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Original Article

Serum Homocysteine Levels in Indian Children on Valproate Monotherapy

Shikha Swaroop[1], Medha Mittal[2], Smita Sandeep Nair[3], Reetika Saini[4], Jyoti Bagla[5]

[1] Assistant Professor, Manipal Tata Medical College, Jamshedpur, India
[2] Associate Professor, Department of Paediatrics, Chacha Nehru Bal Chikitsalaya, Delhi, India
[3] Assistant Professor, SMS and R, Sharda Hospital, Delhi, India
[4] Research Officer, Central Ayurveda Research Institute, Punjabi Bagh, New Delhi, India
[5] Professor and HOD, Department of Paediatrics, ESI Postgraduate Institute of Medical Education and Research, Basaidarapur, Delhi, India

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***Corresponding Author**

Medha Mittal
Associate Professor,
Department of Paediatrics,
Chacha Nehru Bal Chikitsalaya,
New Delhi- 110070, India
Email: goelmedha@gmail.com

Abstract

Introduction: Various research works have reported elevated serum homocysteine levels with the use of antiepileptic drugs. This may lead to an increased risk of atherosclerosis, a higher seizure frequency and may also cause cognitive decline.

Methods: Twenty five children (Two to 12 years) on valproate monotherapy for more than one year and the same number of age and sex-matched healthy controls were enrolled. Venous blood samples were analyzed for serum homocysteine, vitamin B12 and folic acid levels. All biochemical parameters were compared between the groups.

Results: The antiepileptic drug users had significantly lower serum homocysteine levels and higher B12 levels as compared to the controls. But serum folate levels were similar between the groups. No correlation of serum homocysteine levels was observed with either serum folate, B12 or valproate.

Conclusions: The use of valproate monotherapy for epilepsy in the Indian paediatric population does not increase the risk of hyperhomocysteinemia.

Introduction

Antiepileptic drugs (AED) such as carbamazepine (CBZ), valproate, and phenytoin (PHT), routinely prescribed in paediatric epilepsy patients, have been observed to increase levels of serum homocysteine (Hcy).[1,2] High Hcy levels can cause vascular endothelial damage.[3] It increases the atherogenic lipid levels and influences the blood coagulation system resulting in a prothrombotic state.[4] This makes the individual prone to both atherogenesis and venous thromboembolism.[5] An increased risk of cardiovascular disease and a fourfold heightened risk of occlusive cerebrovascular events have been reported.[6] Elevated Hcy levels could also decrease the seizure threshold and this has been attributed to its agonist action on N-methyl-D aspartate type glutamate receptors that are linked to epileptogenesis and sequestration of adenosine, an endogenous anticonvulsant.[7,8] Further, high Hcy levels may adversely affect cognitive function and even association with psychiatric disorders has been reported.[9,10]

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The mechanisms and pathways through which the antiepileptic drugs influence Hcy levels are not clearly known. Carbamazepine and phenytoin are known enzyme inducers and induction of liver enzymes could deplete levels of folic acid, pyridoxal 5'phosphate and vitamin B12 [11,12] These are known co-factors in the metabolism of Hcy to methionine and deficiency of these co-factors could lead to high Hcy levels. Sodium valproate is not an enzyme inducer but can impair intestinal absorption of folic acid and interfere with the metabolism of folic acid coenzymes.[13] However, there is no general agreement on the effects of antiepileptic drugs on Hcy levels and the underlying mechanisms. While some studies have demonstrated a clear association between antiepileptic drug use and high Hcy levels others have reported no change or even a decrease in its level with the use of the same drug.[14-17] Similarly, the effect of antiepileptic drugs on levels of folate and vitamin B12 is also quite variably reported.[11,14-17]

The levels of Hcy and its cofactors are known to be different in different populations.[18] Hence, a comparative control arm to see levels in age and sex-matched healthy population of similar ethnic background is most essential. This was, unfortunately, lacking in most of the reported studies. Data on the Indian population is particularly lacking. The current study was hence planned to evaluate the effect of valproate therapy on levels of Hcy and its cofactors (Folate and B12) in Indian children.-

Methods

The study was conducted in the outpatient department of a tertiary care hospital in India over 10 months (January 2019 to October 2019) with prior approval of the Institutional Ethical Committee meeting held in year 2018 (Reg. No ESIPGIMSR-IEC/20180039). Children between two to 12 years of age, receiving valproate monotherapy for more than a year, were offered participation in the study. Children with any systemic illness, leukemia, severe anemia, those receiving folic acid supplements or antagonists, patients with developmental delay and seizures due to meningitis or head injury, and with poor compliance to prescribed treatment were excluded. Children with weight for height (or body mass index for more than five year olds) less than -3 z score were excluded. For the comparative (or control) arm, age and sex-matched children (those with minor health complaints or coming for vaccination) were offered participation in the study. Written informed consent was obtained from the parents besides verbal assent from those more than seven years old. Endocrinal disorders (such as hypothyroidism or type 1 diabetes) are known confounding factors regarding hyperhomocysteinemia. So, children with such disorders were also excluded. A detailed history taking which included onset and type of seizures, number of breakthrough seizures, dose and duration of sodium valproate, development history and a thorough general examination and central nervous examination for any focal neurological deficit were done, for all the enrolled subjects. A venous sample was drawn for complete blood count, liver function test, kidney function test and lipid profile and levels of valproate. The venous blood sample was drawn in fasting state for serum Hcy, vitamin B12 and folate level. Samples were transported immediately to the laboratory for further processing and analysed on the same day. Hcy was estimated by the enzymatic method with a coefficient of variation of less than 6%.[19] Serum vitamin B12 and folic acid were analysed by electrochemiluminescence technique. Serum valproate levels were assayed by enzyme immunoassay. The laboratory personnel were unaware of the clinical details of the participant. Normal values for vitamin B_{12} were 200 - 900 pg / mL and folic acid 5 - 20 ng / mL.[20] According to the American Heart Association (AHA) advisory statement, normal Hcy concentrations range from 5 - 15 µmol / L.

Statistical analysis - The sample size was calculated using open epi calculator based on a previous study, where the mean level of Hcy in cases was 14 µmol / L and the standard deviation was 6.8 µmol / L and the mean level of Hcy in controls was 9.2 µmol / L with a standard deviation of 2.7 µmol / L.[2] Assuming confidence interval 95% on both sides and power 90%, the sample size calculated in each group was 25. Mean levels of serum Hcy, vitamin B12 and folic acid were compared in the two groups using unpaired t-test. Correlation between Hcy and other parameters was determined using Pearson's correlation coefficient. A P-value of less than 0.05 was taken as significant.

Results

Thirty subjects satisfying the inclusion criteria were screened for inclusion in the study. Of these, five were excluded (Two were receiving supplements that contained folate, one had developmental delay, one had poor compliance and one had hepatitis). Twenty-five subjects were, hence, enrolled and the same number of age and sex-matched healthy subjects were enrolled as controls. The demographic profile of the study population is tabulated in table 1.

Table1. Demographic profile of study population

		Epileptic cases (n = 25)	**Control (n = 25)**
Age in years	2 to 6	7	5
	7 to 12	18	20
Sex	Male	18	16
	Female	7	9
Diet	Veg	10	10
	Non- Veg	15	15
Height in cm		1.279 ± 0.190	1.299 ± 0.158
Weight in kg		9.349 ± 27.952	6.709 ± 25.404

All the epileptic subjects were neurologically normal on clinical examination and well compliant to treatment for various seizure types (figure1).

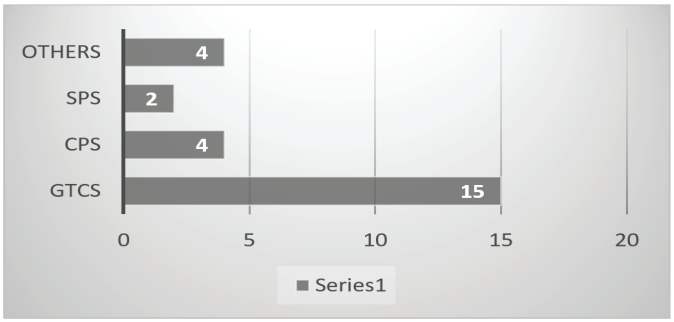

Figure 1. Graph depicting the seizure type in the enrolled cases

There was no history of breakthrough seizure in the previous year in any case. MRI of the brain was done in all epileptic cases. It was normal in 15 subjects, ring-enhancing lesions suggestive of neurocysticercosis were seen in seven and mild ischemic changes due to perinatal insult were seen in three cases. Electroencephalograph (EEG) was performed in 24 subjects; 14 of them showed generalised epileptiform discharges, three had focal discharges and seven had normal EEG. The subjects were receiving sodium valproate at a mean dose of 26.3 ± 5.5 mg / kg / day. The mean serum level of valproate was 69.35 ± 30.8 mcg/ml. Duration of treatment ranged from 368 days to 2008 days with a mean of 862 ± 209 days. The cases had a mean haemoglobin level of 12.6 mg / dl SD ± 1.33 (range 9.3 mg / dl to 14 mg / dl) and the controls had a mean haemoglobin level of range 12.05mg/dl SD ± 1.16 (8.8 mg / dl to 14.4 mg / dl). The liver function test and renal function test results were within normal limits for all participants. The biochemical results of mean serum levels of vitamin B_{12}, folate and Hcy in the cases and controls are mentioned in Table 2.

Table 2. Vitamin B_{12}, folate and homocysteine profile of the study population

Parameter	epileptic cases		Control		p value
	Mean	SD	Mean	SD	
S*. B12 (pg / mL)	584.6	293.6104278	331.1875	213.473795	0.011
S. homocysteine (µmol / L)	7.3495	2.48748774	11.14	5.447247011	0.0027
S. folate (ng / ml)	12.948	5.894497434	10.768	6.584117253	0.2234

*S = Serum

The mean serum vitamin B_{12} in the cases was significantly higher than the controls, though serum folate levels were normal in both groups. The mean Hcy levels were significantly lower in the cases than in the controls. The Hcy level of 96% of cases and 88% of controls were within the reference range of five to 15 µmol / l. One case had a Hcy level of 16.5 µmol / l and three of the controls had Hcy levels above 15 µmol / l. Low vitamin B_{12} levels were observed in 1 / 25 (4%) cases and 7 / 25 (28%) controls while low folate concentrations were seen in one each of cases and controls. No correlation was noted between serum Hcy levels and levels of folate or B_{12} or that of valproate (Table 3).

Table 3. Correlation between serum Homocysteine level and other parameters

	Homocysteine level in cases	Homocysteine in controls
S. B12	0.169	0.219
S. folic acid	0.56	- 0.378
S. valproate levels	- 0.088219	
mean duration of stay	- 0.22	

None of the correlations was significant at the 0.05 level.

Discussion

The study was carried out to evaluate the effect of valproate therapy on serum Hcy levels in Indian children. The levels were compared with age and sex-matched healthy controls as the levels vary with age, gender and ethnicity. Our results show that valproate therapy did not lead to higher homocysteine levels. The mean level in the cases was significantly lower than in the controls. Valproate therapy also did not adversely influence levels of vitamin B_{12} and folate. Mean B_{12} levels were higher in the cases than in the controls while mean folate levels were similar.

Hcy is generated from the demethylation of methionine. The enzyme methionine synthase remethylates Hcy back to methionine with 5-methyltetrahydrofolate as a methyl donor and Vitamin B_{12} as a cofactor. Another metabolic pathway involves conjugation of Hcy with serine that requires pyridoxal 5'-phosphate (a biologically active form of B_6) as a cofactor. Nutritional deficiency of folate, B_{12} and B_6 can result in high Hcy levels. Antiepileptic drug use has been recognized as a cause of folate, B_{12} and B_6 deficiency and influences Hcy levels. In this study, we have estimated Hcy levels along with levels of B_{12} and folate. The enzyme methylenetetrahydrofolate reductase (MTHFR) catalyses the conversion of 5, 10 methylenetetrahydrofolate to 5

methyltetrahydrofolate and reduced activity of this enzyme would lead to high Hcy levels. Genetic variants of this enzyme account for high Hcy levels seen in certain individuals. Yoo and Hong found that a common MTHFR C677T mutation was a determinant of hyperhomocysteinaemia in patients with epilepsy receiving AEDs, which suggests that a gene-drug interaction induced hyperhomocysteinaemia.[21] Valproate treatment has been associated with low folate levels.[16] Guanzhong et al have demonstrated that valproate induces hypomethylation of DNA in folate-deficient states leading to decreased MTHFR activity.[22] This could be the underlying mechanism of valproate use leading to high Hcy levels. However, our observations show that valproate use does not lead to higher Hcy levels. Our patients were folate replete and remained so despite valproate use. The average valproate dose required in our cases was not particularly high and this could also be one reason for valproate not having any adverse effects on folate or Hcy levels. Our results are in agreement with those of Gidal et al who observed that 32-week treatment with valproate was associated with an increase (57%) in serum B_{12} levels and a decline (27%) in Hcy levels as compared to baseline values and no change in folate levels.[17] The mechanisms underlying these changes however could not be elucidated. Apeland et al compared levels of Hcy, folate and B_{12} between AED users (adults) and age-matched controls and their results show no effect of valproate on levels of Hcy and folate.[23] The subjects on valproate had higher B_{12} levels than controls and the triglyceride levels were similar to controls. They too could not attribute any reason for such findings. Other researchers have reported valproate use to be associated with high Hcy values.[2,24] These include Krabiber et al and Verrotti et al who also observed lower folate levels (than controls) in their participants.[2,24] Abd El Dayem et al found no effect of drug use on Hcy levels.[16] Differences in various observations may be due to differences in ethnic origin, diet and duration of medication.

Hcy levels in healthy Indian children 10 to 19 years has been reported as being 11.649 + / - 2.42 µmol / l.[25] Another Indian study has reported baseline Hcy values (in children two to 12 years old) as 11.5 µmol /l.[26] The Hcy values of our control population agree with these studies.

Considering hyperhomocysteinemia by absolute values, levels more than 15 µmol / l are regarded as hyperhomocysteinemia. It was observed in one of the participants on valproate while two of the controls had high values of Hcy. One of the controls with hyperhomocysteineimia had a low serum folate level i.e 2 ng / ml while others had a normal folate level. Vitamin B_{12} was normal in all such patients. The cases in our study population had significantly higher vitamin B_{12} concentrations than the matched controls. This is in agreement with the observations of several other authors.[11,17,27,28] Krabiber et al reported increased vitamin B_{12} in the valproate group though it was not statistically significant.[2] Though there was no clear explanation for this observation, it was suggested that an increase in vitamin B_{12} binding capacity (transcobalamin II) could occur on therapy with valproate.[28] The higher B_{12} levels could be an early indicator of hepatic injury caused by the anticonvulsants.[29] Other researchers have found conflicting results. Verrotti et al noted no change in serum levels of vitamin B_{12} in patients on one year of valproate at an average dose similar to ours (mean dose of 21.7 mg / kg / day).[24] Results of Abd El Dayem SM et al also indicate no effect of valproate on B_{12} levels.[16] A decline in levels has also been observed on valproate therapy.[30] The folate levels were similar in the cases and controls. Our results agree with those of Sener et al and Abd El Dayem who also found that valproate therapy did not affect folate levels.[14,16] Gidal et al also observed that 32-week treatment with valproate did not affect blood folate levels.[17] Other published works have reported reduced folate levels in subjects on valproate as compared to controls.[30]

Our study is one of the rare studies which has tried to explore the effect of valproate therapy on serum Hcy level in Indian children. This study includes a comparison with, age and sex-matched ethnically similar controls as the levels of Hcy, folate and B12 are known to be different in different populations. Further, we have enrolled only those cases who had a sufficiently long treatment period (more than one year) to ascribe any changes in outcome variables to be due to the drug in consideration. However, we acknowledge a few limitations. One of the major limitations of our study include the fact that we did not measure baseline Hcy levels at the initiation of treatment which might be important to assign a causative role to the drug. Methionine loading test, which is more sensitive in identifying hyperhomocysteinemia, was not done and some subjects with hyperhomocysteinemia might have been missed. Genetic variations that determine Hcy concentrations were not studied.

Conclusions

Our study convincingly proves that sodium valproate does not adversely influence Hcy levels and in fact, the levels decrease along with a rise in vitamin B_{12} levels. It may be assumed to be safe concerning this risk. However, the mechanism of the drug inducing the said changes was beyond the scope of this study and would make an interesting research question for further studies.

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