RESEARCH PAPER

Effect of Carbamazepine Therapy on Homocysteine, Vitamin B_{12} and Folic Acid Levels in Children with Epilepsy

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Received: July 27, 2012; Initial review: August 14, 2012; Accepted: October 25, 2012.

Objectives: To compare the levels of homocysteine, vitamin B_{12} and folic acid before and after 6 months of carbamazepine therapy and to correlate them with carbamazepine level at 6 months.

Design: Prospective comparative study.

Setting: Tertiary care centre in North India.

Participants: 51 children (2-12 years of age) presenting with motor partial seizures.

Intervention: Carbamazepine (10-20 mg/μ/day) for 6 months.

Main outcome measure: Change in serum homocysteine, B_{12}, folic acid level.

Methods: Fasting venous samples were collected before carbamazepine therapy and after six months. Homocysteine was analyzed using homocysteine enzyme immunoassay. Vitamin B_{12} and folic acid were estimated using electrochemiluminesence technique. Carbamazepine levels were measured at 6 months.

Results: Of the 51 children, 36 (males-21), were followed up and their data analyzed. Mean homocysteine level was 11.51±3.95 μmol/L at recruitment and 11.77±6.65 μmol/L at six months (*P*=0.785). At recruitment 6(16%) children had homocysteine level above 15 μmol/L which increased to 10(27%) at 6 months. Mean vitamin B_{12} at recruitment was 292.1±111.2 pg/mL and 297.8±82.9 pg/mL at 6 months (*P*=0.764). Mean folic acid at recruitment was 9.98±3.45 ng/mL and 10.66±3.97 ng/mL at 6 months (*P*=0.358). There was no correlation between carbamazepine levels with homocysteine, vitamin B_{12} and folic acid (*P*>0.05). There was no effect of age, sex or dietary pattern on homocysteine levels.

Conclusion: Hence 6 months of carbamazepine therapy did not cause significant change in serum levels of homocysteine, vitamin B_{12} and folic acid.

PII: S097475591200685

Carbamazepine is a commonly used drug for seizures in children [1]. Previous studies measuring a change in homocysteine levels due to cabamazepine therapy are conflicting. Elevation of mean homocysteine level was seen in some studies [2-4]. Few studies showed significant change or demonstrated decrease in homocysteine levels [5]. Most of these studies were case-control or cross-sectional [2,3,5]. Antiepileptic drugs as carmamazepine affect homocysteine, vitamin B_{12} and folic acid levels by microsomal enzyme induction. Hence it may have a role in atherosclerosis through involvement of homocysteine and lipids [6,7].

We conducted this study to prospectively measure the changes in homocysteine, vitamin B_{12} and folic acid in ambulatory children with motor focal epilepsy receiving carbamazepine therapy.

METHODS

The study was conducted in the outpatient and emergency department of a tertiary care hospital in India from January 2010 to February 2011. Approval was taken from the Institutional Ethical Committee. A written informed consent was taken from the parents. Children in age group of 2-12 years presenting within 7 days of occurrence of motor focal seizures were included in the study. Children receiving antiepileptic drugs other than carbamazepine, or taking any drug known to affect homocysteine levels, or vitamin supplements were excluded. Children with development delay, chronic liver disease, kidney disease and severe anemia were excluded from the study. Children with seizures due to meningitis, head injury or febrile seizures were excluded from the study.

A detailed history and examination was carried out. Basic demographic characteristics and anthropometry of the child was recorded. Magnetic resonance imaging or contrast enhanced computed tomography and an electroencephalograph was carried out. These children were prescribed carbamazepine in a dose of 10 mg/kg/day.

The dose was increased by 5 mg/kg/day upto 30 mg/kg/day, if required. If seizure was not controlled then another alternative anticonvulsant was added and child excluded from the study. Patients diagnosed as neurocysticercosis were given prednisolone 2 mg /kg/day in 3 divided dose for 5 days and albendazole 15 mg/kg/day in two divided doses for 28 days.

At recruitment, venous blood sample was collected for hemogram, liver function tests and kidney function tests. Venous sample for homocysteine, vitamin B_{12} and folic acid was collected after 12 hour of fasting, at recruitment and at 6 months. EDTA vial was used for collecting sample for homocysteine. It was immediately centrifuged and kept at –86°C for storage. Homocysteine were estimated using Enzyme Immunoassay (EIA). Precision of the assay was taken as 10%. Vitamin B_{12} and folic acid were estimated by electrochemiluminescence technique. At 6 months, venous sample for carbamazepine levels was collected in morning approximately 12 hours after the evening dose and before the morning dose. The estimation of carbamazepine level was done by photometric analysis in an autoanalyzer [ECHO Auto analyzer (Logotech)] with CEDIA carbamazepine II assay technique. Homocysteine levels of 5-15 µmol/L were taken as normal. Normal values for vitamin B12 were 200-835 pg/mL and folic acid 3-20 ng/mL.

Statistical Analysis

Sample size of 36 was calculated using SYSTAT version-10 based on previous study [2] for a change in homocysteine level of 0.70 µmol/dL, standard deviation of 1.25, power of 90% and alpha error of 0.05. Mean and standard deviation were calculated for quantitative variables. Mean homocysteine, vitamin B_{12} and folic acid at beginning and end of therapy were compared using paired-*t* test. Intragroup comparison was done using F-test (repeated measure ANOVA); if found significant, Tukey's test was applied. Intergroup comparison and interactions were similarly determined. Pearson's co-efficient of correlation was used for determination of the correlation for multiple variables at 6 month. *P* value of less than 0.05 was taken as significant.

RESULTS

51 children (males 27) were recruited. At 6 months, 36 children (male 21) were followed up. Baseline characteristics of children followed up and those lost to follow-up were similar. Of the 15 (6 males) lost to follow up, 5 had a drug-induced rash, one had intractable seizure, one died, and 5 didnot return for follow-up. Among the followed-up group, 29 (80.6) had complex seizures and rest had simple focal seizures (***Table* I**).

Majority of children had neurocysticercosis on neuro imaging (***Table* I**). Electroencephalograph was carried out in 29 children, was normal in 6 children, spike and wave pattern in 13 and focal slowing in 10 children. Mean alkaline phosphatase levels at start of therapy was172.92 (95.33) IU/L and 6 months of carbamazepine therapy was 249.28 (117.67) IU/L ($P<0.001$). ***Table* II** depicts the mean serum levels of homocysteine, vitamin B_{12}, and folic acid at recruitment and after 6 months of therapy.

At the start of treatment, 6 (16%) children had homocysteine level above 15 µmol/L. At 6 months of therapy among 36 children, 10 children (27.8%) had homocysteine level above the normal range. At the start of therapy, vitamin B_{12} level was below 200 pg/mL in 3 children, none at end of therapy. Folic acid levels was within normal range 3-20 ng/mL for all children at recruitment and at 6 months. None had a value above the upper limit of normal.

During follow-up we had to increase the dose of carbamazepine in 5 children due to repeat seizure. The mean carbamazepine dose for the patients was 11.99±2.01 mg/kg/day (range: 10-20 mg/kg/day). At 6 months of carbamazepine therapy mean blood level was 6.57+1.93 mg/L (normal 4-12mg/L). None had a level outside the therapeutic range. There was no correlation between carbamazepine levels and homocysteine levels (Pearson's correlation 0.083, $P=0.631$),Vitamin B_{12} ($P=0.86$) and folic acid ($P=0.776$).

TABLE I CHARACTERISTICS OF THE STUDY SUBJECTS

	No. (%)
Age 24-72 months	16 (44.4)
Family history of seizure	3 (8.3)
Neurocysticercosis	29 (90.6)
Neuroimaging findings ($n=32$)	
Nonspecific hyperintensity	1 (3.1)
Cystic encephalomalacia	1 (3.1)

TABLE II MEAN (SD) SERUM HOMOCYSTEINE, VITAMIN B_{12} AND FOLIC ACID LEVELS AT RECRUITMENT AND FOLLOW-UP.

	Start of therapy	*At 6 months of therapy*	*P value*
Homocysteine (µmol/L)	11.5±3.95	11.8±6.65	0.78
Vitamin B_{12} (pg/mL)	292.1±111.16	297.81±82.93	0.76
Folic acid (ng/mL)	9.98±3.45	10.66±3.97	0.36

WHAT IS ALREADY KNOWN?

- Carbamazepine is an enzyme inducing antiepileptic, has a role in atherosclerosis through involvement of homocysteine. No previous study in Indian subjects. Variable results in other studies

WHAT THIS STUDY ADDS?

- Carbamazepine therapy of six month duration did not cause statistically significant change in homocysteine levels in Indian children.

At recruitment, there was negative correlation between homocysteine level and vitamin B_{12} (P=0.015). Folic acid level at recruitment had significant negative correlation with vitamin B_{12} (P=0.003). After 6 months of carbamazepine therapy, homocysteine level correlated significantly with vitamin B_{12} (P=0.155) or with folic acid (P=0.614) (***Table* III**). Interaction of various factors (age, sex, dietary habits) and change in homocysteine levels was not found to be significant (P>0.05).

DISCUSSION

Hyperhomocysteinemia is an important risk factor for atherosclerotic vascular disease, independent of other risk factors for atherosclerosis [6,7]. Plasma total homocysteine concentrations exceeding the 95th age percentile are related to a four-fold increased risk for ischemic cerebrovascular disease in childhood [8]. Studies suggest that hyperhomocysteinemia is correlated with cognitive decline and brain atrophy [9].

The results of our study were similar to a case control study of 11 children receiving carbamazepine [5]. Similarly no significant change in homocysteine level was seen in a study by Minzter, *et al*. [10].

Attilakos, *et al*. [2] studied the effect of carbamazepine on homocysteine, vitamin B_{12} and folic acid in 52 children in age group 4.5 to 14 years. In their study, mean homocysteine level at start of therapy was 6.9±1.5 µmol/l and after 20 weeks treatment it was 7.6±1.7 µmol/l (P<0.01). The mean folic acid level was significantly decreased (P<0.01). The mean vitamin B_{12} level did not change significantly. Other authors have reported increase in homocysteine level in CBZ treated patients [11,12]. We observed that Homocysteine negatively correlated with vitamin B_{12} initially but no such correlation was seen at end of therapy. This implies that changes in homocysteine level seen in our study was not influenced by vitamin B_{12} or folic acid levels.

TABLE III CORRELATION BETWEEN HOMOCYSTEINE, VITAMIN B_{12} AND FOLIC ACID LEVELS

	Hct_1	FA_1	$VitB_{12}$ (1)	Hct_2	$VitB_{12}$ (2)	FA_2
Hct_1.		0.273	-0.401*	0.552*	-0.408*	-0.102
FA_1			-0.475*	0.250	-0.256	0.302
$VitB_{12}$(1)				-0.357*	0.357*	-0.407*
Hct_2					-0.242	-0.087
$VitB_{12}$(2)						0.088
FA_2				-0.087	-0.088	1

**Correlation is significant at the 0.05 level; Het-Homocysteine; FA=Folic acid; Vit B_{12} = Vitamin B_{12} levels; Subscript 1 and 2 indicate levels at recruitment and follow-up.*

The difference in the study results might be because of different genetic make-up of our population. The methionine loading test seems to identify more patients with hyperhomocysteinemia than fasting levels of plasma total homocysteine [13]. Methionine loading test was not done in our study, hence some cases of hyperhomocysteinemia may have been missed. Individuals with C677T variant in the *MTHFR* (methylenetetrahydrofolate reductase) gene have higher level of homocysteine level as compared to normal *MTHFR* gene variants [14]. In our study, the genetic variation was not determined. Homocysteine levels are known to be different in different populations. We analyzed homocysteine levels in 19 healthy controls and found mean levels similar to our subjects at recruitment, range was also between 5-15 mmol/L.

Neurocysticosis as the comonest neuroimaging abnormality has also been reported previously in our population [15,16]. Increase in artherosclerotic lipids among children on carbamazepine therapy was also demonstrated by one study [16]. Increase in homocysteine may be an additional risk factor for artherosclerosis. Children with neurocysticercosis received prednisolone for 5 days and albendazole for 28 days, but this is unlikely to cause any change in homocysteine levels at 6 months.

The importance of the present study lies in the fact that each child served as his own control and hence number of confounding factors were eliminated, reducing

bias. Since only developmentally normal children were included, chances of influence in homocysteine levels due to dietary deficiencies was eliminated. All the children had a uniform seizure pattern. Focal motor seizures were chosen to eliminate bias in diagnosis, and treated as per hospital protocol. Previous studies in children were case-control studies with a small number of children presenting with different seizure patterns. Drawbacks of this study was that most of the children were with neurocysticercosis only and the duration of follow up was only 6 months, instead of 2 years or longer.

This prospective study highlights that in children aged 2-12 years presenting with motor focal seizures, 6 months carbamazepine therapy did not cause significant change in homocysteine, vitamin B_{12} and folic acid levels. Further studies within different populations of children and longer follow-up are required to demonstrate the generalizability of these finding.

Contributions: AA conceptualized the idea. VK collected data. SS supervised homocysteine estimation. NC supervised folic acid and B_{12} estimation. All authors were involved in study design data analysis, interpretation, manuscript preparation and literature search. AA will act as a guarantor for the paper.
Funding: None; *Competing interests*: None stated.

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