Cognitive Decline due to Impaired Homocysteine Metabolism in Adults on Antiepileptic Monotherapy: A Prospective Study

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ABSTRACT

Aim: Long-term antiepileptic therapy is associated with hyperhomocysteinemia (HHcy), causing cardiovascular risk and subsequent cerebrovascular and cognitive disorders. Objective of our study is to assess the effects of old and new generation drugs on homocysteine (Hcy), folate, and B12 levels in serum and to relate HHcy with cognitive decline.

Materials and methods: Drug-naïve patients recently diagnosed with epilepsy in the age group 18 to 40 years (n = 124) were recruited and grouped according to the drug prescribed: conventional drugs, like phenytoin, carbamazepine, valproate, and newer drugs, like oxcarbazepine and levetiracetam. A pretreatment analysis of Hcy, folate, and B12 was done. Mini-Mental State Examination (MMSE) was used to assess cognitive impairment, if any. After 6 months of continuous monotherapy, the analyses were repeated.

Results: Homocysteine levels were raised significantly in all the groups (p < 0.05 in all groups) after 6 months of therapy. Hyperhomocysteinemia (>15.1 µmoles/L) was found in 64 out of 124 patients, of which 42 were on conventional drugs. Folate and B12 registered a significant decrease in patients on phenytoin, carbamazepine, and oxcarbazepine. Cognitive decline was evaluated as drop of a point or more in MMSE score after 6 months of therapy. A significant positive relation between Hcy and cognitive decline was seen in groups on monotherapy of phenytoin, carbamazepine, and oxcarbazepine. An insignificant positive correlation was observed in sodium valproate and levetiracetam groups.

Conclusion: Both conventional and newer antiepileptic drugs (AEDs) may cause HHcy, leading to cardiovascular complications and cognitive decline. Subjects on sodium valproate and levetiracetam are less likely to show a decline in cognition, regardless of a rise in Hcy levels.

Clinical significance: Folate and B12 deficiency causes HHcy, which correlates positively with cognitive decline.

Keywords: Antiepileptic drugs, Cognitive decline, Hepatic enzyme inducers, Mini-Mental State Examination.

INTRODUCTION

Homocysteine is a sulfur-containing nonessential amino acid derived from an essential amino acid methionine. Hyperhomocysteinemia in blood is indicative of atherosclerotic risk and cardiovascular disorders and is observed in patients treated with AEDs. Both atherosclerosis and cardiovascular disease have been found to be associated with cognitive impairment and dementia. Association of HHcy with cognitive decline may be through direct neurotoxicity or through thrombosis. Accumulation of Hcy is mainly due to deficiency of cofactors B12 and folate required by enzymes involved in remethylation of Hcy to methionine. Methionine is a potent donor of methyl group, which is required for synthesis of myelin, neurotransmitters, and membrane phospholipids. Oral supplementation of folate, B12, and activated methionine S-adenosyl methionine lowers plasma concentrations of Hcy and reduces depressive symptoms. Homocysteine in middle age is also an independent risk factor for progression of dementia and Alzheimer’s later in life. A positive correlation between the rate of cognitive decline and Hcy concentration has been observed in patients of Alzheimer’s disease. Hyperhomocysteinemia has been reported to be associated with cognitive dysfunction measured through several neuropsychological tests.

Older AEDs like phenytoin (PHT), carbamazepine (CBZ), valproate (VPA) may affect folate and B12
metabolism but they are still prescribed due to lower costs, easy availability, and long practical experience. Comparatively newer AEDs like oxcarbazepine (OXC), levetiracetam (LEV), lamotrigine, and topiramate are less likely to affect adversely, reducing the risk of HHcy.19 The risk of cardiovascular disease increases in epilepsy but the association with AEDs is inconclusive.20,21 Studies on efficacy of newer AEDs over old AEDs in Indian population are few and the present study investigates changes in serum levels of Hcy, B12, and folate as an effect of AED therapy and correlation of HHcy with cognitive dysfunction in such patients.

MATERIALS AND METHODS

The study includes 124 drug-naïve patients, recently diagnosed with epilepsy, visiting the outpatient clinic at Department of Neurology, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, Uttar Pradesh, India. The Institutional Ethics Committee had approved final protocol of the study, which was in accordance with the guidelines of Helsinki Declaration. Written informed consent was obtained from subjects or their parents or caregivers.

Inclusion Criteria

Patients newly diagnosed with epilepsy within 18 to 40 years of age were recruited for the study. A baseline assessment of markers was done and treatment with AEDs was initiated thereafter.

Exclusion Criteria

Patients with history of ischemic stroke, hypertension, coronary artery disease, peripheral vascular disease, diabetes mellitus, pregnancy, renal and thyroid disorders, and chronic tobacco users were excluded from the study. Subjects on regular consumption of vitamins or drugs other than AEDs, like levodopa, fibrates, statins, methfor- min, methotrexate, and sulfasalazine, were also excluded.

Measurement of Hcy, Folate, Vitamin B12 Concentration

Following an overnight fasting period, blood was drawn between 8 and 10 AM, from the antecubital vein in sitting position. Serum was immediately separated by centrifugation and assayed for Hcy, B12, and folate. Serum total levels of Hcy folate and B12 concentrations were measured through chemiluminescent immunoassay, using kits available for Advia centaur autoanalyzer (Siemens). Normal reference ranges in fasting conditions were 5 to 15 μM/L for total homocysteine (tHcy), 3 to 17 ng/mL for serum folate, and 178 to 800 pg/mL for vitamin B12. Monotherapy with PHT, CBZ, VPA, OXC, or LEV was initiated and all assessments were repeated after 6 months. Serum Hcy levels ≥15.1 μM/L were indicative of HHcy.

Mini-Mental State Examination

Cognitive impairment was assessed using the MMSE.19 Cognitive function was assessed with the 30-point MMSE, both at baseline and at follow-up.22 The MMSE tests the patient with questions on orientation to time and place, registration, attention and calculation, recall, language, and visual construction. If less than four items (out of 20) were not answered by the patient, they were considered as errors.23 If a subject did not answer four or more individual items, the total MMSE score was considered missing. A score of <26 points on the MMSE at baseline indicated cognitive impairment.24 Cognitive decline was defined as a decrease in the MMSE score from baseline to follow-up of 1 point or more.25

Statistical Analysis

Data were analyzed using statistical software Prism Version 5 program (GraphPad). There were five groups of patients in accordance with the type of drug used for therapy. Discrete variables (gender, seizure type) were compared by the Pearson’s Chi-square test. Continuous data (baseline and follow-up values of tHcy, B12, folate, and MMSE scores) in each group were compared using the paired t-test for normally distributed or log transformed data and the Wilcoxon signed-rank test for skewed data, which failed to exhibit normal distribution after log transformation. We used one-way analysis of variance (ANOVA) for normally distributed data and Kruskal–Wallis test for skewed data followed by Bonferroni correction, to study variation in different markers and MMSE scores between groups. Estimation of RR and 95% CI assessed the association between cognitive decline and HHcy.

RESULTS

Drug naïve subjects (n = 124) were included for the study and divided into five groups according to the drug prescribed: PHT (n = 24), CBZ (n = 22), VPA (n = 34), OXC (n = 28), and LEV (n = 16). Table 1 summarizes the demographic details of the five groups. No significant differences are observed in age (p = 0.1042) and gender (χ² = 4.592, df = 4, p = 0.3318). There was a significant difference in seizure type among the five drug groups (χ² = 22.81, df = 4, p = 0.0001).

All monotherapy groups registered an increase in tHcy levels after 6 months of continuous treatment (p<0.05 in all the groups). A decrease in B12 levels was observed in PHT (p = 0.0084), CBZ (p < 0.0001), and OXC
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(p = 0.0261) groups; similarly serum folate exhibited significant decrease with therapy of PHT (p<0.0001), CBZ (p<0.0001), and OXC (p = 0.0205) drugs. Drop of a point or more in MMSE scores was seen in groups on therapy of both conventional and newer drugs, PHT (p<0.0001), CBZ (p = 0.0009), VPA (p = 0.0308), and OXC (p = 0.0011). Subjects on LEV registered no significant decrease in MMSE scores. One-way ANOVA revealed variation in all parameters among different groups of therapy (Tables 2 and 3).

About 51.6% (64/124) patients had Hcy levels above 15.1 μM/L, out of which 65.6% (42/64) were on therapy of conventional drugs. All drug groups, except LEV exhibited a significant drop in MMSE scores. To assess risk of cognitive decline associated with increased Hcy levels, RR along with CI (95%) was calculated. A significant positive correlation between HHcy and cognitive decline was recorded in the groups on PHT (RR = 4.667; CI = 0.7663, 28.42), CBZ (RR = 2.778; CI = 1.042, 7.403), and OXC (RR = 3.316; CI = 0.9483, 11.59), whereas subjects on VPA and LEV revealed a nonsignificant positive association (Table 4).

**DISCUSSION**

In accordance with previous studies, the present study shows HHcy along with decrease in vitamin levels in epileptic patients started on AED monotherapy. Hyperhomocysteinemia is a major independent risk factor for atherosclerosis, cardiovascular, cerebrovascular, and cognitive disorders. Our study demonstrates significant rise in Hcy levels with therapy of both conventional as well as newer AEDs. This is in accordance with previous studies conducted by Belcastro et al and Kim et al. In our study, vitamin B12 and folate levels show a decrease in patients on PHT, CBZ, and OXC, whereas VPA and LEV treatment shows no significant decrease in folate and B12 levels, which is in accordance with the results demonstrated in earlier studies. Some studies have shown that adults on VPA treatment do not show a substantial decrease in folate levels, while children on VPA show a decrease in folate levels. In a mouse model, PHT directly decreases the activity of enzyme methyl tetrahydrofolate reductase, which is required to remethylate Hcy. More studies reveal that P450 enzyme inducers like PHT and CBZ may lower folate concentrations through enhanced catabolism. Antiepileptic drugs impair folate absorption and gastrointestinal transport and folate may serve as a cofactor in AED catabolism. In pregnant Wistar rats, VPA directly affects the activity of methionine synthase, which remethylates Hcy to methionine. Hence, there are several mechanisms by which AEDs interfere with vitamin B and Hcy metabolism. A study on a transgenic mouse model of Alzheimer’s disease revealed that a diet rich in methionine lowers Hcy, resulting in reduced brain amyloidosis and improved cognition, though they were not confirmed in trials conducted on human patients.

We observed that a higher percentage of subjects with HHcy were on enzyme inducers like PHT, CBZ, and OXC. Likewise, cognitive decline positively correlated with Hcy levels in patients on enzyme-inducing drugs.

The current study is the first to correlate HHcy with cognitive decline in newly diagnosed, drug-naïve patients started on antiepileptic treatment. Previous investigations have associated HHcy with a heightened risk of stroke and other cardiovascular conditions, which are related to cognitive disturbances and dementia. A study on more than 11,000 patients revealed 26% increase of depressive symptoms, with elevation in Hcy levels. We observed a significant correlation between HHcy and cognitive decline in patients on monotherapy of conventional drugs like PHT and CBZ as well as those on newer drugs like OXC. The reliability of cognitive change over a time of 6 months was assessed as suggested in a study conducted by Hensel et al.

This study is a prospective study, investigating the effect of five AEDs, both conventional and newer, on Hcy levels, folate and vitamin B12 levels, and subsequent cognitive decline. All these factors, we believe, are its major strengths. To our knowledge, this is the first study that correlates Hcy levels to cognitive decline as an effect of AED monotherapy.

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**Table 1: Demographic distribution of patients with epilepsy**

<table>
<thead>
<tr>
<th></th>
<th>PHT (n = 24)</th>
<th>CBZ (n = 22)</th>
<th>VPA (n = 34)</th>
<th>OXC (n = 28)</th>
<th>LEV (n = 16)</th>
<th>p-value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.5 (1.525)</td>
<td>20.91 (1.347)</td>
<td>23.12 (1.131)</td>
<td>21.18 (1.069)</td>
<td>26.00 (1.917)</td>
<td>0.1042</td>
</tr>
<tr>
<td>Gender (male:female)</td>
<td>12:12</td>
<td>14:8</td>
<td>24:10</td>
<td>20:8</td>
<td>8:8</td>
<td>0.3318</td>
</tr>
<tr>
<td>Type of seizure</td>
<td>3:21</td>
<td>9:13</td>
<td>0:34</td>
<td>4:24</td>
<td>0:16</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>(partial:generalized)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AED dose (mg/day)</td>
<td>187.5 (15.34)</td>
<td>540.9 (16.98)</td>
<td>682.4 (29.39)</td>
<td>600 (8.909)</td>
<td>968.8 (67.99)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(100–300)</td>
<td>(400–600)</td>
<td>(400–1000)</td>
<td>(450–650)</td>
<td>(500–1,500)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard error (SE); level of significance at p<0.05.
Table 2: Total homocysteine, B12, folate levels, and MMSE scores at baseline and after 6 months of antiepileptic therapy

<table>
<thead>
<tr>
<th>Variable</th>
<th>PHT (Baseline)</th>
<th>PHT (Follow-up)</th>
<th>CBZ (Baseline)</th>
<th>CBZ (Follow-up)</th>
<th>VPA (Baseline)</th>
<th>VPA (Follow-up)</th>
<th>OXC (Baseline)</th>
<th>OXC (Follow-up)</th>
<th>LEV (Baseline)</th>
<th>LEV (Follow-up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy (μM/L)</td>
<td>9.14 (0.843)</td>
<td>18.81 (1.78)</td>
<td>9.01 (0.651)</td>
<td>20.10 (1.27)</td>
<td>7.53 (0.21)</td>
<td>11.76 (0.82)</td>
<td>7.60 (2.080)</td>
<td>16.13 (3.203)</td>
<td>7.53 (0.24)</td>
<td>11.16 (0.64)</td>
</tr>
<tr>
<td>B12 (pg/mL)</td>
<td>293.40 (14.7)</td>
<td>267.80 (13.0)</td>
<td>645.6 (84.86)</td>
<td>359.7 (27.56)</td>
<td>298.1 (10.23)</td>
<td>277.5 (12.41)</td>
<td>327 (11.83)</td>
<td>304.40 (13.4)</td>
<td>315.00 (4.49)</td>
<td>306.30 (6.74)</td>
</tr>
<tr>
<td>Folate (ng/mL)</td>
<td>15.42 (0.69)</td>
<td>6.99 (0.39)</td>
<td>11.77 (2.06)</td>
<td>3.72 (0.06)</td>
<td>5.02 (0.47)</td>
<td>4.67 (0.27)</td>
<td>6.18 (0.39)</td>
<td>5.23 (0.13)</td>
<td>11.91 (2.20)</td>
<td>5.34 (0.27)</td>
</tr>
<tr>
<td>MMSE score</td>
<td>26.13 (0.26)</td>
<td>25.13 (0.33)</td>
<td>26.68 (0.20)</td>
<td>25.64 (0.16)</td>
<td>24.97 (0.32)</td>
<td>24.68 (0.35)</td>
<td>24.46 (0.41)</td>
<td>23.39 (0.38)</td>
<td>25.50 (0.44)</td>
<td>25.38 (0.54)</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard error (SE); reference ranges: 5–15 μM/L for tHcy, 3–17 ng/mL for serum folate, and 178–800 pg/mL for vitamin B12; MMSE is a 30-point score.

Table 3: Variation in parameters after 6 months of monotherapy

<table>
<thead>
<tr>
<th>Measured variable</th>
<th>Change in variable (within group)</th>
<th>p-value</th>
<th>Change in variable (within group)</th>
<th>p-value</th>
<th>Change in variable (within group)</th>
<th>p-value</th>
<th>Change in variable (within group)</th>
<th>p-value</th>
<th>Change in variable (within group)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy (μM/L)</td>
<td>9.6 (1.3)</td>
<td>0.0001*</td>
<td>11.1 (1.1)</td>
<td>&lt;0.0001*</td>
<td>4.7 (0.6)</td>
<td>&lt;0.0001*</td>
<td>8.5 (0.5)</td>
<td>0.0001*</td>
<td>4.4 (0.7)</td>
<td>0.0006*</td>
</tr>
<tr>
<td>B12 (pg/mL)</td>
<td>-25.6 (8.8)</td>
<td>0.0084*</td>
<td>-285.8 (63.7)</td>
<td>&lt;0.0001*</td>
<td>-20.5 (14.4)</td>
<td>0.1621</td>
<td>-22.8 (11.4)</td>
<td>0.0261*</td>
<td>-8.7 (6.2)</td>
<td>0.1790</td>
</tr>
<tr>
<td>Folate (ng/mL)</td>
<td>-8.4 (0.7)</td>
<td>0.0001*</td>
<td>-8.1 (2.0)</td>
<td>&lt;0.0001*</td>
<td>-0.3 (0.4)</td>
<td>0.9318</td>
<td>-0.9 (0.4)</td>
<td>0.0205*</td>
<td>-6.6 (2.1)</td>
<td>0.0721</td>
</tr>
<tr>
<td>MMSE score</td>
<td>-1.0 (0.19)</td>
<td>0.0001*</td>
<td>-1.04 (0.20)</td>
<td>0.0009*</td>
<td>-0.29 (0.10)</td>
<td>0.0308*</td>
<td>-1.07 (0.28)</td>
<td>0.0011*</td>
<td>-0.1 (0.20)</td>
<td>0.5877</td>
</tr>
</tbody>
</table>

*Level of significance at p < 0.05; values are expressed as mean and standard error (SE)

Table 4: Relative risk and 95% CI for longitudinal cognitive decline according to presence or absence of HHcy

<table>
<thead>
<tr>
<th>Variable</th>
<th>PHT TThcy (μmol/L)</th>
<th>CBZ TThcy (μmol/L)</th>
<th>VPA TThcy (μmol/L)</th>
<th>OXC TThcy (μmol/L)</th>
<th>LEV TThcy (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% cases</td>
<td>&lt;15.1</td>
<td>&gt;15.1</td>
<td>&lt;15.1</td>
<td>&gt;15.1</td>
<td>&lt;15.1</td>
</tr>
<tr>
<td>% cases with cognitive decline^a</td>
<td>25</td>
<td>75</td>
<td>45.5</td>
<td>54.5</td>
<td>64.8</td>
</tr>
<tr>
<td>RR (95% CI)</td>
<td>4.667 (0.7663–28.42)</td>
<td>2.778 (1.042–7.403)</td>
<td>1.833 (0.4353–7.721)</td>
<td>3.316 (0.9483–11.59)</td>
<td>4.333 (0.9616–19.53)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0147^b</td>
<td>0.0274^b</td>
<td>0.641</td>
<td>0.0166^b</td>
<td>0.1357</td>
</tr>
</tbody>
</table>

^aCases with a drop of at least 1 point in MMSE score after 6 months of AED therapy
^bLevel of significance at p < 0.05
Vitamins involved as cofactors in remethylation of Hcy show a decrease in concentration as a result of anti-epileptic therapy, resulting in elevated levels of Hcy in serum, though there are studies that confirm that Hcy might affect cognition independently of vitamin B status. Hyperhomocysteinemia may increase risk of cerebral micro- and macroangiopathy through pathological changes in arterial walls and blood coagulation systems. A direct neuronal damage through activation of N-methyl-D-aspartate receptors or apoptosis triggered by deoxyribonucleic acid damage has also been related with HHcy. 

CONCLUSION

Our study is a prospective cohort design, which shows that AEDs, both conventional and newer, are capable of causing HHcy, which may lead to decline in cognitive abilities. A dip in folate and B12 levels is observed with drugs that induce the P450 enzymes. A positive correlation between Hcy levels and cognitive decline is significant in subjects on these enzyme inducers. Subjects on VPA and LEV are less likely to show a drop in cognitive ability, regardless of a rise in Hcy levels in such individuals.

REFERENCES


