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Clinical Science

Evaluation of Homocysteine, Folic Acid and Vitamin B12 Levels among Egyptian Children with Idiopathic Epilepsy

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Abstract

Objective: To evaluate relationship between homocysteine (Hcy), folic acid and vitamin B12 with anti-epileptic drugs in epileptic patients and their role in epilepsy control.

Patient and Methods: The study included 60 patients with idiopathic epilepsy and thirty non-epileptic children of the same age and sex served as controls. All cases were subjected to physical and neurological examination and electroencephalogram (EEG). Serum level of folic acid, homocysteine (Hcy) and vitamin B12 were done for both epileptic patients and controls. Non parametric test, one way ANOVA and Pearson's correlation were used for analysis of data.

Result: 44 patients (73.3%) had generalized epilepsy and the remaining patients had partial epilepsy (simple or complex). 37 patients (61.6%) were in grade I, 3 patients (5 %) were in grade II and the remaining 20 patients (33.3 %) were in grade III epilepsy. Vitamin B12 was significantly higher in epileptic patients. Duration of anti-epileptic drug treatment was correlated significantly to folic acid and Hcy level.

Conclusion: antiepileptic drugs might upset the homeostatic balance of Hcy and its cofactors and cause abnormalities of their serum levels. The duration of anti-epileptic drug treatment was related to decrease of folic acid and increase in homocysteine levels.

Introduction

Vitamin B12 deficiency has been encountered amongst the benign and rare causes of infantile seizures. Common symptoms of Vitamin B12 deficiency in infants include megaloblastic anemia, feeding difficulties, developmental delay, microcephaly, failure to thrive, hypotonia, lethargy, irritability, involuntary movements, seizures and cerebral atrophy [1].

The mechanism of the excitatory properties of folates is uncertain, but there is some evidence that they may do so by blocking or reversing GABA mediated inhibition [2].

Folic acid supplementation was reported to reduce epileptic seizure frequency; it has also been reported to interfere with the action of anticonvulsant medications, resulting in an increase in the frequency and/or severity of seizures [3].

Homocysteine, a sulphur-containing amino acid, has been shown to induce seizures in rats [4]. Patients on anti-epileptic drugs are prone to high concentrations of plasma homocysteine and low status of folate and Vitamin B6 [5].

Increased homocysteine and low folate status may contribute to the development of anti-epileptic drug related side effects, such as impaired cognitive function [6].

We are aiming to evaluate the relationship between the levels of homocysteine and the cofactors involved in its metabolism as folic acid and vitamin B12 with anti-epileptic drugs in epileptic patients and their role in epilepsy control.

Patients and Methods

It is a cross sectional observational study done after obtaining approval from the ethical committee of the National Research Centre, Cairo, Egypt. Written informed consent was obtained from all

patients, their parents and controls after full discussion about the aim of the study.

Patients

The study was conducted on 60 idiopathic epilepsy children selected from pediatric neurology outpatients' clinic, Children's Hospital, Ain Shams University.

Children were divided into two groups: Group I, comprises 20 newly diagnosed epileptic children not receiving anti-epileptic medication (non-AEDs users); their mean age was (7 ± 3.16). They were 11 males and 9 females. Group II, comprises 40 epileptic children on regular antiepileptic medication for at least one year (AEDs users); their mean age was (10.5 ± 3.16), furthermore those were subdivided into: Subgroup A, comprises 20 epileptic children receiving monotherapy treatment, their mean age was (10.3 ± 3.4). it was consisted of 15 patients receiving Valproate (VPA) group and the remaining 5 patients were receiving carbamazepine (CBZ) group. Subgroup B, included 20 patients were receiving polytherapy treatment (combined treatment with VPA and CBZ). Their mean age was (10.6 ± 2.9). Thirty non-epileptic children of the same age and sex group served as control group, their mean age was (11.0 ± 2.4).

Table 1: Descriptive statistics of epileptic patients and controls.

Variables		Patients	Control
Sex	Female	30 (50%)	10 (33.3%)
	Male	30 (50%)	20 (66.7%)
Age	Mean ± SD	9.3 ± 3.5	11 ± 2.4
	Range	(5-15ys)	(5-15ys)
Type of seizures	Generalized	44 (73.3%)	-
	Focal	16 (26.7%)	-
Type of treatment	Non AEDs	20 (33.33%)	-
	AEDs	40 (66.7%)	-
	Monotherapy (VPA)	15 (25%)	-
	Monotherapy (CBZ)	5 (6.3%)	-
	Polytherapy	20 (33.33%)	-

AEDs: Anti-epileptic drugs, VPA: Valproate, CBZ : Carbamazepine.

Inclusion criteria of patients

Children aged from 5 to 15 years old, children with idiopathic epilepsy, duration of antiepileptic drug treatment not less than one year.

Exclusion criteria of patients

Children had other diseases affect serum level of homocysteine as endocrinal, liver, kidney and cardiac diseases, diabetes mellitus and nutritional deficiencies, children receiving vitamin supply or folic acid antagonists as well as vegetarians.

All cases were subjected to full history taking , underlying etiology (according to the commission on Epidemiology and Prognosis, International League against Epilepsy, [7] to exclude other causes of epilepsy than being idiopathic, type of seizure disorder [according to the recommendations of the International League against Epilepsy, [8]. Severity of the attack was assessed by using Chalfont severity score [9]**.**

Complete physical and neurological examination were done. Electroencephalogram (EEG): records will be classified in EEG abnormalities grades "I, II, III" [10].

Estimating of serum level of folic acid, homocysteine (Hcy), vitamin B12 using Chemiluminestence technique were done for both epileptic patients and controls.

Statistical methods

Statistical Package for social science (SPSS) program version 15.0 was used for analysis of data. Data was tested for normal distribution using one-sample Kolomongorov Simirnov test for total sample and subgroups, which revealed that data was not normally distributed.Non parametric (Mann Whitney U) test was used for analysis of two quantitative data as data was not symmetrically distributed. One way ANOVA was done for analysis of more than two quantitative data followed by post HOCC test for detection of significance. Pearson's correlation was also done.

Results

Forty four patients (73.3%) had generalized epilepsy and the remaining patients had partial epilepsy (simple or complex). 37 patients (61.6%) were in grade I, 3 patients (5 %) were in grade II and the remaining 20 patients (33.3 %) were in grade III epilepsy. Table 1 present the descriptive statistics of patients and controls. No significant difference was found between age of epileptic patients and controls (9.3 ± 3.5 vs 11.0 ± 2.4 yrs respectively). Serum levels of vitamin B12 were significantly higher in epileptic patients compared to the controls (p=0.03) (Table 2).

Table 2: Comparison between mean serum levels of homocysteine, folic acid, and vitamin B12 of epileptic patients and the controls.

Variables	Epileptic patients Mean ± SD N=60	Controls Mean ± SD N=30	P-value
Homocysteine (µmol/l)	5.9 ± 2.4	5.5 ± 1.4	0.07
Folic acid (ng/ml)	7.7 ± 3.1	6.4 ± 2.9	0.6
Vitamin B 12 (pg/ml)	495.7 ± 231.1	443.9 ± 150.3	**0.03**

Comparison between mean serum levels of homocysteine, folic acid and vitamin B12 of epileptic patients (with different type of treatment) and control group (Table 3) and its relation to EEG grades (Table 4).

Correlation between serum levels of homocysteine, folic acid and vitamin B12 of epileptic patients with duration of treatment and Chalfont score (Table 5). Correlation between mean serum levels of homocysteine, folic acid and vitamin B12 of all epileptic patients and those with anti epileptic drugs (Table 6).

Table 3: Comparison between serum levels of homocysteine, folic acid, and vitamin B12 of epileptic patients (with different type of treatment) and control group.

Variables	Homocysteine (µmol/l)	Folic acid (ng / ml)	Vitamin B 12 (pg / ml)
Non-AEDs users	5.8 ± 1.4	7.3 ± 1.9	433.5 ±171.2
AEDs users	6.0 ± 2.7	7.9 ± 3.5	526.8 ± 252.1
Controls	5.5 ± 1.4	6.4 ± 2.9	443.9 ± 150.3
P-value	**0.5**	**0.1**	**0.1**
CBZ group	5.5 ± 1.9	6.4 ± 2.7	456.4 ± 252.5
VPA group	5.4 ± 1.6	8.7 ± 2.6	503.2 ± 321.3
P-value	**0.9**	**0.09**	**0.8**
Monotherapy group	5.4 ± 1.6	8.2 ± 2.7	491.5 ± 299.9
-Polytherapy group	6.7 ± 3.3	7.7 ± 4.2	562.0 ± 194.6
P-value	**0.2**	**0.7**	**0.4**
Focal seizures	6.2 ± 1.4	7.8 ± 3.2	533.2 ± 238.2
Generalized seizures	5.6 ± 2.6	7.7 ± 3.0	482.0 ± 229.7
P-value	**0.1**	**0.7**	**0.5**

AEDs: Anti-epileptic drugs, non-AEDs users: non anti-epileptic drugs, VPA: Valproate, CBZ : Carbamazepine.

Discussion

In the current study, mean serum levels of Hcy and folic acid were not significantly higher in all epileptic patients and AEDs users compared to controls. This is in concordant with Schwaninger et al. [11] who observed that patients with normal homocysteine plasma concentrations had folate levels that were not significantly different from controls [11]**.** Previous studies reported significantly higher serum levels of Hcy in epileptic patients when compared to controls [12,13]. In contrast to our finding, Aslan et al., [13] reported that serum level of folic acid were significantly lower in epileptic patients compared to controls.

Table 4: Comparison between mean serum levels of homocysteine, folic acid and vitamin B12 of epileptic patients in relation to EEG grades.

Variables	Grade I Mean ± SD N = 37	Grade II Mean ± SD N = 3	Grade III Mean ± SD N = 20	P - value
Homocysteine (µmol/l)	6.1 ± 2.7	5.4 ± 1.2	5.7 ± 1.7	0.8
Folic acid (ng / ml)	7.6 ± 3.2	10.6 ± 0.7	7.5 ± 2.9	0.3
Vitamin B 12 (pg / ml)	478.8 ± 221.5	630.0 ± 360.4	506.7 ± 235.9	0.5

Our study showed that mean serum level of vitamin B12 was significantly higher in all epileptic patients, whereas, its mean serum level was non-significantly higher in AEDs users when compared to controls. This is in agreement with the study done by Tamura et al. [14] who found that serum vitamin B12 concentration were elevated in all patients. On the contrary, Karabiber et al. [15] and Aslan et al. [13] found low vitamin B12 level in patients; yet, their data were not statistically significant. Increased circulating vitamin B12 level, however, served as a sensitive biochemical index of hepatic damage due to anticonvulsants [16]. Prolonged drug treatment was possibly the cause of the slightly impaired ability of the liver to store vitamin B12 [17].

Table 5: Correlation between serum levels of homocysteine, folic acid and vitamin B12 of epileptic patients in relation to duration of treatment and Chalfont score.

Variables	Duration of treatment (yrs)		Chalfont score	
	r	P-value	r	P-value
Homocysteine (µmol/l)	**0.4**	**0.02**	0.2	0.2
Folic acid (ng/ml)	**-0.5**	**0.03**	-0.02	0.9
Vitamin B 12 (pg/ml)	0.1	0.7	-0.1	0.3

Moreover, no difference was observed between mean serum levels of Hcy, folic acid and vitamin B12 concentration measured in the non-AEDs users and the control group. This is in agreement with Senera et al. [12], who conducted his study on 75 patients with idiopathic epilepsy comprised 18 non-AEDs users and 57 patients receiving anti-epileptic drugs, found no significant difference between the two groups.

Table 6: Correlation between mean serum levels of homocysteine, folic acid and vitamin B12 of all epileptic patients and those with anti epileptic drugs.

Variables	Homocysteine (µmol/l)		Folic acid (ng/ml)		Vitamin B 12 (pg/ml)	
	r	P-value	r	P-value	r	P-value
All epileptic patients:						
Homocysteine (µmol/l)	--	--	**- 0.6**	**0.0001**	- 0.2	0.2
Folic acid (ng /ml)	**- 0.6**	**0.0001**	--	--	0.1	0.3
Vitamin B 12 (pg/ml)	- 0.2	0.2	0.1	0.3	--	--
Patients receiving anti epileptic drugs:						
Homocysteine (µmol/l)	--	--	**-0.5**	**0.002**	0.01	0.9
Folic acid (ng/ml)	**-0.5**	**0.002**	--	--	0.1	0.7
Vitamin B 12 (pg/ml)	0.01	0.9	0.1	0.7	--	--

In the present study, comparing mean serum levels of Hcy, folic acid and vitamin B12 in AEDs users (66.7%) to non-AEDs users (33.3%), no significant difference was found. This is in agreement with Cardo et al. [18] and Senera et al. [12] who found similar results. Data regarding anti-epileptic drugs and their effect on homocysteine metabolism have been controversial [12]. It has been reported that homocysteine level of patients receiving antiepileptic drugs increased by 11.4% to 40% [14, 15, 19].

AEDs interfere with folate metabolism in many ways, and may potentiate its effect. Folate may serve as a cofactor in AEDs catabolism (19). In adults taking VPA, folate depletion is normally not observed (20), whereas in children receiving VPA, folate depletion may develop (21).

Decreased, normal and increased vitamin B12 levels have all been reported in patients under long-term AEDs administration [14, 15]. It is also worth noting that our results showed that mean serum level of Hcy was higher but not significant in patients who were receiving polytherapy treatment compared to those receiving monotherapy treatments. This is in concordant with Huemer et al. [19] and Ono et al. [22].

Hcy showed no significant difference between patients who were receiving CBZ or VPA; this is in agreement with the study done by Vurucu et al. [23] who found similar results. In the contrary, Gidal et al. [24] reported a statistically significant decline in plasma Hcy concentration among patients who received VPA.

Our study showed that mean serum folic acid level were lower but not significant in patients on polytherapy treatment, compared to those on monotherapy treatment. This is in concordant with Ono et al. [22] and Huemer et al. [19].

In the present study, serum folic acid was not significantly higher in patients who were receiving VPA compared to those who were receiving CBZ. This is in agreement with the study done by Geda et al. [25] and Gidal et al. [24].

Various researchers have shown that treatment with enzyme-inducing antiepileptic drugs (AEDs) including phenytoin, phenobarbital and primidone is associated with decreased plasma folate concentration in adult and in children [5, 14, 15, 26]. These AEDs may interfere with both absorption and metabolism of folate [20]. It has also been suggested that AED-mediated decrease in plasma folate concentration may in part represent a drug–gene interaction [22].

Also, mean serum level of vitamin B12 showed no significant difference between patients who were receiving polytherapy treatment, and patients on monotherapy treatment; the same was found between patients who received VPA, or CBZ. This is in accordance to the finding of Vurucu et al. [23] who conducted a study in 2008 on a total of 93 patients with idiopathic epilepsy, 29 were on CBZ and 64 were on VPA, and they reported no difference in that mean serum level of vitamin B12 in patients who were receiving VPA or CBZ. The same was found by Kurul et al. [27] who conducted their study on 25 children with idiopathic epilepsy (8 on VPA, 11 on CBZ, and 6 on oxcarbamazepine); they found no difference between the mean plasma vitamin B12 levels among epileptic patients who received different types of antiepileptic drugs.

In our study there was no significant difference between mean serum levels of Hcy, folic acid and vitamin B12 with different EEG abnormalities (grade I : 61.7% , grade II : 5% , grade III : 33.3%). Similarly, Aslan et al. (13) found that there was no statistically significant relation between mean serum levels of Hcy, folic acid and vitamin B12 with EEG abnormalities.

In the present study, there was no significant difference between mean serum level of Hcy, folic acid and vitamin B12 and different seizure types. No similar study was done studying their level in relation to different seizure types.

Also, we found that duration of therapy correlated significantly with elevated serum homocysteine (P=0.02) and low serum folic acid (P=0.03), which is similar to previous results of Schwaninger et al. [11]; Ono et al. [22] and Huemer et al. [19].

In our study, we found that there was significantly negative correlation between Hcy and folic acid in the patients using AEDs (p=0.002), and all epileptic patients (p=0.0001). This is in accordance to the finding of Senera et al. [12] and Aslan et al. [13] who found a significant negative correlation between the levels of homocysteine and folic acid in patients using AEDs and in entire study population. However, no association was found between homocysteine and vitamin B12. On the contrary, Ono et al. [22] found no correlation between Hcy, folic acid and vitamin B12.

We conclude that, no significant difference was found between epileptic patients and controls regarding mean serum levels of Hcy and folic acid with a significantly higher vitamin B12 levels. Based on the previous observations, we can speculate that AEDs might upset the homeostatic balance of Hcy and its cofactor and cause abnormalities of their serum level as evidenced by nonsignificantly higher homocysteine and vitamin B12 in patients on polytherapy treatment compared to those on monotherapy treatment. Also, folic acid level was lower but not significant in patients on polytherapy treatment compared to those on monotherapy one .The duration of anti-epileptic drug treatment was correlated significantly to the decrease of folic acid and to the increase in homocysteine, yet their serum levels didn't correlate with severity of seizure. Although, our study showed that the plasma homocysteine level between patients and controls were not found significantly different; yet all cases and particularly patients on antiepileptic drugs demonstrated higher levels of homocysteine. This result is compatible with literature findings. As we studied both epileptic patients were not receiving AEDs (non-AEDs users) and controls, we observed that there were no differences in homocysteine levels between them, suggesting that, the increase of homocysteine levels may be due to anti-epileptic drug use, rather than being epileptic in origin. We think that we excluded the effect of being epileptic on homocysteine status which has not been considered in most of the studies. For this reason, we suggest that the drugs, rather than the disease, play a major role in the development of hyperhomocysteinemia in epileptic patients.

Reference

1. Benbira G, Uysalb S, Saltikc S, Aktuglu Zeybekd C, A Aydind A, Derventa A and Yalcinkayaa C. Seizures during treatment of Vitamin B12 deficiency. Euro J Epilepsy. 2007; 16(1): 69-73.
2. Reynolds EH. Benefits and risks of folic acid to the nervous system. J Neurol Neurosurg Psychiatry. 2002; 72: 567-571.
3. Lewis DP, Van Dyke DC, Willhite LA: Phenytoin-folic acid interaction. Ann Pharmacother 1995; 29:726-735.
4. Mares P, Folbergrova J, Langmeier M, Haugvicova R and Kubova H. Convulsant action of d,l-homocysteic acid and its stereoisomers in immature rats. Epilepsia. 1997; 38:767–776.
5. Vilaseca MA, Monros E, Arthuch R. Anti-epileptic drug treatment in children: T mutation of the hyper homocysteinemia. B vitamins and the 667C methyleneterahydrofolate reductase gene. Eur J Paediatr Neurol. 2000; 41:269-277.
6. Loring DW and Meador KJ. Cognitive side effects of anti epileptic drugs in children. Neurology. 2004; 62:872-877.
7. Guidelines for epidemiologic studies on epilepsy. Commission on Epidemiology and Prognosis, International League Against

Epilepsy. Epilepsia. 1993;34(4):592-6.

8. International League against Epilepsy Revised Classification of Epileptic Seizures, 1981.
9. Duncan and Sander JW. Chalfont seizures severity score. J Neurol Neurosurg Psychaitry. 1991; 54(10):873-876.
10. Stern JM and Engel J. Categorization by pattern features. Atlas of EEG patterns. Lippincott Williams and Wilkins, 2005: 13-25.
11. Schwaninger M, Ringleb P, Winter R, Kohl B, Fiehn W, Rieser PA, Walter-Sack I. Elevated plasma concentrations of homocysteine in antiepileptic drug treatment. Epilepsia. 1999; 40(3):345-350.
12. Senera U, Zorlua Y, Karaguzela O, Ozdamarb O, Cokerb I and Topbasc M. Effects of common anti-epileptic drug monotherapy on serum levels of homocysteine, Vitamin B12, folic acid and Vitamin B6. British Epilepsy Association. 2006; 15(2):79-85.
13. Aslan K, Bozdemir H, Unsal C and Guvenc B. The effect of antiepileptic drugs on vitamin B12 metabolism. International Journal of Laboratory Hematology. 2008; 30: 26-35.
14. Tamura T, Aiso K, Johnston KE, Black L and Faught E. Homocysteine, folate, vitamin B12 and vitamin B6 in patients receiving antiepileptic drug monotherapy. Epilepsy Res. 2000; 40:7–15.
15. Karabiber H, Sonmzgoz E, Ozerol E, Yakinci C, Otlu B and Yologlu S. Effects of valproate and carbamazepine on serum levels of homocysteine, vitamin B12 and folic acid. Brain Development. 2003; 25:113–115.
16. Dastur DK and Dave UP. Effect of prolonged anticonvulsant medication in epileptic patients: serum lipids, vitamins B6, B12, and folic acid, proteins, and fine structure of liver. Epilepsia. 1987; 28:147-159.
17. Frank O, Baker H and Leavy CM. Vitamin binding capacity of experimentally injured liver. Nature. 1964; 203: 302-303.
18. Cardo E, Vilaseca MA, Campistol J, Artuch R, Colomé C, Pineda M. Evaluation of hyperhomocysteinemia in children with stroke. Eur J Paediatr Neurol. 1999; 3: 113-117.
19. Huemer M, Ausserer B, Graninger G, Hubmann M, Huemer C, Schlachter K, Tscharre A, Ulmer H, Simma B. Hyperhomocysteinemia in children treated with antiepileptic drugs is normalized by folic acid supplementation. Epilepsia. 2005; 46:1677-1683.
20. Kishi T, Fujita N, Eguchhi T and Ueda K. Mechanism for reduction of serum folate by antiepileptic drugs during prolonged therapy. J Neurol Sci. 1997; 145: 109-112.
21. Verrotti A, Pascarella R, Trotta D, Giuva T, Morgese G and Chiarelli F. Hyperhomocysteinemia in children treated with sodium valporate and carbamazepine. Epilepsy Res. 2000; 41:253-257.
22. Ono H, Sakamoto A, Mizoguchi N and Sakura N. The C677T mutation in the methylenetetrahydrofolate reductase gene contributes to hyperhomocysteinemia in patients taking anticonvulsants. Brain Dev. 2002; 24:223–226.
23. Vurucu S, Demirkaya E, Kul M, Unay B, Gul D, Akin R, Gokçay E. Evaluation of the relationship between C677T variants of methylenetetrahydrofolate reductase gene and hyperhomocytsteinemia in children receiving antiepileptic drug therapy. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2008; 23: 844-848.
24. Gidal BE, Tamura T, Hammer A, Vuong A. Blood homocysteine, folate and vitamin B-12 concentrations in patients with epilepsy receiving lamotrigine or sodium valproate for initial monotherapy. Epilepsy Res. 2005;64(3):161-6.
25. Geda G, Caksen H and Icagasioglu D. Serum lipids, vitamin B12 and folic acid levels in children receiving long-term valproate therapy. Acta Neurol Belg. 2002; 102: 122-126.
26. Apeland T, Mansoor MA and Strandjord RE. Antiepileptic drugs as independent predictors of plasma total homocysteine levels. Epilepsy Res. 2001; 47:27–35.
27. Kurul S, Unalp A and Yis U. Homocysteine levels in epileptic children receiving antiepileptic drugs. J Child Neurol. 2007; 22(12): 1389-1392.