

Association of Serum Homocysteine and hs-CRP with Idiopathic Generalised Epilepsy and Duration of Antiepileptic Drug Therapy

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ABSTRACT

Introduction: Several human and experimental studies have revealed that chronic inflammation may play a vital role in neurodegenerative processes including epilepsy. There is accumulating evidence that inflammatory processes affect the pathophysiology of different epilepsy types.

Aim: To assess the concentrations of Homocysteine (Hcy) and High Sensitivity C-Reactive Protein (hs-CRP) in Idiopathic Generalised Epilepsy (IGE) patients and their association with IGE and duration of the Anti Epileptic Drugs (AEDs).

Materials and Methods: This case-control study consisted of 100 IGE patients (50 tonic-clonic, 15 absence and 35 myoclonic seizures) and equal number of healthy controls. Hcy levels were assayed by Centaur XP using ADVIA centaur Hcy; whereas hs-CRP levels by ELISA method using commercially available kits.

Results: The Hcy and hs-CRP levels were significantly increased in both the patient groups (<18 years and >18 years). Significant

difference in the levels of Hcy was observed between different epilepsy types of <18 years patients ($p=0.01$), whereas hs-CRP in >18 years patients (<0.05). Significantly elevated levels of hs-CRP were noticed in non-responders group compared to responders (<0.05). There was a positive correlation between hs-CRP and Hcy ($R^2=0.44$ and $p<0.001$) and significant difference in the levels of Hcy and hs-CRP was observed in the patient subgroups who were on AEDs for different time periods (≤ 1 year, $1- \leq 5$ years and >5 years) ($p=0.002$ and $p<0.05$ respectively) since, AEDs can induce oxidative stress.

Conclusion: Hyperhomocysteinaemia (Hyper-Hcy) can induce as well as promote oxidative stress and hence, it can be implicated in neurodegeneration in epilepsy. Elevated levels of hs-CRP in non-responders may be resulted by the contribution of inflammatory pathways in ictogenesis in epileptic tissue, causing intractable epilepsy.

Keywords: Anti epileptic drugs, Hyperhomocysteinaemia, Neurodegeneration, Oxidative stress

INTRODUCTION

Homocysteine is a thiol containing non essential amino acid formed from methionine by demethylation and is an intermediate product in One Carbon Metabolism (OCM). Deficiency of vitamin B12 and folic acid may cause serum Hcy concentration to rise. The role of folate in neurodevelopment during early embryonic life has been well documented. Hcy delays the closure of the neural tube in chick embryo by inhibiting the transmethylation pathway [1]. It has been reported that patients with epilepsy experience Hyperhomocysteinaemia (hyper-Hcy) more frequently than general population mainly ascribable to the reduced activity of 5, 10-Methylene Tetrahydrofolate-Reductase (MTHFR) enzyme, deficiency of folate and vitamin B12 [2]. In addition, previous studies have demonstrated that hyper-Hcy is also induced by AEDs [3,4].

Out of four forms of Hcy that exist in plasma, about 1% exists as free thiol, 70-80% of Hcy is bound to plasma proteins and remaining 20-30% combines with itself or with other thiol including cysteine to form Hcy cysteine mixed disulfide [5]. The nutritional deficiency of either B12/folate or B6 is associated with increased levels of Hcy as the metabolism of Hcy requires them in transsulfuration or remethylation pathway [6].

The association of Hcy with various neurological disorders such as epilepsy, Parkinson's disease, Alzheimer's disease and multiple sclerosis has been established by several epidemiological and clinical studies as well [7]. However, the relation between Hcy and epilepsy remains controversial despite a growing evidence of the pro-convulsive effects of the increased Hcy level observed in animal studies [8]. Hcy and homocysteic acid brings about neuronal death by stimulating N-Methyl-D-Aspartate (NMDA)

receptors and hence Hcy may reduce seizure threshold in epileptic patients [9].

Evidences show that Hcy can be produced in brain and transported from plasma into the brain and vice versa via bi-directional cellular transporters [10].

There is not much information available on the influence of AEDs on Hcy levels in epileptic patients. However, AED's like carbamazepine, phenytoin and phenobarbital have been observed to cause folate, vitamin B6 and B12 deficiency [11]. A previous study has also suggested that enzyme inducers can directly modulate the activity of different liver enzymes which in turn may result in depletion of cofactors involved, leading to alteration in Hcy levels [12].

Brain inflammation occurs during epileptogenesis and contributes in determination of the seizure threshold in susceptible brain regions. Thus, it plays a role in seizure initiation and its recurrence [13]. In addition to inflammation, some of the pathways are up regulated immediately after epileptogenic injury and persists during latent phase which precedes spontaneous recurrent seizure. The brain inflammation has gained significance as a crucial contributor in the aetiopathogenesis of various Central Nervous System (CNS) disorders, including epilepsy as the auto inflammatory and autoimmune diseases are the pathological outcome of tissue inflammation [14]. CRP has emerged as an inflammatory marker and its role has been studied in non inflammatory neurological diseases; however, very few studies have been reported in epileptic seizures [15]. Hence, the present study was taken with an aim to find out the association of Hcy and hs-CRP with IGE. In addition, the levels have also been evaluated in association with the duration of AED therapy.

MATERIALS AND METHODS

In this case control study, 100 diagnosed epileptic patients of IGE (as consulted by the statistician), (male:female=65:35) particularly idiopathic generalised tonic clonic seizures were recruited from Nizam's Institute of Medical Sciences, Hyderabad from October 2012 to December 2014 and similarly, equal number of age and sex matched healthy controls were recruited from Institute of Genetics and Hospital for Genetic Diseases, Hyderabad with their written informed consent. Patients with trauma, major renal, hepatic, cardiac disease, cerebrovascular disease/ infection, hypoxic-ischaemic encephalopathy, congenital CNS abnormalities, metabolic disorders, CNS malignant tumors and other degenerative disorders were excluded from the study. The study was approved by the Ethics Committee of the 'Institute of Genetics and Hospital for Genetic Diseases', as well as from the study hospital. The clinical diagnosis was made according to the admitted criteria by International League Against Epilepsy (ILAE) by an expert Epileptologist from Nizam's Institute of Medical Sciences, Hyderabad. In addition, patients had normal intelligence (as evaluated by a psychologist) and the MRI brain was normal. The information on demographic features such as socioeconomic status, nutritional status and geographic area and seizure frequency, type of epilepsy as well as duration was collected in a specially designed proforma. The medical history such as drugs and duration of the therapy was also obtained about each patient. As IGE has an age related onset, we preferred 18 years as cut off age to differentiate "classical" and adult onset IGE on the basis of previous reports [16-18]. Further, the patients were divided into three subgroups viz., ≤ 1 year, 1- ≤ 5 years and >5 years based on the duration of the AED treatment. Patients were classified into responders and nonresponders on the basis of their response to AEDs. Nonresponders included the patients who had one or more seizures in the previous six months despite being treated with at least two AED such as phenytoin, carbamazepine, sodium valproate, lamotrigine etc.

A 3 mL of venous blood was collected from all subjects, in case of patients within 24 hours from the seizure attack, in sterile silicone tubes without addition of anticoagulants and centrifuged at 2,500 rpm for 20 minutes. The serum was collected in sterile tubes and was stored at -80°C until the levels of serum Hcy and hs-CRP were analysed. Serum Hcy levels were estimated by Centaur XP using ADVIA centaur Hcy and assay. Serum hs-CRP levels were assayed by ELISA method using commercially available kits procured from Diagnostics Biochem, Canada.

STATISTICAL ANALYSIS

The difference in the levels of Hcy and hs-CRP between patients and controls was evaluated by Student's t-test, one-way analysis of variance (ANOVA) and the p-value of <0.05 was considered to be significant. Pearson correlation was used to evaluate the correlation between Hcy and hs-CRP.

RESULTS

The mean age of IGE patients was 21.8 ± 13.4 years where as in controls it was 23.4 ± 15.3 . The ratio between male and female was 65:35 and the family history of IGE was observed in 21% of patients. The mean age (in years) of the patients with IGE for age group <18 years and healthy controls was 7.6 ± 1.96 and 10.5 ± 1.85 ; and for patients in the age group >18 years and controls was 36.5 ± 10.4 and 39.0 ± 11.6 respectively. Out of 100 IGE patients, in majority (63%) the age of onset was <18 years and the remaining (37%) patients had the onset above the age of 18 years [Table/Fig-1].

Demographic features	Patients (n=100)	Controls (n=100)	p-value
Age (years) (4-69) (mean \pm SD)	21.8 \pm 13.4	23.4 \pm 15.3	0.43
<18 years (n=63)	7.6 \pm 1.96	10.5 \pm 1.85	0.000*
>18 years (n=37)	36.5 \pm 10.4	39.0 \pm 11.6	0.26
Gender (male: female)	65:35	65:35	
Family history (%)	21%	1%	

[Table/Fig-1]: Demographic profile of idiopathic generalised epilepsy patients and controls.

Note: $p < 0.05$ * significant

The mean time since diagnosis was 14.8 years. The levels of Hcy and hs-CRP in patients belonging to different age groups with their respective controls have been presented in [Table/Fig-2]. The hs-CRP levels in both the patient groups (<18 years and >18 years) were found to be significantly high as compared to their healthy controls (2.10 ± 0.62 , 0.56 ± 0.28 ; 3.22 ± 1.05 , 0.48 ± 0.20 mg/L respectively; $p < 0.05$). Significantly elevated levels of serum Hcy were observed in patients of different age groups compared to their respective controls 14.6 ± 1.8 $\mu\text{mol/L}$, 9.9 ± 2.18 $\mu\text{mol/L}$ ($p < 0.001$) and 14.5 ± 1.50 $\mu\text{mol/L}$, 9.33 ± 1.79 $\mu\text{mol/L}$ ($p < 0.05$) respectively [Table/Fig-2].

Variable		Controls (mean \pm sd)	Patients (mean \pm sd)	p-value
Hcy ($\mu\text{mol/L}$)	Controls vs Patients (n=100)	9.59 \pm 1.98	14.5 \pm 1.63	<0.001 *
	<18 years age group (n=63)	9.9 \pm 2.18	14.6 \pm 1.8	<0.001 *
	>18 years age group (n=37)	9.33 \pm 1.79	14.5 \pm 1.50	<0.001 *
hs-CRP (mg/L)	Controls vs Patients (n=100)	0.53 \pm 0.25	2.52 \pm 0.97	<0.001 *
	<18 years age group (n=63)	0.56 \pm 0.28	2.10 \pm 0.62	<0.001 *
	>18 years age group (n=37)	0.48 \pm 0.20	3.22 \pm 1.05	<0.001 *

[Table/Fig-2]: Data showing the serum homocysteine and hs-CRP levels in patients and controls in different age groups.

Note: $p < 0.05$ significant*

Hcy: Homocysteine, hs-CRP: High Sensitivity C-Reactive Protein

The serum levels of Hcy and hs-CRP in the patients of different epilepsy types have been given in [Table/Fig-3]. A significant difference in the levels of Hcy and hs-CRP was observed among different epilepsy types ($p = 0.007$ and $p < 0.001$ respectively). Serum levels of Hcy and hs-CRP in patient subgroups who were on AEDs treatment for different time periods (≤ 1 year, 1- ≤ 5 years and >5 years) have been presented in [Table/Fig-4]. Significant difference in the levels of Hcy and hs-CRP was observed in the patient subgroups who were on AEDs for different time periods ($p = 0.003$ and $p = 0.000$ respectively). Significantly elevated levels of hs-CRP were noticed in non-responders compared to responders group ($p < 0.05$) whereas, no significance was observed with Hcy levels [Table/Fig-4]. We also found that a positive correlation between the homocysteine, hs-CRP levels [Table/Fig-5,6] and the duration of the AED treatment. However, no significant difference in the levels of Hcy and hs-CRP was noted in the patients of either monotherapy (phenytoin, carbamazepine, sodium valproate) or polytherapy ($p = 0.99$ and $p = 0.95$ respectively) [Table/Fig-7].

Parameter	Epilepsy types			p-value
	Absence (n=15)	Myoclonic (n=35)	TC seizures only (n=50)	
Hcy ($\mu\text{mol/L}$)	14.05 \pm 1.16	14.4 \pm 1.48	15.05 \pm 1.03	0.007*
hs-CRP (mg/L)	2.02 \pm 0.34	2.38 \pm 0.94	2.9 \pm 0.77	0.000*

[Table/Fig-3]: The serum levels of hs-CRP and homocysteine in different epilepsy types.

Note: $p < 0.05$ significant*

Hcy: Homocysteine, hs-CRP: High Sensitivity C-Reactive Protein, TC: Tonic Clonic

Variable	<1 year (mean±SD) (n=23)	1-5 years (mean±SD) (n=32)	>5 years (mean±SD) (n=45)	p-value	Non-responders (n=33)	Responders (n=67)	p-value
Hcy (µmol/L)	13.9±1.94	14.7±1.46	15.2±1.14	0.003*	14.7±0.96	14.5±1.88	0.56
hs-CRP (mg/L)	1.92±0.54	2.10±0.59	3.12±1.02	0.000*	3.38±0.99	2.09±0.61	0.000*

[Table/Fig-4]: Levels of homocysteine and hs-CRP in patients with different time duration of AEDs treatment and in responders vs non-responders.

Note: p<0.05 significant*

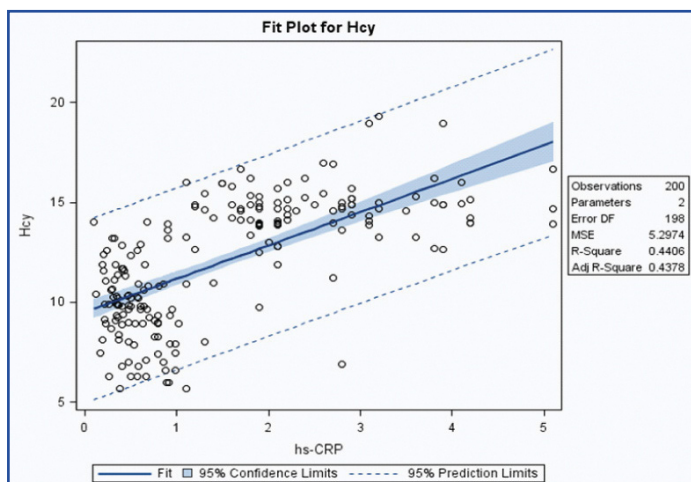
Hcy: Homocysteine, hs-CRP: High Sensitivity C-Reactive Protein

Variable	With variable	Sample size (n)	Sample correlation	Odds ratio (95% CI)	R ²	p-value
Hcy	hs-CRP	200	0.6638	0.6628 (0.57-0.73)	0.44	<0.0001*

[Table/Fig-5]: Correlation of homocysteine and hs-CRP in idiopathic generalised epilepsy patients.

Note: p<0.05 significant*

Hcy: Homocysteine, hs-CRP: High Sensitivity C-Reactive Protein



[Table/Fig-6]: Scatter plot with trendline showing correlation between homocysteine and high sensitivity C-reactive protein.

Parameter	Monotherapy			Polytherapy (n=25)	p-value
	Phenytoin (n=25)	Carbamazepine (n=25)	Valproate (n=25)		
Hcy (µmol/L)	14.5±1.6	14.56±1.62	14.62±1.59	14.6±1.58	0.99
hs-CRP (mg/L)	2.48±0.9	2.50±0.78	2.52±0.62	2.4±0.8	0.95

[Table/Fig-7]: Comparison of results among idiopathic generalised epilepsy patients on monotherapy and polytherapy.

Note: p>0.05 not significant*

Hcy: Homocysteine, hs-CRP: High Sensitivity C-Reactive Protein

DISCUSSION

It is well documented that Hcy levels are elevated both in brain as well as Cerebrospinal Fluid (CSF) of several neurological disorders [19]. Our study showed significantly elevated levels of Hcy in patients with IGE (p<0.05) and also in patients of different age groups (p<0.05) when compared to controls. In addition, a significant difference in levels of Hcy were noticed among the patient subgroups who were on AED treatment for different duration (p<0.05). Our results are in accordance with the previous studies in which elevated Hcy levels have been noticed in epileptic patients and also positively correlated with the duration of AED treatment [20,21]. The higher levels of Hcy in epileptic patients have been attributed to the AEDs inducing microsomal liver enzymes as well as increasing cytochrome P450 enzyme activities that may lead to a decrease in serum folate concentration resulting in Hcy accumulation [22].

It is known from previous studies that Hcy inhibits neurite outgrowth from primary cultured dopaminergic neurons and cerebellar purkinje neurons indicating that Hcy can inhibit communication between neurons [23]. Baldelli E et al., have reported that increased Hcy levels can enhance seizure activity and neurodegeneration [24].

Hyper-Hcy results in 10-40% of the epileptics particularly those on AEDs pharmacotherapy in both children and adults [25,26]. It has been observed that the patients on Enzyme-Inducing Anti Epileptic Drugs (EIAEDs) such as carbamazepine and valproic acid presented low levels of folate or B12 vitamin and higher levels of Hcy. However, supplementation with folate 5 mg/day or Vitamin B12 900 µg/day or with both, gained the normal levels of both vitamins and reduced plasma Hcy levels in three months [27]. In another study, a positive correlation was noticed between multidrug AED treatment, age and duration of treatment with elevated Hcy levels and low folate levels [24]. Similarly, Schwaninger M et al., and Ono H et al., also reported increased Hcy levels in the epileptic patients on polytherapy [28, 29]. In contrast to it, no correlation was found between the levels of Hcy, pyridoxal 5-phosphate, vitamin B12 and the duration of treatment [11]. Similarly, the results of monotherapy were also controversial. Some studies have reported the elevated Hcy levels in the patients on carbamazepine and valproic acid treatment [2,30,31]. Though, the mechanism by which AEDs bring about a decrease in serum folate is unknown but it has been postulated that AEDs may cause a decrease in intestinal absorption of folate or an induction of liver enzymes [32]. However, in the present study we did not find any significant difference of Hcy levels between the patients of mono and polytherapy.

Oxidative stress directly influences Cystathionine Beta Synthase (CBS) and methionine synthase causing disruption of redox homeostasis and contributes to neuronal damage [33]. Hcy also induces neurological dysfunction by means of oxidative stress and increases the neurotoxicity of amyloid beta (Aβ) by promoting oxidative stress [34].

Hyper-Hcy is an established risk factor for vascular diseases such as stroke, myocardial infarction and peripheral arterial disease [35]. Chuang YC et al., have reported that the long term use of older generation AEDs such as carbamazepine, phenytoin, valproate may contribute to the atherosclerosis in epileptic patients due to changes in the lipid profile and also due to the increased serum Hcy levels [36]. Major congenital malformations have also been noticed in children whose mothers receive AEDs. This has been attributed to teratogenic effects of high Hcy levels [37].

Several human and experimental studies have revealed that chronic inflammation may play a vital role in neurodegenerative processes. Proinflammatory cytokines are increased in rodent models of epilepsy and their activation has been well correlated with the frequency of seizures [38,39]. The contribution of specific inflammatory pathways to the mechanism of ictogenesis in epileptic tissue has been demonstrated in experimental models, but the exact contribution of these pathways in epileptogenesis is still under assessment. Elevated CRP levels have been reported in various diseases including atherosclerotic cardiovascular disease for which it is an important biomarker as well [40]. Higher levels of inflammatory markers such as CRP and Interleukin 6 (IL-6) have been observed in various neurological diseases [41]. Xu G et al., also claimed that elevated levels of CRP were detected in mild cognitive impairment patients and significantly higher levels of plasma IL-6 and CRP were noticed in nondemented children with Down's Syndrome [42,43]. Increased levels of IL-6 and CRP have been demonstrated in patients with undiagnosed and untreated tonic clonic seizures without any

demonstrable systemic or CNS infection [44]. In the present study, elevated hs-CRP levels were observed in IGE patients compared with healthy controls and our results were in accordance with the previous study where the higher concentrations of CRP have been noticed in epileptic patients and in paediatric epilepsy patients with frequent, refractory generalised motor seizures [15,45]. In another study, the base line concentrations of CRP were higher in patients with refractory epilepsy but no difference was found in comparison with healthy controls [15,46]. Significantly elevated hs-CRP levels were noticed in non-responders (refractory) when compared to responders ($p < 0.05$). Further, the levels of hs-CRP positively correlated with the duration of the disease as well the duration of AED treatment ($p < 0.05$). Our results are in accordance with a recent study in which elevated CRP levels have been demonstrated in paediatric epilepsy patients with frequent, refractory generalised motor seizures [15]. The elevated levels of hs-CRP in non-responders can be attributed to the contribution of inflammatory pathways in ictogenesis in epileptic tissue and thereby, causing neurodegeneration and intractable epilepsy.

CRP levels have also been associated with different epilepsy types. Patients with tonic clinic seizures showed higher levels of IL-6 compared with controls and also correlated with peripheral blood leucocyte count and CRP levels [44,47]. In the present study also, we found a significant difference in the hs-CRP concentrations between different epilepsy types particularly in the age group > 18 years of tonic clonic seizures ($p < 0.05$). Our data suggests that generalised tonic clonic seizures may stimulate CRP production. Similarly, Hcy levels were positively correlated with hs-CRP levels in IGE patients ($p < 0.05$). It is assumed that inflammation is mediated by two different mechanisms i.e., through activation of nuclear factor- $\kappa\beta$ or oxidative stress. The significant relation between Hcy and hs-CRP suggests that the association of Hcy with IGE may be dependent on inflammation related mechanisms.

LIMITATION

A more extensive study with a large sample size and for longer duration is required to attain a firm conclusion. We have not done the follow up study with individual AEDs which could be more informative.

CONCLUSION

Hyper-Hcy may be a known phenomenon in the epilepsy patients, who were on AEDs, either mono or polytherapy. It has been claimed that supplementation with cofactors of Hcy metabolism such as folic acid, Vitamin B12 and B6 can reduce the Hcy levels to normal in such patients. Since, chronic AED therapy can induce oxidative stress, we suggest that all the epileptic patients who are on AEDs whether mono or polytherapy should be screened for Hcy levels to avoid adverse effects of hyper-Hcy and be treated accordingly if the levels are found to be high. Hyper-Hcy can induce as well as promote oxidative stress and hence, it can be implicated in neurodegeneration in epilepsy. Elevated levels of hs-CRP in non-responders may be resulted by the contribution of inflammatory pathways in ictogenesis in epileptic tissue and thereby, causing neurodegeneration and intractable epilepsy.

ACKNOWLEDGEMENTS

We would like to thank Dr. NTR University of Health Sciences, Vijayawada, Andhra Pradesh.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **May 10, 2017**
Date of Peer Review: **Jun 19, 2017**
Date of Acceptance: **Aug 24, 2017**
Date of Publishing: **Feb 01, 2018**