L Prater. “Quality in dual-energy X-ray absorptiometry scans.” Bone vol. 104 (2017): 13-28.
19. Nakhaeymoghadam M, Teimouri A, Khaje A, hoseini SB. Bone Metabolism Disorder in Epileptic Children. Iran J Child Neurol. Spring 2018; 12(2): 17-24
20. Oner N, Kaya M, Karasalihoglu S, Karaca H, Celtik C, Tutunculer F. Bone mineral metabolism changes in epileptic children receiving valproic acid. J Paediatr Child Health. 2004;40(8):470 –3.

21. *Pack AM, Morrell MJ. Epilepsy and bone health in adults. Epilepsy & Behavior. 2004; 5:24-9.*
22. Peichl P, Griesmacherb A, Marteau R. et al. Serum cross laps in comparison to serum osteocalcin and urinary bone resorption markers. Clinical Biochemistry 2001; 34:131-9.
23. *Petty SJ, O'Brien T, Wark J. Anti-epileptic medication and bone health. Osteoporosis international.2007; 18(2):129-42.*
24. Razazizan, Nazanin et al. "Comparison of 25-hydroxy vitamin D, calcium and alkaline phosphatase levels in epileptic and non-epileptic children." *Acta neurologica Taiwanica* vol. 22,3 (2013): 112-6.
25. Rifai Nader et al. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier 2018.Reference information for clinical laboratory:2258-302
26. Sato Y, Kondo I, Ishida S, Motooka H, Takayama K, Tomita Y, et al. Decreased bone mass and increased bone turnover with valproate therapy in adults with epilepsy [RETRACTED]. Neurology. 2001 Aug 14;57(3):445.
27. Serin HM, Koç ZP, Temelli B, Esen İ. The bone mineral content alterations in pediatric patients medicated with levetiracetam, valproic acid, and carbamazepine. Epilepsy & Behavior. 2015 Oct 1;51:221-4.
28. Seydewitz H, Henschen M, Kühnel W, Brandis M. Pediatric Reference Ranges for Osteocalcin Measured by the Immulite Analyzer. 2001;39(10): 980-82.
29. Shi K-L, Guo J-X, Zhao H-M, Hong H, Yang C-Z, Wu Y-H, et al. The effect of levetiracetam and oxcarbazepine monotherapy on thyroid hormones and bone metabolism in children with epilepsy: A prospective study. Epilepsy Behav [Internet|. 2020;113(107555):107555.
30. Suljic EM, Mehicevic A, Mahmutbegovic N. Effect of Long-term Carbamazepine Therapy on Bone Health. Med Arch. 2018 Oct;72(4):262-266.
31. Takeshita N, Seino Y, Ishida H et al. Increased circulating levels of γ-carboxyglutamic acid containing protein and decreased bone mass in children on anticonvulsant therapy. Calcif. Tissue Int. 1989; 44: 80–5.
32. Tatum, W O 4th et al. "The neurosurgical treatment of epilepsy." Archives of family medicine vol. 9,10 (2000): 1142-7.
33. Tripathi, K. D. Essentials of Medical Pharmacology. 8th ed., Jaypee Brothers Medical, 2018.Antiepileptic drugs :444-445
34. Tripathi, K. D. Essentials of Medical Pharmacology. 8th ed., Jaypee Brothers Medical, 2018.Antiepileptic drugs :442-47
35. Verrotti A, Agostinelli S, Coppola G, Parisi P, Chiarelli F. A 12-month longitudinal study of calcium metabolism and bone turnover during valproate monotherapy. Eur J Neurol. 2010 Feb;17(2):232-7.
36. Verrotti A, Coppola G, Parisi P, Mohn A, Chiarelli F. Bone and calcium metabolism and antiepileptic drugs. Clinical neurology and neurosurgery. 2010;112(1):1-10.
37. Yang L, Grey V. Pediatric reference intervals for bone markers. Clinical biochemistry. 2006 Jun 1;39(6):561-8.
38. Zhang Y, Zheng YX, Zhu JM, Zhang JM, Zheng Z. Effects of antiepileptic drugs on bone mineral density and bone metabolism in children: A meta-analysis. J Zhejiang Univ Sci B. 2015;16(7):611–21. 5.