INT. J. BIOL. BIOTECH., 18 (3): 441-445, 2021.

HOMOCYSTEINE SERUM LEVELS IN ADULT NON-OBESE WOMEN WITH EPILEPSY

Ahmed Almalki[1], Kausar A. Saldera[2], M. H. Hussain[3], Gisela H. Maia[4], Abdulhalim S. Serafi[5] and Zahir Hussain[5, *]

[1]*King Faisal Hospital, Makkah, Ministry of Health, Saudi Arabia*
[2]*Department of Physiology, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, Pakistan*
[3]*Biomedical, Computational and Theoretical Research (BCTR) Lab, Karachi, Pakistan*
[4]*EEG Department, Medibrain-Center for Neurophysiology Studies, Neurofeedback Therapy & Brain Research Institute, Porto, Portugal.*
[5]*Department of Physiology, Faculty of Medicine, Umm Al-Qura University (UQU), Makkah, Saudi Arabia*
***Corresponding Author's email:** zhakbar@uqu.edu.sa

ABSTRACT

Epilepsies are common chronic neurological disorders that affect approximately 500 million people world-wide, and are mainly characterized by recurrent seizures. There are a variety of factors that may influence the neural excitability and cause epilepsies. One of those factors is the increased serum homocysteine (Hcy). Hence, we planned to determine serum Hcy using enzyme linked immunosorbent assay (ELISA) in well age control matched non-obese adult epilepsy women (Ep; n: 41; age: 26-40 years) with tonic-clonic seizures so that we may predict the influence of seizure disorders on serum levels of Hcy. The Hcy serum levels (mean± SD) for Ep group (11.99 ± 2.97 μmol/L) were highly significant ($p < 0.0001$) as compared to those for control group (C; n: 39; age: 26-40 years; Hcy: 9.01 ± 3.09 μmol/L). Plot of age vs. serum Hcy in C group showed a positive linear relationship that indicated a highly significant correlation ($R = 0.5101$, $p < 0.001$). The plot of age vs. serum Hcy in Ep group showed a positive linear relationship that indicated a significant correlation ($r = 0.3146$, $p < 0.05$), though, this correlation was not so high significant in Ep group compared to one obtained for the C group subjects. Hence, significantly high serum Hcy levels and less association of Hcy with age in Ep group was investigated. The less association of Hcy with age in Ep group might be the effect of aging, antiepileptic drugs (AEDs), or seizures. The results in the present investigation provide newer and potential information for exploring further insights since hyperhomocysteinemia condition has a multifactorial origin.

Key-Words: Serum homocysteine, epilepsy, age, adult non-obese women with epilepsy, seizures

INTRODUCTION

Epilepsies are the common chronic neurological disorders that affect approximately 500 million people world-wide, and are mainly characterized by recurrent seizures (Megiddo *et al*., 2016). Epilepsy requires long-term management with anticonvulsants/ antiepileptic drugs (AEDs), and is prevalent more in developing countries (WHO, 2018).

There are a variety of factors that may influence the neural excitability and cause epilepsies. One of those factors is the increased level of serum homocysteine (Hcy) mainly due to the deficiency of folic acid and/or vitamin B-12 exposing to various risks (Tamura *et al*., 2000; Karabiber *et al*., 2003; Fruchart *et al.*, 2004; Pezzini *et al.,* 2007; Naz *et al.*, 2009; Holmes *et al*., 2011; Linnebank *et al*., 2011; Attia *et al.*, 2019). The Hcy appears as a toxin, and promotes a variety of disorders. It is an amino acid that is produced by the body, usually as a by-product of consuming the meat. It is originally a thiol amino acid synthesized during methionine metabolism.

It was investigated that gene polymorphism in 5,10-methylenetetrahydrofolate reductase (MTHFR) and deficiency of vitamin B-12 (methylcobalamin), folic acid and vitamin B6 (pyridoxal phosphate) often cause epilepsy and other associated disordered conditions (Pezzini *et al.*, 2007; Linnebank *et al.*, 2011; Holmes *et al.*, 2011). Vitamin B-12 and folic acid are the cofactors involved in the conversion of homocysteine to methionine. (Verrotti *et al*., 2000; Hoffer, 2004). In this regard, we carried out several studies in epilepsy and other neurological disorders (Hussain, 1991; Mahmood *et al.*, 1998; Fatima *et al.*, 2007; Hussain *et al.*, 2007; Khan and Hussain, 2008; Khan *et al.*, 2009; Naz *et al*., 2009; Hussain, 2010; Hussain and Zahir, 2012; Attia *et al*., 2019). It was revealed that the main focus eventually seems free Hcy for therapeutic considerations (Naz *et al.*, 2009). There is a possibility that free Hcy is more harmful than the protein-bound Hcy (Chambers *et al*., 2000), though few studies on Hcy and disease have distinguished between the two at present.

Long-term AEDs are used for the treatment of epilepsies (Tolou-Ghamari *et al*., 2013). Those patients who take long-term AEDs, may show a variety of changes including the increased levels of Hcy (Belcastro and Striano, 2012; Chuang *et al.,* 2012; Fontes *et al.,* 2013; Mintzer *et al.,* 2016; Mahdavi *et al.,* 2019), and related complications (Homocysteine Studies C, 2002; Chuang *et al.,* 2012; Furness *et al.,* 2013). Enzyme-inducing AEDs e.g., carbamazepine and phenytoin (PHT) may cause complications in patients with epilepsy via increasing the serum Hcy (Linnebank *et al.,* 2011; Chuang *et al.,* 2012). The PHT has been shown to reduce the serum folic acid and vitamin B-12 that suggests the occurrence of hyperhomocysteinaemia under long-term use of PHT (Mintzer *et al.,* 2009; Linnebank *et al*., 2011).

However, there are other AEDs including levetiracetam that do not show any considerable effect on Hcy serum levels, although levetiracetam is used with high dosages for long-term (Mahdavi *et al.,* 2019). Considerable increased levels of Hcy in patients receiving PHT were not obtained in a case-control study (Sener *et al.,* 2006).

In view of the prevailing situation, we planned to study adult non-obese epilepsy subjects with predominant tonic-clonic seizures and lower seizure severity, and having 1-3 per month seizure frequency with not more than two years of the duration of illness, and who take low dosages of AEDs due to less seizure severity/ frequency, so that we may predict the influence of seizure disorders on the serum levels of Hcy.

MATERIALS AND METHODS

Age-matched adult female controls (C; n: 39; age: 26-40 years) with normal body mass index (BMI) were selected from the healthy volunteer subjects. Non-obese female epilepsy patients (Ep; n: 41; age: 26-40 years) studied for the present report were those having predominant grand mal (generalized tonic-clonic) seizures, mild severity of seizure occurrence, and the duration of illness not more than two years. Furthermore, the subjects in Ep group used lower dosages of antiepileptic drugs (AEDs). Both C and Ep group subjects were not having premenstrual syndrome (PMS). It was confirmed that samples drawn were from ovulatory cycles. Samples collected from anovulatory cycles were not included in the study. The frequency of seizure occurrence was in the range of 1-3 seizures per month. The blood was drawn after taking consent of the subjects and serum was collected and stored for analysis.

Serum Hcy was determined by enzyme linked immunosorbent assay (ELISA) for studying the age-related Hcy variations in patients with epilepsy following the methods in our previously published work (Mahmood *et al.,* 1998; Naz *et al.,* 2009), whereas the results were analysed/ compared statistically obeying general statistical principles (Zahir *et al*., 2014). Inter-assay variations and intra-assay variations for ELISA method were 12% and 10%, respectively.

MS Excel was used for data entry. Descriptive values were expressed as mean ± SD. Comparison of Ep and C groups was carried out employing the students *t*' test. The spreadsheets (written for Excel; workable easily with Calc program) were used to analyse the data.

RESULTS

The results for age (mean± SD) of Ep (33.10 ± 4.54; n: 41) and C subjects (33.23 ± 4.56 years; n: 39) showed non-significant difference ($p > 0.05$) since the groups were age-matched. Hcy serum levels (mean ± SD) for Ep group (11.99 ±2.97 μmol/L; n: 41) were highly significant (t: 4.34; p: 0.0001) as compared to those for C group (9.01 ±3.09 μmol/L).

Relationship of age and serum homocysteine levels in control subjects:

Plot of age vs. serum Hcy (Fig.1) showed a positive linear relationship (slope: 0.35; df: 37) that indicated a highly significant correlation ($R = 0.5101$, $p < 0.001$).

Relationship of age and serum homocysteine levels in epilepsy subjects:

The plot of age vs. serum Hcy (Fig.2) showed a positive linear relationship (slope: 0.21; df: 39) that indicated a significant correlation ($r = 0.3146$, $p < 0.05$). However, this correlation was not so high significant as the one obtained for the C group subjects. The other difference was that the individual values of serum Hcy in Ep group are quite high in level and express a specific dispersion pattern as compared to low level values of Hcy in C group.

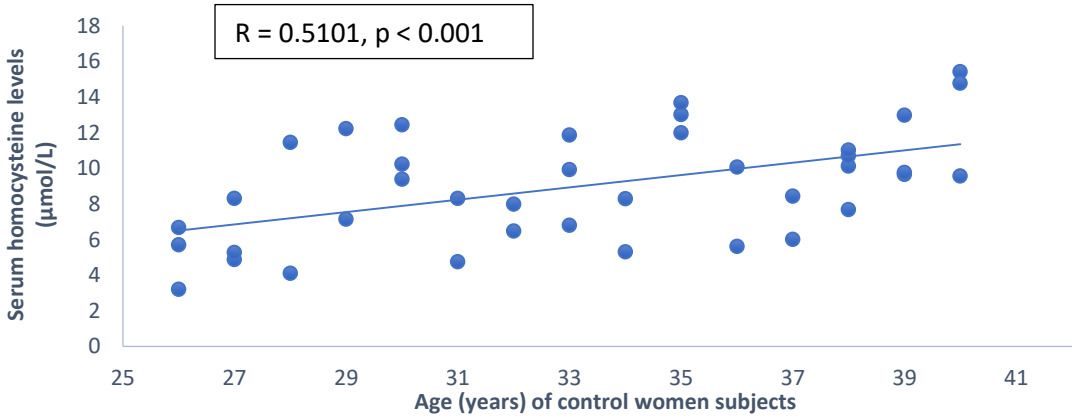

Fig.1. Relationship of age and serum homocysteine levels in control subjects.

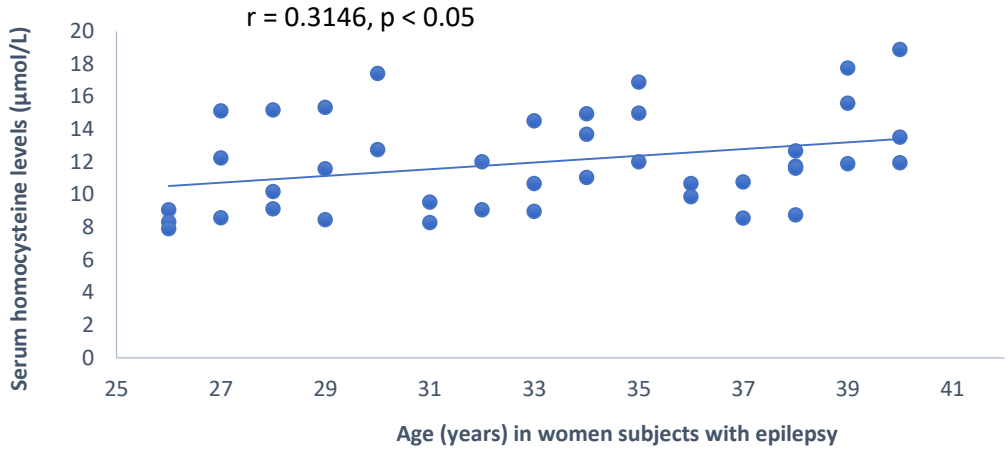

Fig.2. Relationship of age and serum homocysteine levels in epilepsy subjects.

DISCUSSION

Increased levels of Hcy in women patients with epilepsy investigated in the present study is in accordance with the previous studies that provide evidence that 20% of patients with homocystinuria had seizures and increased serum levels of Hcy (Schwarz and Zhou, 1991), and it was found that effective control of seizure cannot be attained if Hcy serum level increases too much (Ono *et al.*, 2002). Our present study further confirms the relationship of increased serum levels of homocysteine- a potent agonist for NMDA (*N*-methyl-D-aspartate) receptor with epilepsy (Lipton *et al.*, 1997).

An indirect interpretation of our current report is that whether the increased level of serum Hcy in patients with epilepsy has a link with the pathogenesis of brain atrophy leading to seizures, or has a link with the effects of AEDs, it is known that increase in serum Hcy and decrease in serum folic acid associated with the AEDs treatment lead to seizure occurrence and brain damage in patients with epilepsy (Gorgone *et al.*, 2009).

Significantly high serum Hcy levels and lesser association (though significant) of Hcy with age in Ep group was investigated in the present report. The less association of Hcy with age in Ep group might be the effect of aging, antiepileptic drugs (AEDs), or seizures. Results in the present investigation, hence, provide newer and potential information for exploring further insights since hyperhomocysteinemia condition has a multifactorial origin

incorporating genetic, pharmacological, nutritional, and pathological factors (Naz *et al*., 2009). 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 Asian population, and hence, it is not suitable to introduce a definite cut off value for Hcy serum levels as significant without incorporating widely the larger population analysis (Naz *et al*., 2009).

It is recommended that the proper management of patients with epilepsy indeed requires new relevant drug products that may additionally minimize the risk of increased serum Hcy. However, proper amount of folic acid and vitamin-12 as supplements for the patients with epilepsy is essentially required for maintaining the physiological level of Hcy.

REFERENCES

Attia, S.M.A., Y.H. Bahakeem, N.I. Khan, Z. Hussain, M.H. Kalakatawi, H. Faidah, L. Naz, G. Yasmeen, S. Mushtaq, A. Alkhotani, H. Zahir, A.A.S. Allahaibi, T.H.A. Alhassani, M.H.A. Al-Harbi, M.A. Bafail and A.S. Serafi (2019). LDL-cholesterol serum variations relate to serum homocysteine in female patients with ischemic stroke. *Int. J. Biol. Biotech.*, 16 (2): 341-344.

Belcastro, V. and P. Striano (2012). Antiepileptic drugs, hyperhomocysteinemia and B-vitamins supplementation in patients with epilepsy. *Epilepsy Res*, *102*:1–7.

Chambers, J.C., P.M. Ueland, O.A. Obeid, J. Wrigley, H. Refsum and J.S. Kooner (2000). Improved vascular endothelial function after oral B vitamins: An effect mediated through reduced concentrations of free plasma homocysteine. *Circulation*, 102(20): 2479-83.

Chuang, Y.C., H.Y. Chuang, T.K. Lin, C.C. Chang, C.H. Lu, W.N. Chang *et al*. (2012). Effects of long-term antiepileptic drug monotherapy on vascular risk factors and atherosclerosis. *Epilepsia,* 53(1): 120-8.

Fatima, S., N.I. Khan, G. Yasmeen, B. Hajir and Z. Hussain (2007). Antiatherogenic effects of Nigella Sativa (Kalonji) in rabbits with experimentally induced hypercholesterolemia. *Int. J .Biol. Biotech.* 4(4): 437-441.

Fontes, L.P., M.P. Fontes, P.Q. Jiménez, J.M. Pérez and M.M. Iriarte (2017). Comparative case-control study of homocysteine,vitamin B12,and folic acid levels in patients with epilepsy. *Neurología (English Edition),* 32: 440–5.

Fruchart, J.C., M.C. Nierman, E.S. Stroes, J.I. Kastelein and P. Duriez (2004). New risk factors for atherosclerosis and patient risk assessment. *Circulation,* 109 (23 Suppl 1): III15-9.

Furness, D., M. Fenech, G. Dekker, T.Y. Khong, C. Roberts and W. Hague (2013). Folate, vitamin B12, vitamin B6 and homocysteine: impact on pregnancy outcome. Matern Child *Nutr,* 9(2):155-66.

Gorgone, G., D. Caccamo, L.R. Pisani, M. Currò, G. Parisi and R. Oteri, *et al*. (2009). Hyperhomocysteinemia in patients with epilepsy: does it play a role in the pathogenesis of brain atrophy? A preliminary report. *Epilepsia.* 50 Suppl 1:33-6.

Hoffer, L J. (2004). Homocysteine remethylation and trans-sulfuration. *Metabolism,* 53: 1480–3.

Holmes, M.V., P. Newcombe, J.A. Hubacek, R. Sofat, S.L. Ricketts and J. Cooper *et al*. (2011). Effect modification by population dietary folate on the association between MTHFR genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. *Lancet,* 378(9791): 584-94.

Homocysteine Studies C (2002). Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA*, 288: 2015–22.

Hussain, Z. (2010). Menstrual Cycle and Epilepsy: Premenstrual Reproductive Study. 1st Edn. Germany (Printed in USA & UK), P.239.

Hussain, Z. (1991). *Clinical, electroencephalographic and hormonal study in menstruation related seizures*. PhD Thesis, Department of Physiology, University of Karachi. Pages: 236.

Hussain, Z. and H. Zahir (2012). Sodium channels, neurosteroid receptors and physiochemical and ischemic changes in a kindling model of epilepsy in rat. *Annual Symposium JPMC,* Karachi.

Hussain, Z., K.Z. Hasan, H. Aziz and M.A. Qureshi (2007). Clinical and neurological study of women with precatamenial epilepsy. *J. Coll. Physicians Surg. Pak.,* 17 (4): 211-214.

Karabiber, H., E. Sonmezgoz, E. Ozerol, C. Yakinci, B. Otlu and S. Yologlu (2003). Effects of valproate and carbamazepine on serum levels of homocysteine, vitamin B12, and folic acid. *Brain Dev*, 25: 113–5.

Khan, N.I. and Z. Hussain (2008). Pathophysiology of ischemic disorders: 1- LDL cholesterol and Ischemic stroke. *Int. J. Biol. Biotech.,* 5 (1-2): 1-16.

Khan, N.I., L. Naz, S. Mushtaq, L. Rukh, S. Ali and Z. Hussain (2009). Ischemic stroke: Prevalence of modifiable risk factors in male and female patients in Pakistan. *Pak J Pharmaceut Sci*., 22 (1): 62-67.

Linnebank, M., S. Moskau, A. Semmler, G. Widman, B. Stoffel-Wagner, M. Weller and C.E. Elger (2011). Antiepileptic drugs interact with folate and vitamin B12 serum levels. *Ann Neuro,* 69 (2):352-9.

Lipton, S.A., W.K. Kim, Y.B. Choi, S. Kumar, D.M. D'Emilia, P.V. Rayudu, D.R. Arnelle and J.S. Stamler (1997). Neurotoxicity associated with dual actions of homocysteine at the N-methyl-D-aspartate receptor. *Proc Natl Acad Sci U S A*, 94(11):5923-8.

Mahmood, A., S. Anjum and Z. Hussain (1998). C-reactive protein- inflammatory marker in atherosclerotic conditions in ischemic heart disease and ischemic stroke. *The Med Intern*, 1 (7): 13-15.

Mahdavi, A., A.A. Naeini, M. Najafi, M. Ghazvini and M. Maracy (2019). Vitamin B12 and Folate Status in Patients with Epilepsy under Levetiracetam Monotherapy. *Int J Prev Med*, 10:32.

Megiddo, I., A. Colson, D. Chisholm, T. Dua, A. Nandi and R. Laxminarayan (2016). Health and economic benefits of public financing of epilepsy treatment in India: An agent-based simulation model. *Epilepsia.* 57:46–74.

Mintzer, S, C.T. Skidmore, C.J. Abidin, M.C. Morales, I. Chervoneva, D.M. Capuzzi and M.R. Sperling (2009). Effects of antiepileptic drugs on lipids, homocysteine, and C-reactive protein. *Ann Neurol*, 65(4):448-56.

Mintzer, S., R. Miller, K. Shah, I. Chervoneva, M. Nei, C. Skidmore and M.R. Sperling (2016). Long-term effect of antiepileptic drug switch on serum lipids and C-reactive protein. *Epilepsy Behav.* 58:127-32.

Naz, L., Z. Hussain and T. Husain (2009). Risk factors and biochemical variations in patients with ischemic stroke. *Int. J. Biol. Biotech*., 6 (1-2): 83-87.

Ono, H., A. Sakamoto, N. Mizoguchi and N. Sakura (2002). The C677T mutation in the methylenetetrahydrofolate reductase gene contributes to hyperhomocysteinemia in patients taking anticonvulsants.*Brain Dev.*, 24 (4):223-6.

Pezzini, A., E. Del Zotto and A. Padovani (2007). Homocysteine and cerebral ischemia: pathogenic and therapeutical implications. *Curr Med Chem.*, 14(3):249-63.

Schwarz, S. and G.Z. Zhou (1991). N-methyl-D-aspartate receptors and CNS symptoms of homocystinuria. *Lancet*, 337: 1226–7.

Sener, U., Y. Zorlu, O. Karaguzel, O. Ozdamar, I. Coker and M. Topbas (2006). Effects of common anti-epileptic drug monotherapy on serum levels of homocysteine, vitamin B12, folic acid and vitamin B6. *Seizure.*15 (2): 79-85.

Tamura, T, K. Aiso, K.E. Johnston, L. Black and E. Faught (2000). Homocysteine, folate, vitamin B-12 and vitamin B-6 in patients receiving antiepileptic drug monotherapy. *Epilepsy Res*, 40: 7–15.

Tolou-Ghamari, Z., M. Zare, J.M. Habibabadi and M-R. Najafi (2013). Antiepileptic drugs: A consideration of clinical and biochemical outcome in patients with epilepsy. *Int J Prev Med.*, 4 (Suppl. 2): S330–7.

Verrotti, A., R. Pascarella, D. Trotta, T. Giuva, G. Morgese and F. Chiarelli (2000). Hyperhomocysteinemia in children treated with sodium valproate and carbamazepine. *Epilepsy Res.*, 41(3):253-7.

WHO (2018). WHO information kit on epilepsy: what you can do?
https://www.who.int/mental_health/neurology/epilepsy/epilepsy_global_toolkit.pdf?ua=1
Accessed September, 2018.

Zahir, H., A. Javaid, R. Rehman and Z. Hussain (2014). Statistical concepts in biology and health sciences. *J Ayub Med Coll Abbottabad*., 26(1): 95-7.

(*Accepted for publication July 2021*)