Pravara Med Rev; December 2022, 14 (04), 28 - 33
DOI: 10.36848/PMR/2022/99100.51040

Original article

Correlation of serum homocysteine level with antiepileptic drugs in pediatric population

Dr. Randhir Khurana, Assistant Professor* , Dr. Monika Bairagi, Senior Resident* , Dr. Sonal Jindal, Associate Professor , Dr. Luna Sinha, Assistant Professor*****

*Department of Pediatrics, N.C. Medical College and Hospital, Israna, Panipat, Haryana (India)
**Department of Microbiology, LLRM Medical College, Meerut
***Department of Biochemistry, Santosh Medical College and Hospital, Ghaziabad, U.P.
Correspondence: Dr. Luna Sinha

ABSTRACT

Introduction: Homocysteine is a sulphur containing amino acid and it is derived from dietary methionine. Increased homocysteine levels induced by anti-epileptic drugs increased the risk of vascular occlusive diseases along with risk of resistance to anti-epileptics and development of refractory epilepsy.
Aim and Objectives: To correlate serum homocysteine level with antiepiletic drugs in pediatric population.
Material and Methods: Prospective observational and comparative study was conducted in 60 children with age range of 5-15 years out of which 30 epileptic children were on antiepileptic medication for atleast one year (Cases) who was further divided into (i) 14 children receiving monotherapy (Sodium valproate) and (ii) 16 children received polytherapy (Sodium valproate plus other drugs) and 30 epileptic children were not on antiepileptic medication (Control).
Results: Mean age in Case group was 8.9±2.83 and in Control group was 8.2±2.83 years. Generalized epilepsy was found in 44 patients (73%) and focal seizures in 16 patient (27%). Mean serum level of homocysteine showed significant difference between Cases and Control was 23.56±21.46 micromol/L vs. 11.62±3.77 micromol/L which was found to be highly significant ($p < 0.004$). Further with regard to monotherapy it was 19.16±14.57 micromol/L and in polytherapy it was 27.4±25.92 micromol/L ($p < 0.005$).
Conclusion: Study concluded that after one year of anti-epileptic drugs, there was a significant change in serum levels of homocysteine. Study also suggests that AED's, rather than the disease play a major role in the development of homocystenemia in epileptic patients.
Keywords: Serum homocysteine, Epilepsy, Antiepileptic drugs,

INTRODUCTION

Homocysteine (Hcy) is a sulfur-containing amino acid involved in methionine metabolism. An elevated total plasma Homocysteine concentration is a risk factor for vascular disease. In the last decade, a relationship between increased total Homocysteine plasma levels and antiepileptic treatments has been recognized[1-9]. The duration of therapy has also been recognized as a potential risk factor[6]. There are conflicting results about the role of methylenetetrahydrofolate reductase (MTHFR) polymorphisms particularly the C677T and A1298C) as determinants of high homocysteine levels in this patient group[10]. MTHFR is a key enzyme in the production of 5-methyltetrahydrofolate, which is required as the methyl donor for homocysteine remethylation to methionine. The C677T mutation of the MTHFR gene decreases the activity of this enzyme. High homocysteine blood levels for age have been found to have potential NMDA-mediated proconvulsant effects and are acknowledged as a vascular risk factor linked to toxic effects on the arterial endothelium[11].

Homocysteine metabolism stands at the intersection of two pathways and its remethylation requires two key enzymes: methionine synthase (MS) and methylenetetrahydrofolate reductase (MTHFR). The MS uses vitamin B_{12} as a cofactor and 5-methyltetrahydrofolate as a methyl donor. When there is an excess of protein or methionine, a larger proportion of homocysteine is metabolized by irreversible transsulfuration pathway, which degrades

homocysteine to cysteine. In this, homocysteine is first conjugated to cystathionine by cytathionine beta-synthase (CBS). Cystathionine is further cleaved into cysteine by cystathionine γ-lyase. Both enzymes need Vitamin B6 as a cofactor. Hyperhomocysteinemia acts via various mechanisms: such as increased tissue factor expression, attenuated anticoagulant processes, enhanced platelet reactivity, increased thrombin generation, augmented factor V activity, impaired fibrinolytic potential via impairing with the synthesis of collagen and flbrillin and vascular injury including endothelial dysfunction which lead on to increased potential for venous thrombosis. Molecular mechanisms undergoing prothrombotic actions of homocysteine are incompletely understood and include oxidative stress, DNA hypomethylation and pro inflammatory effects.[12]

With regard to pediatric population, Hyperhomocysteinemia is reported in 15.5 % of children receiving AEDs. There is little information available on the influence of AEDs on homocysteine level in paediatric age group. Hyperhomocysteinemia leads to increased risk for atherosclerosis, cerebrovascular disease, decreased seizure threshold and increased AEDs related side effects.

The relationship between increased homocysteine level and epileptic seizure remains controversial in humans despite a growing evidence of the pro-convulsive effect of the hyperhom-ocysteinemia observed in the animal studies. The mechanism of this association with epileptogenesis has not been clearly understood, but some evidence is available. Homocysteine and its oxidative product, homocysteic acid, are potent agonists of the N-methyl-Daspartate (NMDA)-type glutamate receptor, which are linked with epileptogenesis.[13] Furthermore, there is some data from animal studies demonstrating that homocysteine sequesters adenosine, an endogenous anti-convulsant and thereby reduces the seizure threshold.[14]

Keeping in view the above mentioned facts, the present study was conducted to correlate serum homocysteine level with antiepiletic drugs in pediatric population.

MATERIAL AND METHODS

The present prospective observational and comparative study was conducted in the Department of Pediatrics, N.C. Medical College and Hospital, Israna, Panipat, Haryana (India). A total of 60 children were included with age range of 5-15 years out of which 30 epileptic children were on antiepileptic medication for atleast one year (Cases) who was further divided into (i) 14 children receiving monotherapy (Sodium valproate) and (ii) 16 children received polytherapy (Sodium valproate plus other drugs). A total of 30 epileptic children were not on antiepileptic medication (Control). The study was conducted from 15th September 2021 to 14th September 2022.

Estimation of homocysteine:

Homocysteine was estimated by immuno assay technology.[15]

Specimen collection and handling

Homocysteine was measured through a routine blood test in all the patients in both groups. After overnight fasting for 12 hours, two ml of venous blood sample was taken.

The following recommendations for handling and storing blood samples were followed by us as given by the Clinical and Laboratory Standards Institute (CLSI).[16]

* All blood samples were collected following universal precautions for venipuncture.
* Blood samples were collected in vaccutainers without any preservations and sent to Department of Biochemistry. There, the vacutainer tubes containing blood samples were kept capped and upright all the time.
* Serum samples were allowed to clot adequately before centrifugation
* Samples were frozen only once and then mixed thoroughly after thawing.

The following additional recommendations were followed for handling and storing blood samples for homocysteine assay:

* Samples were centrifuged to remove serum or plasma from red blood cells at the earliest to ensure accurate measurements. Samples that were not separated soon after collection were stored on ice until centrifugation.

Reagents

All primary reagent pack was mixed by hand before loading them onto the system. Visually inspect the bottom of the reagent pack was inspected to ensure that all particles are dispersed and re-suspended.

PMR P ISSN: 0975-0533, E ISSN: 0976-0164

	Reagent	Ingredients
Primary reagent pack	Lite Reagent	Monoclonal mouse anti-SAH antibody (~0.4µg/mL) labeled with acridinium ester in phosphate buffer with bovine serum albumin and preservatives.
	Solid Phase	SAH (~2.1µg/mL) covalently coupled to paramagnetic particles in phosphate buffer with bovine serum albumin and preservatives
	Enzyme Reagent	Bovine derived S-adenosylhomocysteine hydrolase enzyme (~60 mU/mL) in TRIS buffer with preservatives
Ancillary reagent	Reducing Reagent	Dithiothreitol (~1.5 mg/mL) in citrate buffer with preservatives
	Homocysteine Diluent	Phosphate buffer with bovine gamma globulin and preservatives

Assay Principle

Homocysteine assay is a competitive immunoassay using direct, chemiluminescent technology. The different forms of homocysteine in the patient sample are reduced to free homocysteine by the Reducing Reagent. Free homocysteine is then converted to S-adenosylhomocysteine (SAH) by the Enzyme Reagent. Converted SAH from the patient sample competes with SAH covalently coupled to paramagnetic particles in the Solid Phase for a limited amount of acridinium ester-labelled anti-SAH in the Lite Reagent.

The system automatically performs the following steps:

- Dispenses 20 µL of sample into a cuvette
- Dispenses 50 µL of Reducing Reagent and incubates for 4.7 minutes at 37°C
- Dispenses 50 µL of Enzyme Reagent and incubates for 3.0 minutes at 37°C
- Dispenses 250 µL of Solid Phase and incubates for 3.0 minutes at 37°C
- Dispenses 100 µL of Lite Reagent and incubates for 3.0 minutes at 37°C
- Separates, aspirates, and washes the cuvettes with Wash 1
- Dispenses 300 µL each of Acid Reagent (R1) and Base Reagent (R2) to initiate the chemiluminescent reaction.
- Reports results according to the selected option.

An inverse relationship exists between the amount of Hcy present in the patient sample and the amount of relative light units (RLUs) detected by the system.

Sample volume

This assay requires 20 µL of sample for a single determination. The system reports tHcy results in µmol/L.

Reference value:

Homocysteine normal value = 3.7-13.3µmol/L

Vit. B12 normal value = 200-900 pg/ml

Folic acid normal value = 2-20 ng/ml

Methodology

After an overnight fasting 2ml venous blood was drawn; plasma was immediately separated and stored at -20 degree celsius until measurement of total homocysteine level. Homocysteine was measured with an enzyme linked immunosorbant **(ELISA**) method. The upper limit of normal level of homocysteine provided by the company was 15 micromol/lt (Figure 1).

Figure 1:

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In group A patients serum homocysteine levels and B_{12} and folic acid levels were estimated in all the samples by immuno assay. All the patients were given vitamin B_{12}, 1500 µg once a day and folic acid 5 mg once a day along with standard treatment of venous stasis. After 12 weeks of B_{12} and folic acid therapy the homocysteine levels were again estimated. In group B (control group) only homocysteine levels was studied.

STATISTICAL ANALYSIS:

At the end of the study, mean comparison between the two groups were made by using 't'-test and ANOVA test. A p value of <0.05 was considered as statistically significant. Statistical software package SPSS v. 20.0 was used to analyze the data.

RESULTS

In the present study, the mean age in Case group was 8.9±2.88 years and in Control group, it was 8.2±2.83 years. Generalized epilepsy was found in 44 patients (73%) and focal seizures in 16 patients (27%).

Table 1 Demographic profile

Parameters	Cases (n=30)	Control (n=30)	P value
Age	8.9 ± 2.88	8.2 ± 2.83	0.3463
Male	(20) 0.66 ± 0.479	(21) 0.7 ± 0.466	0.8242
Female	(10) 0.33±0.499	(9) 0.3 ± 0.466	0.8891

Table 2 Mean comparison of homocysteine among two groups

Parameters	Cases (n=30)	Control (n=30)	t-test	P value
Homocysteine	23.56 ± 21.46	11.62 ± 3.76	2.999	0.004

Table 3 Multigroup comparison

Parameters	Control	Monotherapy	Polytherapy	One –way Anova	p value
HOEMOCYSTEINS	11.62±3.77	19.16± 14.57	27.4±25.92	5.595	0.005

Table 3 demonstrates that mean homocysteine in control group was 11.62 ±3.77 micromol/L and further with regard to monotherapy it was 19.16±14.57 micromol/L and in polytherapy it was 27.4±25.92 micromol/L. Statistical analyses revealed a significant difference among these group (p <0.005).

DISCUSSION

Many studies in the literature are available regarding levels and prevalence of homocysteinemia in western countries. But there are only few studies or literature available regarding the levels of homocysteine and anti epileptic drugs in pediatric Indian population. So this study was conducted to correlate the serum homocysteine levels and anti epileptic drugs pediatric patients in this region of India.

Present study showed significant differences of serum homocysteine level between patients and control. Mean serum level of homocysteine showed significant difference between group A i.e. 23.56±21.46 micromol/L and in group B it was 11.62±3.77 micromol/L which was found to be highly significant (p <0.004).

Study reported by Kurul et al investigated the homocysteine, folic acid, and vitamin B12 levels in epileptic children who received antiepileptic drugs. In their study, 25 children with idiopathic epilepsy out of which 8 received valproate, 11 received carbamazepine, and 6 received oxcarbazepine. A total of 10 healthy children were also included. The mean homocysteine, folic acid, and vitamin B_{12} levels in

PMR P ISSN: 0975-0533, E ISSN: 0976-0164

their study group were 7.57±3.78 micromol/L, 10.19±4.05 ng/mL and 428.20±256.12 pg/mL respectively. The differences between the mean plasma homocysteine, folic acid, and vitamin B12 levels of the study and control groups were not significant (P=0.522; P=0.855; P=0.798, respectively). But in their study, plasma homocysteine levels were higher than the normal cutoff point accepted for childhood in 4(16%) of the study patients. Out of these 4 children, 3 were from the carbamazepine group and 1 was from the valproate group.[7]

In the present study finding of higher mean serum levels of homocysteine in patients receiving polytherapy compared to those receiving monotherapy. Mean homocysteine in control group was 11.62 ±3.77 micromol/L and further with regard to monotherapy it was 19.16±14.57 micromol/L and in polytherapy it was 27.4±25.92 micromol/L. Statistical analyses revealed a significant difference among these group ($p < 0.005$).

Similar study was reported by Huemer et al in which Hyperhomocysteinemia was present in 19 of 123 patients. Patients with hyperhomocysteinemia were older 13.7±4 vs. 11.0±3.9 years and had significantly lower folate and cobalamin concentrations. In their study, multidrug (two or more) AED treatment and duration of therapy correlated significantly with elevated total homocysteine (tHcy) and low folate. In contrast, polymorphisms in the methylene tetrahydrofolate reductase gene (MTHFR 677 C-->T, 1298 A-->C, 1793 G-->A) had no significant impact on tHcy. They reported nine of 19 patients with hyperhomocysteinemia who were randomized to placebo, whereas the remaining 10 patients received folic acid supplementation. Folic acid supplementation resulted in a significant increase of folate and decrease of tHcy, whereas both parameters remained unchanged in the placebo group.[17]

Another study by Ono et al reported use of Plasma total homocysteine (tHcy) and serum folate (FA) concentrations in 130 epileptic patients. In their study significant inverse correlation was found between FA and tHcy which was greater in the older age group >15 years than in the younger group <1-14 years.[18]

The mechanism of the association between homocysteine and epilepsy is not fully understood, it is recommended that AED'S increase serum homocysteine level by decreasing the blood folate level, due to antifolate properties.

According to the present study, antiepileptic drugs can induce hyperhomocysteinemia through various mechanisms such as (i) Reinhibition in vitamin absorption (ii) Homocysteine metabolism dysfunction (iii) Accelerated vitamin absorption and (iv) Modulation of renal function.

RECOMMENDATIONS

Folic acid supplementation is highly recommended in low preventive doses, particularly with elevated Hcy levels to prevent vascular complications. Strict monitoring of lipid profiles and advising low cholesterol diet, or consider prescribing lipid lowering drugs. Antioxidants plus multivitamin therapy could be beneficial through their protective effect on endothelial function and hence, reducing the risk to develop atherosclerosis among patients with epilepsy.

CONCLUSION

Present study concluded that after one year of anti-epileptic drugs, there is a significant change in serum levels of homocysteine. Present study suggests that AED's, rather than the disease play a major role in the development of homocystenemia in epileptic patients. Homocystenemia in blood is linked with a great risk of heart disease, stroke and complication of diabetes such as neuropathy. So, present study recommend to routinely screening of homocysteine and its cofactor level in epileptic patients, and be treated when their levels are found to be disturbed.

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