Epilepsy, Hyperhomocysteinemia and Mutant Methylenetetrahydrofolate Reductase Gene

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ABSTRACT

Rationale and Background: Recent evidences mount that homocysteine level significantly increased in epileptic patients particularly those taking anticonvulsant drugs. Several previous studies revealed that a 677C→T transition in the methylenetetrahydrofolate reductase (MTHFR) gene is related to hyperhomocysteinemia and might increase risk of vascular occlusive pathology. However other publications negate this relationship. This study aimed to evaluate homocysteine level in epileptic patients in comparison to control group, and to investigate the possible relation between hyperhomocysteinemia and (MTHFR) gene and clinical data in these patients.

Patients and Methods: Twenty five epileptic patients [13 males (52%), 12 females (48%)], their mean age is 15.08±13.7 years divided into two groups according to medications Group 1: Newly diagnosed patients who had not received medications yet. Group 2: patients who received valproate. Fifteen healthy sex- and age-matched controls were also recruited. After thorough neurological evaluation and EEG study, plasma total homocysteine (tHcy) level was determined by specific immunoassays (IMX, Abbott Laboratories). MTHFR 677 C→T mutation using a polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis with HinfI digestion were investigated.

Results: Mean of homocysteine level was significantly higher in the epileptic patients than in the controls (10.23 ± 5.9 V 5.35 ± 1.64, P < 0.05). The prevalence of hyperhomocysteinemia (≥11.4 micromol/L) was significantly higher in the epileptic patients than in the controls [15 patients (60%) Vs one volunteer (6.6%) P< 0.05]. No significant relation was found between clinical data (Age, sex, age of onset, seizure type, seizure frequency, duration of illness, valproate medication) and homocysteine level. The MTHFR gene was normal in 14 (56%), hetero in 10 (40%), homo in one (4%) of the patients. Mean homocysteine level was statistically significantly higher in hetero mutant patients as compared to patients with normal gene (12.64±6.64 V 8.50±4.68 P value < 0.01).

Conclusion: Hyperhomocysteinemia is more common in the epileptic patients than in the control. The C677T mutation in MTHFR gene contributes to hyperhomocysteinemia. Measuring homocysteine in epileptic patients is recommended as early management of hyperhomocysteinemia help to avoid its devastating consequences. (Egypt J. Neurol. Psychiat. Neurosurg., 2006, 43(1): 495-505)

INTRODUCTION

Over the last years, there has been an explosion of interest in homocysteine (Hcy)1. The methyl donor for the conversion of homocysteine to methionine is provided by the reduction of 5, 10-methylene tetrahydrofolate to 5-methylene tetrahydrofolate by the enzyme 5, 10-methylenetetrahydrofolate reductase (MTHFR)2. A common mutation in methylenetetrahydrofolate reductase gene (MTHFR) is a missense mutation a cytosine to thymine substitution at nucleotide 677, which converts an alanine to a valine codon that results in a thermolabile variant with reduced activity and consequently hyperhomocysteinemia (HCA)3.

Hyperhomocysteinemia is believed to induce endothelial dysfunction and promote atherosclerosis through complex oxidative and excitatory neurotoxic molecular mechanisms4. Consequently hyperhomocysteinemia is becoming an important risk factor for atherosclerotic disease, venous thrombosis as well as stroke, carotid wall thickening and cardiovascular diseases, independent of long-recognized factors5. In addition homocysteine may play a role in...
neurodegenerative disorders. Furthermore, hyperhomocysteinemia have been consistently reported in patients with epilepsy on phenytoin (PHT), phenobarbital (PB), primidone (PRD) and carbamezapine while data on valproate (VPA) are conflicting.

The C677T mutation in MTHFR is thought to be related to hyperhomocysteinemia in epileptic patients taking anticonvulsants. Furthermore the polymorphism in the gene encoding 5, 10-methylenetetrahydrofolate reductase (MTHFR) influences not only the Hcy concentration but also the response to Hcy-lowering therapy.

These information forms the rationale for future routine screening of homocysteine level and MTHFR gene mutation and correction of such metabolic alterations in epileptic patients in order to prevent atherosclerosis and its consequences in epileptic patients.

This study aimed to evaluate homocysteine level in epileptic patients in comparison to control group, and to investigate the possible relation between hyperhomocysteinemia and (MTHFR) gene and clinical data in these patients.

**PATIENTS AND METHODS**

**Patients**

This is a cross-sectional case control study, investigated twenty five epileptic patients [13 males (52%), 12 females (48%)]; with a mean age 15.08±13.7 years and fifteen healthy age and sex matched controls. The patients were recruited from neurology outpatient clinic and department Cairo University Fayoum branch hospital.

**The inclusion criteria:**

Any age or sex was inclusive. Idiopathic epilepsy, newly diagnosed cases and those on valproate. Routine laboratory test (CBC, liver and kidney functions) within normal.

**The exclusion criteria:**

Symptomatic epilepsy, presence of metabolic diseases such as hypertension, diabetes, renal, liver, thyroid disease. Or neurological diseases related to Hyperhomocysteinemia as stroke, dementia or other risk factors of hyperhomocysteinemia as Smoking, pregnancy and lactation as this related to folate deficiency, regular vitamin supplementation in the previous 4 months. Patients on any antiepileptic other than valproate were also excluded.

The patients were divided into two groups according to medication:

**Group 1:** Newly diagnosed patients who did not received medications yet. They were 16 patients [7 males (43.8%), 9 females (56.2%)] with a mean age 18.31±14.75 years.

**Group 2:** patients received valproate. They were 9 patients [6 males (66.7%), 3 females (33.3%)], with a mean age 9.33±10.17 years.

The control group was recruited from healthy persons of patients’ relatives after detailed history taking, examination and lab investigation to rule out any risk factor or diseases that affect homocysteine (exclusion criteria as in patients group).

**Methods**

All patients and control group were subjected to:

**Clinical Evaluation:**

Detailed history taking: With special emphasis on factors known to be associated with hyperhomocysteinemia as hypertension, diabetes..., cardiological diseases or other neurological disease, smoking and vitamin supplementation.

**Epilepsy sheet of Cairo University hospital** (for the patients groups), including age of onset, description of fits, auras, type of seizure, presence of postictal confusion, Todd’s paralysis, post ictal headache, precipitating factors, frequency, duration of illness, timing of last seizure, history of status epilepticus, family history, history of head trauma.

**Through general and Neurological Examination**

The type of seizures were diagnosed in our patients according to Commission on Classification and Terminology of the International League Against Epilepsy.
Mini Mental State Examination (MMSE)\textsuperscript{14}:

MMSE grossly assess cognitive function namely; orientation, attention, calculation, registration, immediate and short-term memory, recall and language. Scoring $\leq 23$ was considered abnormal.

 EEG study: (for the patients groups)

EEG electrodes were placed according to 10-20 system recording with 14-channel Nihon Kohden equipment. EEG was carried out under standard conditions and with hyperventilation, photic stimulation as provoking factors. EEG data were analyzed for background activity, presence of epileptogenic activity. Localization was based on the site of phase reversal in bipolar montages.

 MRI brain: MRI brain with contrast was done for all patients on a 1.5 Tesla Phillips intra® scanner to exclude symptomatic causes of epilepsy.

Biochemical measurement

Overnight fasting (12 h) blood samples were drawn from all participants by venepuncture. Blood was collected into EDTA-containing tubes, kept on ice. Plasma was promptly separated by centrifugation at 4 °C, divided into aliquots, and stored at -20 °C until analysis. Plasma tHcy (protein-bound plus free oxidized and reduced species) were determined by specific immunoassays (IMX, Abbott Laboratories).

Genetic analysis

Genomic DNA was extracted from white blood cells using the Qiagen blood mini kit (Qiagen, Hilden, Germany). The DNA samples were analyzed for the C677T missense mutation by polymerase chain reaction with locus-specific primers and a subsequent analysis of a restriction fragment length polymorphism that was created by the mutation\textsuperscript{15}. The primers for PCR amplification of the region spanning the 677 locus were 677F (5'-TGAAGGAGAAGGTCTGCGGGA-3') and 677R (5'-AGGACCGGTGCGGTAGTGTG-3').

The PCR reactions were conducted in a 50 ul reaction mixture containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl\textsubscript{2}, 0.2 mM of each dNTP, 50 pmol 677F, 50 pmol 677R, 2.5 units DNA Taq Polymerase (MBI Fermentas, Hanover, USA), and 0.5-1.0 of genomic DNA. The 677 CT substitutions created a HinfI recognition sequence, which digested the initial polymerase chain recognition product of the 198 base-pair (bp) into 175 and 23 bp fragments. Presence of the mutation was determined by enzymatic digestion of the initial polymerase chain reaction product with HinfI (MBI Fermentas) at 37°C for 24 h. The digested DNAs were separated on 3% agarose gel in 1x Tris borate EDTA buffer, followed by staining by an ethidium bromide solution. The MTHFR C677T genotypes were typed by visualization under ultraviolet light\textsuperscript{15}.

Statistical analysis

Software package S.P.S.S 13 was used in statistics analysis. Means and standard deviation (SD) were calculated for quantitative data. Frequency & percent were calculated for qualitative variables. Student t test, modified Chi square and ANOVA tests were used for comparisons. $P<0.05$ was considered statistically significant.

RESULTS

This study included 25 epileptic patients their mean age is 15.08±13.7 years. They are [13 males (52%), 12 females (48%). In addition to 15 age and sex matched volunteers served as a control group.

The clinical presentations of the patients:

The clinical presentation of the patients was generalized epileptic attacks in 14 patients (56%); Generalized tonic clonic in 12 patients, myoclonic in 2 patients. Partial epilepsy was detected in 11 patients (44%); simple partial in 2 patients, complex partial in 3 patients, and partial with secondary generalization in 6 patients.

Frequency of epileptic attacks:

The frequency of epileptic attacks is presented in table (1).

The duration since last attack:

The duration since last attack was > month in 4 patients (16%). A month to > week in 9 patients (36%) and ≤ week in 12 patients (48%). History of status was found in one patient.
The patients were classified into 2 groups according to medications

**Group 1:** Newly diagnosed patients who did not received medications yet. They were 16 patients [7 males (43.8%), 9 females (56.2%)] with a mean age 18.31±14.75 years.

**Group 2:** patients received valproate. They were 9 patients [6 males (66.7%) and 3 females (33.3%)], with a mean age 9.33±10.17 years.

**Biochemical results:**

**Mean of homocysteine level in patients and control group:**

Mean of homocysteine level was significantly higher in patients groups (10.23±5.9) than in control group (5.35±1.6), P<0.05 as in represented figure (2).

The number of patients had hyperhomocysteinemia (≥11.4 micromol/L) was significantly higher in the patients groups than in the controls [15 patients (60%) Vs one of the control group (6.6%) P<0.05] (Fig. 3).

**Relation between mean of homocysteine level and patients clinical data:**

No significant relation was found between mean of homocysteine level and patients; age, gender, duration of illness, type and frequency of seizures, medications and EEG findings (Table 2).

**Genetic results:**

**MTHFR gene in patients and control group**

The MTHFR gene was normal in 14 (56%), heterozygous mutant in 10 (40%), homozygous mutant in one (4%) of the patients. In the control group MTHFR gene was normal in 13 (86.7%), heterozygous mutant in 2 (13.3%), none of the control group had homozygous mutant gene as shown in figure (4). No statistically significant difference is noticed in gene distribution between patients and control group.

Genotyping for the C677T polymorphism in the MTHFR gene is shown in figure (5).

**The homocysteine mean in comparisons to MTHFR gene**

Patient with homozygote T-T 677 MTHFR gene mutation had hyperhomocysteinemia. Heterozygote C-T mutation epileptic patients had significantly higher mean homocysteine level than patients with normal MTHFR gene as shown in table (3).

Heterozygote C-T mutation epileptic patients had significantly higher mean homocysteine level than the two heterozygote in the control group (12.6±6.64 V 7.9±2.54, P<0.05).

![Fig. (1): Types of epileptic seizures the patients.](image)

**Table 1.** Frequency of epileptic attacks.

<table>
<thead>
<tr>
<th>Frequency of epileptic attacks</th>
<th>Patients Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once in ≥ month</td>
<td>11 (44%)</td>
</tr>
<tr>
<td>From 1-4 in &lt; a month</td>
<td>6 (24%)</td>
</tr>
<tr>
<td>≥ once a week</td>
<td>8 (32%)</td>
</tr>
</tbody>
</table>
**Fig. (2):** Mean of homocysteine level in patients and control group.

**Fig. (3):** Homocysteine level in patients and control groups.
Table 2. Relation between mean of homocysteine level and patients data.

<table>
<thead>
<tr>
<th>Patients clinical data</th>
<th>Homocysteine</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child ≤ 14 years</td>
<td>8.66±4.9</td>
<td>NS</td>
</tr>
<tr>
<td>Adult &gt; 14 years</td>
<td>9.21±6.14</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8.82±5.2</td>
<td>NS</td>
</tr>
<tr>
<td>Female</td>
<td>11.41±6.37</td>
<td></td>
</tr>
<tr>
<td>Duration of illness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ One year</td>
<td>11.37±6.23</td>
<td>NS</td>
</tr>
<tr>
<td>&lt; One year</td>
<td>8.73±5.19</td>
<td></td>
</tr>
<tr>
<td>Seizure frequency</td>
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</tr>
<tr>
<td>Once in a month or more</td>
<td>8.34±3.81</td>
<td>NS</td>
</tr>
<tr>
<td>&gt; once in less than a month</td>
<td>10.96±6.1</td>
<td></td>
</tr>
<tr>
<td>Seizure Type</td>
<td></td>
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<tr>
<td>Generalized</td>
<td>11.24±6.7</td>
<td>NS</td>
</tr>
<tr>
<td>Partial</td>
<td>9.68±5.1</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
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<tr>
<td>No medication</td>
<td>9.81±6.24</td>
<td>NS</td>
</tr>
<tr>
<td>Medication</td>
<td>10.98±5.57</td>
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<tr>
<td>EEG</td>
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<tr>
<td>Normal</td>
<td>5.83±0.84</td>
<td>NS</td>
</tr>
<tr>
<td>Sub cortical</td>
<td>11.1±6.1</td>
<td></td>
</tr>
<tr>
<td>Focal</td>
<td>10.04±6.2</td>
<td></td>
</tr>
</tbody>
</table>

Fig. (4): MTHFR gene in patients and control group.
Discussions

Hyperhomocysteinemia is critically implicated in the vessel wall pathology. It is becoming not only an independent risk factor for cerebrovascular, cardiac diseases but also it is involved in cognitive dysfunction and neural tube defects in increasing the risk of resistance to anti-epileptics leading to development of refractory epilepsy. Hyperhomocysteinemia can result from decreased methylenetetrahydrofolate reductase (MTHFR) enzyme that catalyzes the reaction supplies 5-methyltetrahydrofolate, needed to remethylate homocysteine to methionine. Decreased (MTHFR) enzyme activity can result from MTHFR gene mutation as (C677T). Recent evidences mount that homocysteine level significantly increased in epileptic patients particularly those taking anticonvulsant drugs. Hyperhomocysteinemia have consistently been reported in patients with epilepsy on phenytoin, phenobarbital, primidone and carbamezapine while data on valproate are conflicting.

This study aimed to evaluate homocysteine level in epileptic patients in comparison to control group, and to investigate the possible relation between hyperhomocysteinemia and (MTHFR) gene and clinical data in these patients.

Several studies reported that homocysteine level significantly increased in epileptic patients. In this study in accordance to D’Angelo et al. and McQuillan et al., mean of homocysteine level was significantly higher in patients than in the control group (10.23±5.9 V 5.35±1.6, P<0.05) but in contrast to Tumer et al., who had not found significant differences in Hcy between the control group and the epileptic. This difference could be explained by: Tumer et al. made this comparison on the patients before the beginning of therapy and there is a postulated drug effect on homocysteine level.
In this study hyperhomocysteinemia was detected in 15 (60%) of epileptic patients. In a previous study hyperhomocysteinemia was present in 15.5% of children. This percentage difference could be attributed to the difference between patients' age, this study include adult patients whereas the other study all patients were children. Higher homocysteine level was reported more in adult than in children.

In this study in agreement with Castro et al., no correlation was found between patients' age and homocysteine level. But in contrast Sazci et al. reported greater homocysteine level in the older group (> 14 years) than in the younger group (up to 14 years). This may be attributed to by age there is accumulations of other conditions associated with hyperhomocysteinemia. The exact relation between homocysteine and age is one of areas that need further researches. Some studies relate resistance to antiepileptic and development of refractory epilepsy to hyperhomocysteinemia.

In this study in accordance to Sener et al., no relationship was found between seizure frequency or type and homocysteine levels in epileptic patients. The elevation of homocysteine levels may be due to antiepileptic drug use.

Several previous studies support the unfavorable effects of some anti-epileptic drugs on the plasma homocysteine concentrations. The effect of phenytoin, carbamezapine on homocysteine elevation was confirmed but the results about valproate effect are conflicting.

In this study no significant relation was found between mean of homocysteine level in patients without treatment and those on VPA medication. Gidal et al. reported that the administration of a mean daily dose of 2070 mg of VPA resulted in a 27% decline in plasma Hcy concentrations, although the mechanism of such change is unknown. Their data indicate that hyperhomocysteinemia may not be a serious clinical problem among patients with epilepsy, who receive VPA. Conversely Ono et al. showed that mean values of Hcy levels was higher in patients group on VPA than in the control group (P<0.05). In Tumer et al. study after 1 year of therapy, patients treated with VPA showed a significant increase of the plasma concentrations of Hcy when compared to baseline data and controls values.

Causes of changes observed in the patients using valproic acid remain unclear. Additionally, in this study the number of treated group was too small to give conclusive results.

In this study in accordance to Sener et al., no significant correlation was found between duration of medication and mean of homocysteine level. But Huemer et al. reported significant correlation between duration of therapy and homocysteine level. The Huemer patients received carbamezapine an enzyme inducer drug that decreases vitamin B6, B12, and folate. It is supposed that the longer the duration of medication the higher the decrease in these vitamins.

Though several previous studies revealed that C677T mutation in the MTHFR gene is related to hyperhomocysteinemia, the effect of MTHFR genotypes on homocysteine levels was not confirmed.

In this study the MTHFR gene was normal in 14 (56%), heterozygous mutant in 10 (40%), homozygous mutant in one (4%) of the patients. In the control group MTHFR gene was normal in 13 (86.7%), heterozygous mutant in 2 (13.3%), none had homozygous mutant gene. No statistically significant difference is noticed in gene distribution between the patients and control groups. These results were comparable to Caccamo et al. results who found that the frequency of CT677 polymorphic allele was similar between epileptics and controls.

In this study mean homocysteine level was significantly higher in patients with 677C--)T heterozygous mutant gene than those with normal gene. This was in agreement with Vilasca et al. who reported that 677C--)T mutation in MTHFR was independent determinants of hyperhomocysteinemia. Also Sazci et al. reported that the (MTHFR) gene polymorphisms C677T cause mild hyperhomocysteinemia, not only in homozygous mutant for C677T, but also in heterozygous mutant and Y00 et al. found that MTHFR TT genotype is an independent predictor of hyperhomocysteinein in epileptic patients receiving anticonvulsants suggesting that gene-drug interactions induce hyperhomocysteinemia.
In contrast Huemer et al.\textsuperscript{21} found that the polymorphisms in the methylene tetrahydrofolate reductase gene MTHFR 677 C→T had no significant impact on tHcy level. In other study patients receiving monotherapy showed no difference in occurrence of hyperhomocysteinemia between homozygotes for C677T and heterozygotes or patients with no mutant MTHFR. But among patients receiving multidev therapy, hyperhomocysteinemia in homozygotes for C677T occurred significantly more often than in heterozygotes or patients with no mutant enzyme. They conclude that C677T mutation is closely related to hyperhomocysteinemia in epileptic patients taking multiple anticonvulsants.\textsuperscript{18,32} 

There is still a dilemma whether hyperhomocysteinemia in epileptic patients is a drug related or gene determinant or most probably interaction of all.

**Conclusions:**

Hyperhomocysteinemia is common in Epileptic patients. The C677T mutation of MTHFR is a determinant of hyperhomocysteinemia. No significant relation between hyperhomocysteinemia and clinical data.

**Recommendations:**

Measurement and early management of hyperhomocysteinemia is recommended in epileptic patients, particularly those mutants for MTHFR is a determinant of hyperhomocysteinemia in epileptic patients receiving anticonvulsants. Further studies including large number of patients, looking for other gene mutations and assessing vitamin B12, folate level are needed to establish the relationship between epilepsy, homocysteine and MTHFR gene mutation.

**REFERENCES**

3. Apeland, T; Mansoor, M A; Strandjord, R E; Kristensen, Plasma homocysteine and serum folate in patients with epilepsy on carbamazepine or valproate monotherapy. Carbamazepine and valproate have different effects on folate and homocysteine metabolism. Acta Neurologica Scandinavica May 2000; Vol 101, Issue 5.


