

# Age-Related Homocysteine Serum Levels Elevate in Men with Epilepsy

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## Abstract

**Objectives:** The purpose of the present study was to determine the correlation of age with serum homocysteine (Hcy) levels in male non-obese epilepsy (Ep) patients and non-obese male control (C) subjects.

**Methodology:** This case-control study was conducted in non-obese male epilepsy patients (Ep; n: 44; age: 26-40 years) having predominant grand mal (generalized tonic-clonic) seizures and in non-obese male control subjects (C; n: 42; age: 26-40 years). The Ep and C subjects were further subdivided into age-based subgroups: 26-30, 31-35, 36-40 years. Serum Hcy was determined by enzyme-linked immunosorbent assay (ELISA). SPSS software version 24 was used for the purpose of data entry/ statistical analysis.

**Results:** The mean± SD values for serum Hcy (µmol/L) in non-obese male Ep (n:44) and non-obese control male C subjects (n:42) were 12.34±3.34 and 9.16±3.38 respectively that showed a highly significant increase in the level of Hcy (p< 0.001) in Ep compared to C subjects. Serum Hcy levels showed significantly increased serum levels of Hcy and positive linear correlation in all age groups (26-30, 31-35, 36-40, 26-40) in Ep subjects compared to control subjects. However, significantly elevated serum Hcy and lesser association (though significant) of Hcy with age in the Ep group as compared to C subjects were found in the present report.

**Conclusion:** The present study provides evidence of the significant impact of age on serum homocysteine levels in non-obese male epilepsy patients compared to non-obese male control subjects. However, it is essentially required to carry out well-controlled studies comprising wider age range data and a large number of samples in each age subgroup to have a better idea about the potential impact of Hcy in epilepsy and to investigate the comparative effects of antiepileptic drugs (AEDs) and dosages of AEDs on serum levels of Hcy and vice versa.

**Keywords:** Epilepsy, Serum Homocysteine, Age, Non-obese Men, Seizures, Antiepileptic Drugs.

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## Introduction

Epilepsies are a group of the third most common non-communicable chronic neurological disorders characterized by recurrent epileptic seizures<sup>1,2</sup> that affect approximately 500 million people worldwide or about 1% of the world population<sup>2</sup>. Epilepsy is more in developing countries and mainly requires proper long-term management with antiepileptic drugs (AEDs)/anticonvulsants<sup>2</sup>.

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Neuronal excitability is affected by a number of factors that may lead to epilepsy. Some of the major causes are a head injury, brain infections like meningitis or encephalitis, brain tumors, birth defects, stroke, and even altered levels of blood components like sodium or blood sugar. Homocysteine (Hcy), a non-proteinogenic α-amino acid that is produced during methionine metabolism, is one blood component that may elevate in concentration mainly due to the deficiency of folic acid and/or vitamin B-12 leading to various risks<sup>3-5</sup>.

Independent risk factors for elevated Hcy levels in patients with epilepsy were found to influence the Hcy homeostasis<sup>5</sup>. Furthermore, it has been documented that epilepsy and related disorders may occur as a result of gene polymorphism in 5,10-methylenetetrahydrofolate reductase (MTHFR), along with the deficiency of vitamin B-12 (methylcobalamin), folic acid, and vitamin B6 (pyridoxal phosphate)<sup>5</sup>.

Association between hyperhomocysteinemia and epilepsy was found, and it was revealed that an increase in Hcy level is essentially associated with low folate levels<sup>6,7</sup>. However, some of the electrophysiological investigations did not confirm the role of elevated Hcy levels in epilepsy<sup>8</sup>.

Several of our studies<sup>3,4,9,10</sup> convinced us to view the free Hcy for therapeutic considerations<sup>3</sup>. The free Hcy might be more harmful than the protein-bound Hcy<sup>3</sup>, though few studies have distinguished between the two forms of Hcy. Epilepsies showing a variety of alterations, including elevated levels of Hcy<sup>11</sup> and related disorders, are treated by long-term AEDs<sup>12</sup>. Some of those, e.g., phenytoin (PHT) and carbamazepine that are enzyme-inducing AEDs, may cause disorders with elevated blood levels of Hcy<sup>12</sup>. However, a case-control study in patients with epilepsy receiving PHT does not confirm considerable elevated Hcy levels<sup>13</sup>. Hyperhomocysteinemia is also demonstrated in patients with epilepsy treated with anticonvulsant drugs. But it is still not fully known how significant is this relationship between hyperhomocysteinemia and epilepsy or AEDs<sup>14</sup>.

Another AED levetiracetam does not cause considerable change in the levels of Hcy, though used with high dosages for long-term treatment<sup>11,12</sup>. It has further been found that sodium valproate, oxcarbazepine, and levetiracetam are all effective as the first-line choice for the treatment of epilepsy, though levetiracetam and oxcarbazepine showed a lower incidence of adverse effects and did not increase blood Hcy<sup>12</sup>.

No age-based change in Hcy levels could be found except after age 50 years<sup>15</sup>. However, other studies found an increase in Hcy levels with an increase in age<sup>16,17</sup>. Hence, further, well-controlled studies must be conducted to clarify the precise role of homocysteine with aging in control and epilepsy subjects.

Given controversial results in the literature, we planned to conduct the present study for elaborating the role of serum Hcy in healthy control and epilepsy subjects.

## Material and Methods

The study was conducted in the Physiology Department of Basic Medical Sciences Institute (BMSI) of Jinnah Post-graduate Medical Centre (JPMC) Karachi with the collaboration of the Neurology Department of JPMC, from May 03, 2019, to Aug 22, 2019. The Subjects under consultation in the present study were non-smokers and without diabetes, ischemic stroke, and cardiovascular or other diseases. The group of non-obese male epilepsy patients (Ep; n: 44; age: 26-40 years) with normal range body mass index (BMI) studied for the present report were those having predominant grand mal (generalized tonic-clonic) seizures, with seizure frequency in the range of 1-3 seizures per month, mild severity of seizure occurrence, and the duration of illness not more than two years. Furthermore, the subjects in the Ep group used lower dosages of AEDs. The second group of control subjects (C) selected from the normal healthy volunteers comprised age-matched adult non-obese male subjects (n: 42; age: 26-40 years) with normal range BMI.

The data of the subjects/ patients was recorded in a Questionnaire including age, body weight and height, body temperature, education level, habits, nutrition, and detailed history and previously used medication and other important information. However, the subjects were well informed about the present research study, and their willingness/ consent was taken. Only those subjects/ patients were taken in the study who were willing to participate. Blood serum was collected and stored for laboratory analysis. Determination of serum Hcy ( $\mu\text{mol/L}$ ) was carried out by enzyme-linked immunosorbent assay (ELISA) for studying the age-based Hcy changes in patients with epilepsy. Previously published report<sup>3</sup> was followed for laboratory methods, and the results were analyzed/ compared statistically employing general biostatistical concepts<sup>18</sup>. Inter-assay variations and intra-assay variations for the ELISA method were 11% and 9%, respectively.

The mean  $\pm$  SD, students t-test, and p-value were used by comparing two variables, and the strength of correlation was obtained. The SPSS software version 24 was used for the purpose of data entry/ statistical analysis.

Regression was used to find the cause-and-effect relation existing between two variables by applying the relevant equations. Analysis of the coefficient of determination ( $r^2$ ) was used to find the correlation between two variables.

## Results

The mean $\pm$  SD results for serum Hcy ( $\mu\text{mol/L}$ ) in male non-obese Ep group (n:44) and male non-obese C subjects group (n:42) were  $12.34\pm 3.34$  and  $9.16\pm 3.38$  respectively with their corresponding age (years; age range:26-40; mean $\pm$  SD for Ep vs. C:  $33.23\pm 4.54$

vs.  $33.36\pm 4.55$ ) respectively (Table 1). This showed a highly significant elevation of Hcy in Ep compared to C subjects, whereas the age of both groups did not differ significantly ( $p>0.05$ ).

Serum Hcy levels for individual age range subgroups are given in Table 1, which shows significantly increased serum levels of Hcy in all age groups (26-30, 31-35, 36-40, 26-40) in Ep subjects compared to control subjects. Age in all sub-groups of Ep and control subjects did not differ significantly ( $p>0.05$ ) (Table 1).

**Table 1:** Age-based comparison of serum Hcy in men with epilepsy and control subjects.

	Hcy serum levels ( $\mu\text{mol/L}$ )							
	26-30 years		31-35 years		36-40 years		26-40 years	
	Ep n:15	C n:14	Ep n:14	C n:13	Ep n:15	C n:15	Ep n:44	C n:42
<b>Comparison</b>	$10.45\pm$ 2.65	$6.98\pm$ 2.96	$12.67\pm$ 3.21	$9.60\pm$ 3.21	$13.92\pm$ 3.32	$10.81\pm$ 2.92	$12.34\pm$ 3.34	$9.16\pm$ 3.38
<b>*P-value</b>	0.0025		0.0202		0.0110		0.0001	

\*Values are mean $\pm$ SD, Hcy: homocysteine, Ep: epilepsy subjects, C: control subjects, n: number of subjects/ samples, #two-tailed p-value analyzed by unpaired t-test.

**Table 2:** Age-based comparison of serum Hcy in men with epilepsy and control subjects.

Subject groups	Correlation* of age (years) and serum Hcy ( $\mu\text{mol/L}$ ) in various age range groups			
	26-30	31-35	36-40	26-40
<b>Epilepsy (Ep)</b>	0.587* (n:15)	0.652* (n:14)	0.672* (n:15)	0.403** (n:44)
<b>Control (C)</b>	0.740** (n:14)	0.848** (n:13)	0.590* (n:15)	0.475** (n:42)

\*Coefficient of determination ( $r^2$ ), n: number of subjects/ samples, #:  $p<0.001$ , ##:  $p<0.0001$

Results for age of subjects against serum Hcy levels gave the values of coefficient of determination ( $r^2$ ). Both Ep and C subjects showed highly significant positive linear correlation (Table 2). It was found that C subjects have higher level of correlation ( $p<0.0001$ ) in most of the groups compared to Ep subjects. However, the results of whole data (age: 26-40 year) indicated quite similar level of correlation ( $p<0.0001$ ) in Ep and C subjects.

## Discussion

The results obtained in the present study for the elevated Hcy serum levels in non-obese men with epilepsy are similar to a report wherein a group of patients had seizures and increased serum levels of Hcy<sup>19</sup>. Another report also confirms the association between hyperhomocysteinemia and epilepsy, and it was suggested that an increase in Hcy level is

essentially associated with low folate levels<sup>6</sup>. It was revealed that seizures could not be controlled effectively if the Hcy serum level elevates too much<sup>20</sup>. In this perspective, our present study confirms the relationship of increased serum homocysteine in epilepsy patients suggested previously<sup>21</sup>.

Furthermore, the present study confirms whether the increased level of serum Hcy in patients with epilepsy has a link with the pathogenesis of seizures or has a link with the effects of AEDs, and provides evidence that an increase in serum Hcy and hence, decrease in serum folic acid may lead to seizure occurrence in patients with epilepsy<sup>22</sup>.

There are studies that do not confirm the association we obtained in the present report<sup>8,13</sup>, and some of the electrophysiological investigations also do not show an

increase in the prevalence of interictal epileptiform discharges in seizure-free and asymptomatic subjects with methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms related to epilepsy development, and no change in Hcy levels was obtained<sup>8</sup>.

However, there is evidence that shows serum Hcy in epilepsy patients significantly higher than those in the control subjects and that Hcy may be recommended as a biomarker for distinguishing the etiology of epilepsy and may serve in some specific way for clinical diagnosis and treatment<sup>7</sup>.

Significantly elevated serum Hcy and lesser association (though significant) of Hcy with age in the Ep group as compared to C subjects were found in the present report. The less association of Hcy with age in the Ep group might be the effect of antiepileptic drugs (AEDs), seizures, or both. However, it is still not clearly known whether Hcy levels elevate in response to epilepsy disorders, AEDs, a collective effect, and/ or other factors. Though it has further been found that sodium valproate, oxcarbazepine, and levetiracetam are all effective as the first-line choice for the treatment of epilepsy and that levetiracetam and oxcarbazepine show lower incidence of adverse effects and do not increase blood Hcy<sup>12</sup>, it is still confusing to interpret the alterations in Hcy in patients with epilepsy<sup>14</sup>.

The present report is quite interesting since hyperhomocysteinemia has a multifactorial origin involving genetic, pharmacological, nutritional, and pathological factors<sup>3,23</sup>. Effects of MTHFR C677T polymorphisms upon Hcy and folate levels with folic acid intervention and evaluate approaches for overcoming folic acid deficiency, and related symptoms were investigated<sup>22</sup> that help to understand the role Hcy in epilepsy.

Furthermore, in view of the differences in ethnic, dietary, and genetic factors, the data from the West might not be suitable to be applied to the Asian population, and hence, it is not suitable to introduce a definite cut off value for Hcy serum levels as significant without incorporating the larger population analysis with wider age range values<sup>3</sup>. The genetic components

of folate and homocysteine variability are providing the way towards understanding optimal folate status in every individual and, consequently, to decrease their risk of developing epilepsy and other diseases<sup>24</sup>.

There are studies not providing evidence for the increase in serum Hcy that we obtained in our present report. It was found that Hcy levels are higher in males than in females in each age-range of 20 to 30, 30 to 40, 40 to 50, 50 to 60, 60 to 80, and over 80 years, but are not related to age except the occurrence of Hcy increase after age 50 years<sup>14</sup>. Further subgroup analyses suggested that no significant differences were present when grouped by ethnicity and age.

However, other studies confirm our findings in the present report, as it was revealed that the serum levels of Hcy increase by age, and this increasing trend in Hcy levels continues with advancing age<sup>16</sup>. A previous study also documented that the levels of Hcy increase with age throughout the whole adulthood<sup>15</sup>.

In view of the mentioned reports, it is essentially required to carry out well-controlled studies comprising wider age range data and a large number of samples in each age subgroup to have a better idea about the potential impact of Hcy in epilepsy. It is also needed further to investigate the comparative effects of AEDs and dosages of AEDs on serum levels of Hcy and vice versa.

## Conclusion

The present study provides evidence of the significant impact of age on serum homocysteine levels in non-obese control subjects and non-obese epilepsy patients. However, since the role of Hcy in male non-obese patients with epilepsy has scarcely been studied, further studies will clarify the precise involvement of Hcy in non-obese patients with epilepsy.

## Conflict of Interest

Authors have no conflict of interest and no grant/funding from any organization.

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