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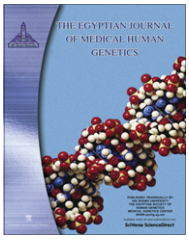

ORIGINAL ARTICLE

Homocysteine, folic acid and vitamin B12 levels in serum of epileptic children

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Abstract The relationship between increased homocysteine (Hcy) level and epileptic seizure remains controversial in human, despite a growing evidence of the pro-convulsive effect of the hyperhomocysteinemia (HHcy) observed in the animal studies. The mechanism of this association with epileptogenesis has not been clearly understood, although there is emerging evidence to support the unfavorable effects of some anti-epileptic drugs (AEDs) on the plasma homocysteine (Hcy) concentrations. The aim of this study was to uncover the relationship between the levels of homocysteine (Hcy), the cofactors involved in its metabolism as folic acid and vitamin B12 and anti-epileptic drugs (AEDs) in epileptic patients. Serum level of homocysteine (Hcy), folic acid and vitamin B12 was measured in 60 patients with idiopathic epilepsy; and its level was compared to 30 healthy children serving as control group. No significant difference was found regarding the plasma homocysteine (Hcy) levels between patients (both receiving anti-epileptics and non anti-epileptic drug users) and controls. Epileptic patients on polytherapy showed higher mean serum levels of homocysteine (Hcy) and lower mean serum levels of folic acid compared to those on monotherapy. However, the mean serum levels of homocysteine (Hcy), vitamin B12 and folic acid showed non significant differences between patients using valproic acid (VPA) or carbamazepine (CBZ). Duration of AED therapy showed a significant positive correlation with mean serum levels of homocysteine (Hcy) and a significant negative correlation with mean serum levels of folic acid. To conclude;

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AEDs upset the homeostatic balance of homocysteine (Hcy) and its cofactors and cause abnormalities in their serum levels.

1. Introduction

Homocysteine (Hcy), is a sulfur-containing amino acid that is formed by de-methylation of methionine [1]. Plasma Hcy concentrations vary with ethnic background, increase with age, are higher in adult men and postmenopausal women, and lowest in children [2]. In children, hyperhomocysteinemia (HHcy) is defined according to age and (after puberty) sex-specific percentiles [3].

Folic acid and vitamin B12 have roles in the metabolism of Hcy and defects in the metabolism of any of them may lead to increased serum Hcy levels, resulting in atherosclerosis [4,5].

It has been reported that Hcy induces neuronal cell death by stimulating N-methyl D aspartate (NMDA) receptors mediating excitotoxicity, as well as by producing free radicals [6] and induction of apoptosis [7]. Its metabolites, homocysteic acid and L-Hcy sulfinic acid, also exhibit high excitotoxic potency by interacting with different glutamate receptor subtypes [8]. They are potent agonists of the NMDA-type glutamate receptor, which are linked with epileptogenesis [9].

Also, stimulation of NMDA receptors by Hcy with excessive calcium influx causes reactive oxygen generation and neurotoxicity that contribute to the cognitive changes and the marked increased risk of cerebrovascular disease in children and young adults with homocysteinuria. In addition, disruption of blood–brain barrier (BBB) in patients with stroke and HHcy exposes the brain to near plasma levels of Hcy and increases neurotoxicity [6].

In animals, systemic administration of high doses of Hcy produces convulsive seizures, a fact that has been exploited in models of experimental epilepsy [10]. Furthermore, there are some data from animal studies demonstrating that Hcy sequesters adenosine, an endogenous anticonvulsant, and thereby reduces the seizure threshold [11].

Therefore, Hcy through these mechanisms, may reduce seizure threshold and increase seizure frequency in patients treated with AEDs. Other mechanisms, such as oxidative stress, DNA damage, inhibition of NA/K-ATPase and activation of caspases, could be involved in Hcy-induced neuronal excitotoxicity [12].

There is little information on the influence of antiepileptic drugs on Hcy levels in pediatric patients. HHcy was reported in 15.5% of children receiving AEDs [13]. Little is known on how phenytoin (PHT), valproic acid (VPA) and carbamazepine (CBZ) exert their effect on Hcy metabolism. It has been suggested that enzyme inducers, can directly modulate the activity of different liver enzymes which in turn may cause depletion of the cofactors involved, leading to the alternations observed in Hcy status [13].

In fact, an independent predictor of HHcy in patients treated with PHT or CBZ is the presence of a homozygous thermolabile genotype of 5,10 methyl tetrahydrofolate reductase (MTHFR), suggesting a gene–drug interaction as a cause of HHcy, this hypothesis is supported by Ono et al. [14]. Additionally, increased Hcy and low folate status may contribute to the development of AEDs related side effects, such as impaired cognitive function, and fetal malformation [15].

The aim of this study was to uncover the relationship between the levels of Hcy and the cofactors involved in its metabolism as folic acid and vitamin B12 and AEDs in epileptic patients.

2. Subjects and methods

2.1. Subjects

This study was a cross-sectional study, conducted on 60 epileptic children diagnosed with idiopathic epilepsy according to the guidelines of The Classifications of the International League Against Epilepsy (1998) [16], with age ranging from 5 to 15 years old. They were recruited from the Pediatric Neurology outpatients' clinic of Children's Hospital, Ain Shams University.

Patients were divided into two groups: Group (I), comprised 20 newly diagnosed epileptic children not receiving anti-epileptic medication (non-AED users). Group (II), comprised 40 epileptic children on regular antiepileptic medication for at least one year (AED users). Group II was further subdivided into: Subgroup (A) which comprised 20 epileptic children receiving monotherapy. Therapy consisted of either valproic acid (VPA) or carbamazepine (CBZ). VPA group comprised 12 children and CBZ group comprised 8 children. Subgroup (B), receiving polytherapy treatment (combined treatment with VPA and CBZ) comprised 20 patients. Serum concentrations of both VPA and CBZ were maintained within the therapeutic range throughout the study (71.4 ± 13.09 μg/ml and 7 ± 1.9 μg/ml, respectively).

Thirty non-epileptic children of the same age and sex as patients served as controls. Children with diseases affecting the serum level of Hcy as endocrinal, liver, kidney and cardiac diseases, diabetes mellitus and nutritional deficiencies or those receiving vitamin supply or folic acid antagonists as well as the vegetarians were excluded from the study. Moreover, patients with uncontrolled seizures were excluded from the study to limit the variables and to make sure not to change the dose of AEDs throughout the study.

2.2. Methods

After obtaining the approval of the Ethics Committee at the Pediatric Hospital, Ain Shams University, consents for participating in the study were signed by the parents or caregivers. Children were subjected to the following: Detailed history laying stress on age of onset of seizure, milestones of development, symptoms of neurological deficits as well as symptoms suggestive of an underlying etiology (according to the commission on Epidemiology and Prognosis, International League against Epilepsy, 1989) [16], type of seizure disorder (according to the recommendations of the International League against Epilepsy, 1981) [17] and duration of the disease. Care should

be taken that AEDs' dose was not changed in last three months before the study, and we checked their dose, duration of treatment, preparations and response to treatment. Complete physical and neurological examinations were also done for all patients.

2.3. Sampling

Estimation of serum levels of Hcy, folic acid and vitamin B12 were done using Chemiluminescent technique (Immulite 2000). Patients must be in a fasting state [18]. Blood was drawn by venipuncture in sterile plain vacutainer tubes; incubated in water bath at 37 °C for 15 min, centrifugated at 10,000 RPM then serum was separated in sterile Epindorph tubes. To prevent erroneous results due to the presence of fibrin ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

2.4. Statistical methods

Statistical package for social science (SPSS) program version 9.0 was used for analysis of data. Data were summarized as mean and SD. Non parametric test (Mann Whitney U) was used for analysis of two quantitative data as data were 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. Simple linear correlation (Spearman's correlation) was also done. "*r*" value was considered weak if <0.25, mild if $\geqslant 0.25 - <0.5$, moderate if $\geqslant 0.5 - <0.75$ and strong if $\geqslant 0.75$.

3. Results

Regarding the epileptic patients included in the study their mean age was 9.3 ± 3.5 years. Thirty (50%) were males and 30 (50%) were females. Generalized epilepsy was found in 44 (73.3%) and focal epilepsy in 16 (26.7%) of the studied patients. Out of these patients, 20 epileptic children were not receiving anti-epileptic medication (non-AED users). Their mean age was 7 ± 3.16 years, and 40 epileptic children were on regular antiepileptic medication (AED users), their mean age was 10.5 ± 3.16 years. They were further subdivided into: 20 epileptic children receiving monotherapy of mean age 10.3 ± 3.4 years, and the other 20 patients receiving polytherapy treatment of mean age 10.6 ± 2.9 years. They were compared to 30 non-epileptic children of mean age 11.0 ± 2.4 years.

Our study showed that the mean serum levels of Hcy and folic acid showed non significant differences between epileptic patients (both AED users and non AED users) and the controls ($p = 0.07$, $p = 0.6$, respectively). However, the mean serum level of vitamin B12 was significantly higher in epileptic patients compared to the controls ($p = 0.03$) (Table 1). The mean serum levels of Hcy, folic acid and vitamin B12 showed comparable results in non AED users, AED users and the controls (Table 2).

Epileptic patients on polytherapy treatment showed higher mean serum levels of Hcy and vitamin B12 and lower mean serum levels of folic acid compared to those on monotherapy treatment (Table 3). However, the mean serum levels of Hcy, vitamin B12 and folic acid showed non significant differences between patients using VPA or CBZ (Table 4).

The mean serum levels of Hcy, vitamin B12 and folic acid did not show significant difference among epileptic patients with different seizure types.

A significant positive correlation was detected between the duration of therapy and the serum Hcy levels ($r = 0.4$, $p = 0.02$) (Fig. 1), however there was a significant negative correlation between duration of therapy and serum folic acid levels ($r = -0.5$, $p = 0.003$) (Fig. 2).

A negative correlation was detected between the mean serum levels of Hcy and folic acid in all epileptic patients as well as in those receiving AEDs. ($r = 0.6$ and $r = 0.5$, respectively; $p = 0.0001$ and $p = 0.002$, respectively) (Figs. 3 and 4).

4. Discussion

The mechanism of the association between homocysteine and epilepsy is not fully understood, it is recommended that AEDs increase serum Hcy by decreasing the blood folate levels, due to antifolate properties, and depletion of other vitamins like B2 and B6 [19,20].

Our study showed that Hcy and folic acid serum levels showed non significant differences between patients (both AED users and non AED users) and controls and this is in accordance with the study of Schwaninger et al. [21]. Moreover, when comparing serum levels of Hcy, folic acid and vitamin B12 in patients receiving AEDs, non-AED users and controls, they showed comparable results. These results are in agreement with previous studies of Sener et al. [20] and Kurul et al. [22].

Our finding of higher mean serum level of Hcy in patients receiving polytherapy compared to those receiving monotherapy is partly supported by evidence from previous literature [13,14], and is further reinforced by the *MTHFR* 677TT genotype; where among patients receiving multidrug therapy,

Table 1 Comparison between mean serum levels of homocysteine and its co-factors among epileptic patients (both AED users and non AED users) and 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*

* *P* value is significant <0.05.

Table 2 Comparison between levels of homocysteine and its co-factors among epileptic patients AED users, non AED users and controls.

Variables	Non-AED users $N = 20$	AED users $N = 40$	Controls $N = 30$	*P*-value
Homocysteine (μmol/l)	5.8 ± 1.4	6.0 ± 2.7	5.5 ± 1.4	0.5
Folic acid (ng/ml)	7.3 ± 1.9	7.9 ± 3.5	6.4 ± 2.9	0.1
Vitamin B 12 (pg/ml)	433.5 ± 171.2	526.8 ± 252.1	443.9 ± 150.3	0.1

Table 3 Comparison between levels of homocysteine and its co-factors among epileptic patients receiving polytherapy and those receiving monotherapy.

Variables	Monotherapy Mean ± SD $N = 20$	Polytherapy Mean ± SD $N = 20$	*P*-value
Homocysteine (μmol/l)	5.4 ± 1.6	6.7 ± 3.3	0.2
Folic acid (ng/ml)	8.2 ± 2.7	7.7 ± 4.2	0.7
Vitamin B 12 (pg/ml)	491.5 ± 299.9	562.0 ± 194.6	0.4

Table 4 Comparison between levels of homocysteine and its co-factors among epileptic patients receiving VPA and those receiving CBZ.

Variables	CBZ Mean ± SD $N = 8$	VPA Mean ± SD $N = 12$	*P*-value
Homocysteine (μmol/l)	5.5 ± 1.9	5.4 ± 1.6	0.9
Folic acid (ng/ml)	6.4 ± 2.7	8.7 ± 2.6	0.09
Vitamin B 12 (pg/ml)	456.4 ± 252.5	503.2 ± 321.3	0.8

VPA: valproic acid, CBZ: carbamazepine.

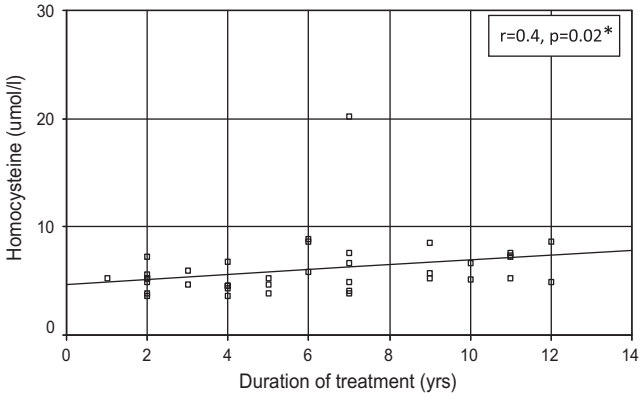

Figure 1 Positive correlation between mean serum levels of homocysteine and duration of treatment of epileptic patients.

HHcy in homozygotes for C677T occurred significantly more often than in heterozygotes or patients with no mutant enzyme [14]. However, Hcy levels showed no significant difference between patients who were receiving CBZ or VPA and this is in agreement with the studies done by Apeland et al. [23] and Vurucu et al. [24], yet this is in contrast to Gidal et al. [25] results, who reported a statistically significant decline in plasma

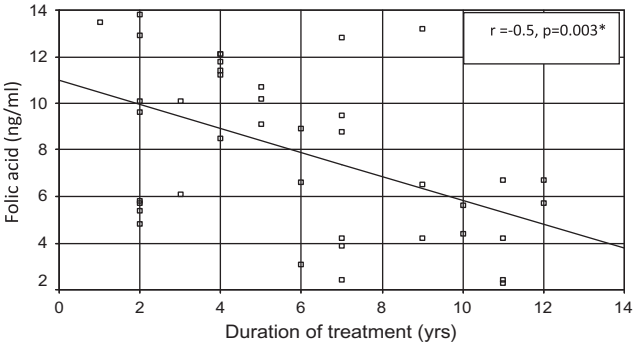

Figure 2 Negative correlation between mean serum levels of folic acid and duration of treatment in epileptic patients.

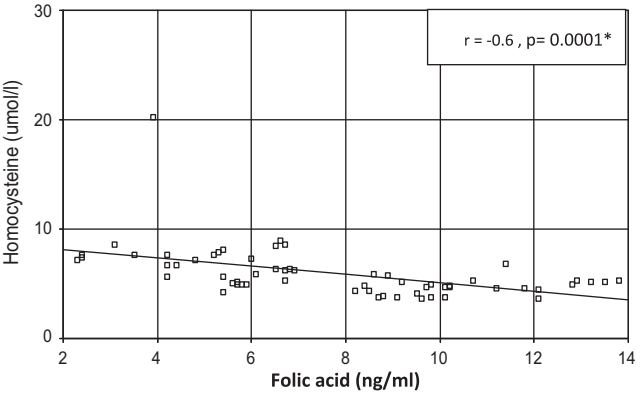

Figure 3 Negative correlation between mean serum levels of homocysteine and folic acid in epileptic patients (both AED users and non AED users).

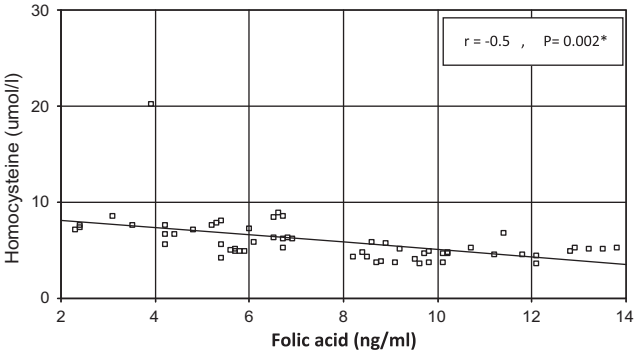

Figure 4 Negative correlation between mean serum levels of homocysteine and folic acid in anti-epileptic drug users.

Hcy concentration among patients receiving VPA. Also, previous studies reported elevated Hcy levels with CBZ and VPA use [26,19,5].

Antiepileptic drugs could be able to induce HHcy through the following mechanisms: reinhibition in vitamin absorption, Hcy metabolism dysfunction, accelerated vitamin metabolism, and modulation of renal function [27].

Although our study showed that the serum Hcy levels between patients and controls were not significantly different, yet all cases and particularly patients on antiepileptic drugs demonstrated higher levels of Hcy. This result is compatible with literature findings [13,20,27]. However, data regarding AEDs and their effect on Hcy metabolism have been controversial [19]. It has been reported that Hcy levels of patients receiving AEDs increased by 11.4–40% [5,13]. As we studied both epileptic patients who were not receiving AEDs (non-AED users) and controls, we observed that there were no differences in Hcy levels between them, suggesting that, the increase of Hcy levels may be due to AEDs use, rather than being epileptic in origin.

The mean serum level of vitamin B12 was significantly higher in patients compared to controls and non significantly higher in patients on polytherapy than those on monotherapy. This is in agreement with the study done by Tamura et al. [4]. This could be explained by the fact that prolonged drug treatment may possibly cause slightly impaired ability of the liver to store vitamin B12, thus increasing the circulating vitamin B12 levels, which served as a sensitive biochemical index of hepatic damage due to anticonvulsants [21]. On the contrary, Karabiber et al. [5] and Aslan et al. [28] detected low vitamin B 12 levels in patients; yet, their data were not statistically significant. It is worth noting that in patients receiving monotherapy treatment the type of AED used (VPA or CBZ) did not alter the serum level of vitamin B12. This is in accordance to the finding of Kurul et al. [22].

Our study showed that mean serum folic acid levels were significantly lower in patients on polytherapy than those on monotherapy. This is in accordance with the studies of Ono et al. [14] and Huemer et al. [13]. However, serum folic acid showed comparable results in patients who were receiving VPA and those receiving CBZ. This is in agreement with the studies of Gidal et al. [25] and Geda et al. [29]. AEDs may interfere with both absorption and metabolism of folate. It has also been suggested that AED-mediated decrease in plasma folate concentrations may in part represent a drug–gene interaction [30,14]. It is worth noting that in the study done in 2005 by Huemer et al. [11] intervention with folic acid resulted in significantly higher folate and lower tHcy concentrations at weeks 6 and 12 compared with patients receiving placebo [13].

Also, we found that the duration of therapy correlated significantly with elevated serum Hcy and low serum folic acid, which is similar to previous results [21,11,12]. A negative correlation was detected between mean serum levels of Hcy and folic acid in all epileptic patients as well as in those receiving AEDs.

Based on the previous observations, we can speculate that AEDs might upset the homeostatic balance of Hcy and its cofactors and cause abnormalities of their serum levels.

Furthermore, the duration of AEDs is correlated significantly to the decrease of folic acid levels and to the increase in Hcy levels. We suggest that the AEDs, rather than the disease, play a major role in the development of homocysteinemia in epileptic patients. So, we recommend to routinely screen Hcy and its cofactors levels in epileptic patients, and be treated when their levels are found to be disturbed.

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The patients in the current study are among those admitted and followed up freely in the Children's Hospital, Faculty of Medicine, Ain Shams University including the use of the laboratory. No external funds were received from a third party during the performance of this study.

Authors' contributions

Professors Osama N. Saleh and Soha M. Abd Eldayem had primary responsibility for the idea and the protocol development, patient screening, enrollment, outcome assessment and revision of the manuscript.

Dr. Rania H. Shatla shared the idea and protocol development, supervised patient screening, enrollment and their dietary protocols. She was responsible for the preliminary data analysis and editing of the manuscript.

Professor Dr. Nahed A. Omara supervised the execution of the laboratory work up and participated in the data analysis.

Dr. Sara S. Elgammal was responsible for patient screening, enrollment, examination and follow up.

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