Original Article

Vitamin B12 and Folate Status in Patients with Epilepsy Under Levetiracetam Monotherapy

Atena Mahdavi, Amirmansour Alavi Naeini, Mohammadreza Najafi[1], Mohammadreza Ghazvini[2], Mohammadrea Maracy[3]

Department of Community Nutrition, School of Nutrition and Food Sciences, Isfahan University of Medical Sciences, Isfahan, Iran, [1]Department of Neurology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Isfahan Center of Health Research, National Institute of Health Research, Isfahan, Iran, [3]Department of Biostatistics and Epidemiology, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: Antiepileptic drugs (AEDs) may lead to an increase in the plasma concentration of homocysteine. There is limited information, especially from Iran, regarding the risk in patients who are treated with levetiracetam as a new type of AED. The aim of the present study was to investigate the effect of levetiracetam on plasma homocysteine, vitamin B12, and folate levels in adult patients with epilepsy. **Methods:** We conducted a case-control study and enrolled adult patients with epilepsy who had received monotherapy with levetiracetam for at least 6 months at some time prior to the study. homocysteine serum, vitamin B12, and folate were measured, and folate and vitamin B12 intake was determined by the food frequency questionnaire (FFQ). **Results:** Thirty-three patients on levetiracetam and 35 control subjects aged between 18 and 60 years were enrolled. No statistically significant differences in the means of the serum markers of vitamin B12, FA, and homocysteine levels were found between the two groups. In the first model, i.e., the crude model, no significant differences were observed in the serum concentrations of homocysteine, vitamin B12, and folate. In the second model, education was considered, and body mass index and folate intake was controlled with no significant difference being observed in the mean homocysteine serum level. **Conclusion:** Treatment with levetiracetam in patients with epilepsy has no effect on the serum levels concentrations of homocysteine, vitamin B12, and folate. This medication is suggested for patients who use AEDs on a long-term basis and at high dosages.

Keywords: *Folic acid, levetiracetam, monotherapy, patients with epilepsy, vitamin B12*

Background

Epilepsy is a noncommunicable chronic brain disorder that affects people all over the world. It is characterized by recurrent seizures whose frequencies may differ from less than once a year to several times a day.[1] The average prevalence of epilepsy in the world is reported to be 8%,[2-5] making it one of the most prevalent neural diseases worldwide.[6,7] Its prevalence is rather higher in developing countries than it is in the developed countries.[8-10] The prevalence of this disease in Iran is reported to be 5%, whereas it is 1% and 4% in the north and east of the country, respectively.[11] Its treatment is mainly based on long-term epileptic drugs (AEDs).[12] Some studies show that patients who take AEDs over a long period of time will be exposed to cardiovascular diseases, especially myocardium infarction and heart stroke. Recent studies have shown increase in the level of markers for cardiovascular risks, such as homocysteine levels, in these patients.[13,14]

Folic acid and vitamin B12 play prominent roles in homocysteine metabolism, and deficiency of any or both of them could lead to elevated serum homocysteine level, resulting in atherosclerosis.[15,16] Earlier antiepilepsy drugs such as carbamazepine, phenytoin, and phenobarbital are associated with the emergence of atherosclerosis.[17] The aim of treating epilepsy is controlling the convulsions without, or with least number of, side effects.[18-22] Levetiracetam is a new drug for the treatment of epilepsy used for partial and generalized seizures. This drug has appropriate medicinal properties that include negligible composition with protein and little effects on liver metabolism.[23]

Most studies are done on earlier AEDs, and their effects are properly identified, but only few studies are being done on new drugs such as levetiracetam, and no studies have been done in this respect in Iran. This study was carried out by determining the serum level of vitamin B12, folate, and homocysteine in patients with epilepsy receiving levetiracetam.

How to cite this article: Atena M, Amirmansour AN, Mohammadreza N, Reza GM, Mohammadreza M. Vitamin B12 and folate status in patients with epilepsy under levetiracetam monotherapy. Int J Prev Med 2018;XX:XX-XX.

Address for correspondence:
Dr. Alavi N. Amirmansour, Department of Community Nutrition, School of Nutrition and Food Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: am.alavi@nutr.mui.ac.ir

Access this article online

Website: www.ijpvmjournal.net/www.ijpm.ir

DOI: 10.4103/ijpvm.IJPVM_71_18

Quick Response Code:

Materials and Methods

This case-control study was conducted among 68 people aged between 18 and 60 years, that included 33 (21 females and 12 males) epileptic patients who has been treated with levetiracetam monotherapy and 35 healthy people (21 females and 14 males) among whom 33 people were affected with generalized or focal epileptic seizures with no definite reasons, their disease having been diagnosed according to the International League Against Epilepsy (ILAE).[24] The patients were selected from those of Massih Epileptic Research Association and the patients of a number of neurologists in private practice. The remaining 35 people were healthy individuals selected as the control group and matched with patients with respect to age and gender. After giving consent, the individuals participated in the study. The inclusion criteria were: (1) aged between 18 and 60 years; (2) At least six months of levetiracetam monotherapy; (3) Confirmed diagnosis of epilepsy by a neurologist. The exclusion criteria of the study were: (1) being a patient with any disease other than epilepsy and taking any other medications; (2) addiction to alcohol and smoking; (3) being affected by diabetes, hypertension, hypercholesterolemia, myocardial infarction, stroke, and other renal, hepatic, thyroid diseases, cancer, anemia, and mental disorders; (4) being pregnant or breast-feeding; (5) taking supplements of folate, vitamin B12; (6) being vegetarian; (7) having a history of genetic deficiencies in homocysteine, cobalamin, and folate metabolism; (8) having a ketogenic diet.

The required information from the participants, including demographic information, was obtained through a devised questionnaire. The rate of nutrients consumption was also determined using the food frequency questionnaire (FFQ) and evaluated, and the validity and reliability of the questionnaire were calculated.[37]

Laboratory analysis

A 5 mL blood sample of fasting participants was taken and centrifuged for 10 min at 6000 rpm. Serum sections were separated by centrifugation and stored at −20°C until biochemical analysis. Folate and vitamin B12 levels were measured using kits from Monobind Co. (USA) based on the competitive binding method. Homocysteine level was measured using the enzyme immunoassay kit (EIA) from Axis-Shield Co. (UK). In this method, the homocysteine bonded to protein is primarily restored in free form, and in the next stage, transformed to s-adenosyl homocysteine by the enzyme; the resulting product is measured by enzyme immunoassay method using an exclusive monoclonal antibody. The assays had sensitivities of 51.74 (pmol/L), 1.178 (nmol/L) and 0.89 (μmol/L) for vitamin B12, folate, and homocysteine, respectively.

Statistical analysis

The one sample Kolmogorov–Smirnov test was used to analyze normality, and the Leven's test was used for the equality of variances. Frequency descriptive statistics (percentage) and central and dispersion indices were used for classification and data report and the covariance analysis test was applied for data analysis. SPSS (Ver. 22) software (IBM) was used to analyze the data; *P* values less than 0.05 were considered statistically significant.

Results

Thirty-three patients affected by epilepsy with age mean and standard deviation (30.2 ± 11.7) under monotherapy treatment with levetiracetam, and 35 healthy people (29.9 ± 11.4) were included in the study. Their demographic and clinical specifications are shown in Table 1. The average duration of medication intake and the daily dose of the medicine in the group of patients under treatment with levetiracetam was 2.2 ± 2.2 within a range of 6 months to 10 years and 992.4 ± 477.9 within a range of 250 to 2500 mg/day, respectively; the mean ± SD specifying the nutrients that can affect homocysteine level was determined by the FFQ questionnaire. Table 2 presents the mean and standard deviations of the vitamin B12, and folate serum markers, and the homocysteine level in the treatment and control groups showed no significant difference. Table 3 shows the homocysteine level, folate,

Table 1: Demographic and basic clinical characteristics in levetiracetam treated and normal controls

Variable	Patients (*n*=33)	Healthy (*n*=35)	*P*
Education *n* (%)			
School student Elementary education	8 (24.2)	7 (20)	0.94
School graduate Diploma	14 (42.4)	14 (40)	
Higher graduate Bachelor and higher	11 (30.6)	14 (37.4)	
Gender *n* (%)			
Female	21 (63.3)	21 (60)	0.762
Male	12 (36.4)	14 (40)	
Age (Y)			
Mean±SD	30.2±11.7	29.9±11.4	0.9
BMI (kg/m^2)			
Mean±SD	24.51±5.56	25.3±4.34	0.486
Duration of using the medicine (year)			
Mean±SD	2.24±2.21	-	-
Dosage of medicine (mg/day)			
Mean±SD	992.42±477.97	-	-
Intake of vitamin B12 (μg/day)			
Mean±SD	3.21±3.22	3.62±5	0.688
Intake of Folate* (μg/day)			
Mean±SD	212.6±92	252.5±100.56	0.093

*: *P* value was calculated for qualitative variable: Chi-square for quantitative variable: independent *t* test

and vitamin B12 conditions in two different models. Model 1 is a crude model. Education, BMI, and folate are controlled in model 2, and no significant difference was observed in the mean homocysteine serum level. The mean folate serum level in model 1 showed no significant difference, but it showed significant difference in model 2 (P = 0.017). The mean difference of the folate serum level between the groups of patients and healthy individuals was −2.33 in model 1, whereas it was −3.43 in model 2.

The mean vitamin B12 serum level in model 2 is very close to the significance level (P = 0.054), with regards to reduction of 61 units in model 1 and 41 units in model 2 in the study group compared to the control group.

Discussion

According to Table 3, we controlled the effects of education, BMI, and folate in model 2 in our study, and it was observed that the P value approached the significance level in both models, such that it was significant for folate, and quite close to the significance level for vitamin B12. It can be said that diet in individuals in anthropometric conditions and even educational status can affect significance. Nutritional conditions have not been specified in previous studies, but it should be noted that diet can be effective on the serum conditions of folate, vitamin B12, and homocysteine, as observed for folate and vitamin B12 in this study.

Table 2: Serum level of vitamin B12, folic acid and homocysteine in levetiracetam treated and normal controls

Variable	Patient	Healthy	*P*
Serum homocysteine (µmol/L)			
Mean±SD	10.06±4.86	10.02±3.16	0.967
Serum folate (nmol/L)			
Mean±SD	21.55±11.22	26.85±12.53	0.071
Serum vitamin B12 (Pmol/L)			
Mean±SD	281.2±99.04	326.22±108.32	0.079

Table 3: Analysis of the serum condition in homocysteine, folate, and vitamin B12 in two different models

Variable	F-test *P*	Difference *P*	95% confidence interval
Serum homocysteine (µmol/L)			
Model 1 *	0.967	0.041 (0.967)	1.936 to 2.517
Model 2 **	0.703	1.6 (0.336)	−1.68 to 4.84
Serum folate (nmol/L)			
Model 1	0.071	−2.33 (0.71)	−4.887 to 2.9
Model 2	0.017	−3.43 (0.15)	−8.16 to 1.26
Serum vitamin B12 (pmol/L)			
Model 1	0.079	−61.018 (0.079)	−1129.259 to 7.224
Model 2	0.054	−41.77 (0.529)	−173.8 to 90.25

*: Crude, **: Controlled for BMI, education, folate intake

Previous studies show that patients under long-term treatment with previous AEDs are at a higher risk of increased homocysteine and reduced folate levels than normal people.[25-29] The nutritional circumstances of vitamin B12, folate, and Hcy was measured in the patients under treatment with levetiracetam for a minimum of 6 months. Levetiracetam is a new medicine that is quite distinct as compared to other antiepileptic medicines due to its pharmacological properties and practical mechanisms, although its mechanism is not yet properly known.[30] It was shown in a study by Aydin and Varoglu that using levetiracetam in epileptic patients cannot be related to hyperhomocysteinemia or reduction of vitamin B12 and folate.[31] Another study done to analyze homocysteine levels in epileptic patients under treatment with new antiepileptic medication showed that lamotrigine and levetiracetam levetiracetam do not increase homocysteine.[32]

Previous AED medicines affect homocysteine metabolism by reducing methylenetetrahydrofolate reductase (MTHFR) activities; these drugs can intervene with the absorption and metabolism of folate.[33] The relation between homocysteine and epilepsy is still not known.[34] However, most studies have shown that using earlier medicines such as carbamazepine and valproic acid increases homocysteine levels,[15,16,33] and long-term treatment with these medicines reduces the storage capabilities of the liver for vitamin B12.[35] In fact, it can be said that these medicines stimulate the P450 (CYP) of liver cells.[17] Recent studies have shown that increase in homocysteine level can be observed in patients who use the newer drugs (new-AEDs) such as topitamate and oxacarbamazepine.[32] The increase in atherogenicity created by the new medicines cannot be entirely due to the CYP system or the deficiency of cofactors for homocysteine metabolism; there are additional mechanisms that are not still known.[17] Nevertheless, the present and previous studies show that people under monotherapy treatment with levetiracetam have a normal homocysteine level.[32,36] In any case, in the study by Kim *et al*., patients under levetiracetam treatment showed significant increase in their homocysteine level, whereas unchangeable levels were observed for folate and vitamin B12.[17]

One of the limitations in our study was the few number of patients; Linnebank *et al*. recruited 2730 patients,[36] and that carried out by Belcastro *et al*. included 480 patients.[32] Also, we had no information about the status of the patients before levetiracetam consumption. Thus, we could not compare the factors with or without treatment by anti-epilepsy medicines. Finally, we did not observe the long-term effects of levetiracetam.

The strong point of our study was the investigation of the patient's diet, which was done using the FFQ, and was shown to be effective on the significance or insignificance of the serum factors.

Conclusion

Our findings show that treatment of epileptic patients exposed to the risk of cardiovascular diseases with the new medicine levetiracetam is possible as the medicine had no effects on homocysteine levels. However, patients who take high doses of the medicine on a long-term basis and patients harboring the risk factors such as obesity who have a family history of cardiovascular diseases should be under observation for the assessment of the relevant markers (vitamin B12, folate, and homocysteine). Another significant point is the diet of the patient as reduction in the level of folate and vitamin B12 in patients, especially those taking the medicine on a long-term basis in high dosages may be due to an inadequate diet; this can be compensated for by diet modification or the use of supplements where necessary.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Received: 13 Feb 18 **Accepted:** 19 Jun 18
Published: ???

References

1. Megiddo I, Colson A, Chisholm D, Dua T, Nandi A, Laxminarayan R. Health and economic benefits of public financing of epilepsy treatment in India: An agent-based simulation model. Epilepsia 2016;57:46-74.
2. Bell G, Sander J. CPD—Education and self-assessment the epidemiology of epilepsy: The size of the problem. Seizure 2001;10:306-16.
3. Ngugi AK, Bottomley C, Kleinschmidt I, Wagner RG, Kakooza-Mwesige A, Ae-Ngibise K, *et al*. Prevalence of active convulsive epilepsy in sub-Saharan Africa and associated risk factors: Cross-sectional and case-control studies. Lancet Neurol 2013;12:253-63.
4. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, *et al*. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2197-223.
5. Mac TL, Tran D-S, Quet F, Odermatt P, Preux P-M, Tan CT. Epidemiology, aetiology, and clinical management of epilepsy in Asia: A systematic review. Lancet Neurol 2007;6:533-43.
6. Najafi MR, Najafi MA, Safaei A. Association of family history of epilepsy with earlier age onset of juvenile myoclonic epilepsy. Iran J Child Neurol 2016;10:10-5.
7. Ebrahimi A, Barekatain M, Bornamanesh A, Najafi MR, Salehzadeh M, Maracy MR. Psychometric properties and validation of Persian version of quality of life in epilepsy inventory (QOLIE-89). J Res Med Sci 2013;18:990-4.
8. Debrock C, Preux P-M, Houinato D, Druet-Cabanac M, Kassa F, Adjien C, *et al*. Estimation of the prevalence of epilepsy in the Benin region of Zinvié using the capture-recapture method. Int J Epidemiol 2000;29:330-5.
9. Bittencourt PD, Adamolekum B, Bharucha N, Carpio A, Cossio O, Danesi M, *et al*. Epilepsy in the tropics: I. Epidemiology, socioeconomic risk factors, and etiology. Epilepsia 1996;37:1121-7.
10. Preux P-M, Druet-Cabanac M. Epidemiology and aetiology of epilepsy in sub-Saharan Africa. Lancet Neurol 2005;4:21-31.
11. Sayehmiri K, Tavan H, Sayehmiri F, MohammadiI I, Carson KV. Prevalence of epilepsy in Iran: A meta-analysis and systematic review. Iran J Child Neurol 2014;8:9-17.
12. Tolou-Ghamari Z, Zare M, Habibabadi JM, Najafi M-R. Antiepileptic drugs: A consideration of clinical and biochemical outcome in patients with epilepsy. Int J Prev Med 2013;4(Suppl 2):S330-7.
13. Fontes LP, Fontes MP, Jiménez PQ, Pérez JM, Iriarte MM. Comparative case-control study of homocysteine, vitamin B12, and folic acid levels in patients with epilepsy. Neurología (English Edition) 2017;32:440-5.
14. Najafi MR, Bazooyar B, Zare M, Aghaghazvini MR, Ansari B, Rajaei A, *et al*. The Investigation of Insulin Resistance in Two Groups of Epileptic Patients Treated with Sodium Valproate and Carbamazepine. Adv Biomed Res 2017;6:25.
15. Tamura T, Aiso K, Johnston KE, Black L, Faught E. Homocysteine, folate, vitamin B-12 and vitamin B-6 in patients receiving antiepileptic drug monotherapy. Epilepsy Res 2000;40:7-15.
16. Karabiber H, Sonmezgoz E, Ozerol E, Yakinci C, Otlu B, Yologlu S. Effects of valproate and carbamazepine on serum levels of homocysteine, vitamin B12, and folic acid. Brain Dev 2003;25:113-5.
17. Kim DW, Lee SY, Shon YM, Kim JH. Effects of new antiepileptic drugs on circulatory markers for vascular risk in patients with newly diagnosed epilepsy. Epilepsia 2013;54:146-9.
18. Guerrini R. Valproate as a mainstay of therapy for pediatric epilepsy. Pediatr Drugs 2006;8:113-29.
19. Najafi MR, Ansari B, Zare M, Fatehi F, Sonbolestan A. Effects of antiepileptic drugs on sexual function and reproductive hormones of male epileptic patients. Iran J Neurol 2012;11:37-41.
20. Najafi MR, Sonbolestan F, Sonbolestan SA, Zare M, Mehvari J, Meshkati SN. The course and outcome of pregnancy and neonatal situation in epileptic women. Adv Biomed Res 2012;1:4.
21. Chitsaz A, Mehvari J, Salari M, Gholami F, Najafi MR. A comparative assessment the efficacy of intravenous infusion of sodium valproate and phenytion in the treatment of status epilepticus. Int J Prev Med 2013;4(Suppl 2):S216-21.
22. Tolou-Ghamari Z, Zare M, Habibabadi JM, Najafi MR. A quick review of carbamazepine pharmacokinetics in epilepsy from 1953 to 2012. J Res Med Sci 2013;18(Suppl 1):S81-5.
23. Alsaadi TM, Koopmans S, Apperson M, Farias S. Levetiracetam monotherapy for elderly patients with epilepsy. Seizure 2004;13:58-60.
24. Engel J. A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: Report of the ILAE Task Force on Classification and Terminology. Epilepsia 2001;42:796-803.
25. El-Farahaty RM, El-Mitwalli A, Azzam H, Wasel Y, Elrakhawy MM, Hasaneen BM. Atherosclerotic effects of long-term old and new antiepileptic drugs monotherapy: A cross-sectional comparative study. J Child Neurol 2015;30:451-7.
26. Verrotti A, Pascarella R, Trotta D, Giuva T, Morgese G,

Chiarelli F. Hyperhomocysteinemia in children treated with sodium valproate and carbamazepine. Epilepsy Res 2000;41:253-7.
27. Tan TY, Lu CH, Chuang HY, Lin TK, Liou CW, Chang WN, *et al*. Long-term antiepileptic drug therapy contributes to the acceleration of atherosclerosis. Epilepsia 2009;50:1579-86.
28. Caccamo D, Condello S, Gorgone G, Crisafulli G, Belcastro V, Gennaro S, *et al*. Screening for C677T and A1298C MTHFR polymorphisms in patients with epilepsy and risk of hyperhomocysteinemia. Neuromol Med 2004;6:117-26.
29. Belcastro V, Gaetano G, Italiano D, Oteri G, Caccamo D, Pisani LR, *et al*. Antiepileptic Drugs and MTHFR Polymorphisms Influence Hyper- Homocysteinemia Recurrence in Epileptic Patients. Epilepsia 2007;48:1990-4.
30. de Albuquerque Oliveira A, Linhares MI, Chaves Filho AJ, Maia e, Rios ERV, de Carvalho Lima CN, *et al*. Antioxidant properties of antiepileptic drugs levetiracetam and clonazepam in mice brain after in vitro-induced oxidative stress. Afr J Pharm Pharmacol 2016;10:278-88.
31. Aydin A, Varoglu AO. "Methylenetetrahydrofolate reductase" gene polymorphism and clinical importance in epilepsy patients using valproic acid, carbamazepine and levetiracetam. Laboratoriums Medizin 2017;41:147-51.
32. Belcastro V, Striano P, Gorgone G, Costa C, Ciampa C, Caccamo D, *et al*. Hyperhomocysteinemia in epileptic patients on new antiepileptic drugs. Epilepsia 2010;51:274-9.
33. Bochyoska A, Lipczyoska-Łojkowska W, Gugała-Iwaniuk M, Lechowicz W, Restel M, Graban A, *et al*. The effect of vitamin B supplementation on homocysteine metabolism and clinical state of patients with chronic epilepsy treated with carbamazepine and valproic acid. Seizure 2012;21:276-81.
34. Eldeen ON, Eldayem SMA, Shatla RH, Omara NA, Elgammal SS. Homocysteine, folic acid and vitamin B12 levels in serum of epileptic children. Egyptian J Med Hum Genet 2012;13:275-80.
35. Schwaninger M, Ringleb P, Winter R, Kohl B, Fiehn W, Rieser PA, *et al*. Elevated plasma concentrations of homocysteine in antiepileptic drug treatment. Epilepsia 1999;40:345-50.
36. Linnebank M, Moskau S, Semmler A, Widman G, Stoffel-Wagner B, Weller M, *et al*. Antiepileptic drugs interact with folate and vitamin B12 serum levels. Ann Neurol 2011;69:352-9.
37. Esfahani FH, Asghari G, Mirmiran P, Azizi F. Reproducibility and relative validity of food group intake in a food frequency questionnaire developed for the Tehran Lipid and Glucose Study. J Epidemiol 2010;20:8-150.

Author Queries???

AQ7: Kindly note reference citations not in chronological order. Please check and cite.

AQ9: Kindly provide complete manufacturer details.