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Effect of Antiepileptic Drugs on Serum Vitamin B12 and Folic Acid Among Children with Epilepsy in Bangalore

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In India, there are more than 12 million people diagnosed with epilepsy, and contributes nearly to the one sixth of global burden. Over 60% of diagnosis for epilepsy is done in childhood; hence, it is of major importance to pediatricians. we aimed to assess the levels of Vitamin B12 and Folic acid among children with epilepsy receiving antiepileptic drugs like Carbamazepine, Sodium Valproate and Levetiracetam monotherapy for >1 year. A total of 77 children with epilepsy (<18 years), on monotherapy with SV, CBZ, LEV, for minimum one year, were enrolled after obtaining consent from the parent/guardian. Children with co morbid condition like chronic kidney disease, chronic liver disease, those children who were on multiple AED's, and children receiving treatment for <1year were excluded from the study. Out of the 77 patients, who were selected for the study, 46 were males and 31 were females. There has been a significant association between duration of treatment and Vitamin B12 and levels of Folic acid. There is also enough evidence to show that age of the patient and Folic acid levels are dependent variables. The proportion of children developing deficiency of Vitamin B12 or Folic acid after treatment with LEV is greater than the proportion treated with CBZ. The proportion of patients developing deficiency of Folic acid after treatment with LEV is greater than the same proportion after treatment with CBZ. Though there were a few significant findings as reported, we, the authors, feel that the study should be continued to ascertain if the AED's have a significant role in Vitamin B12 and Folic acid metabolism and supplementation should be advised along with the AED's.

Keywords: Antiepileptic drugs; Children; Epilepsy; Folic acid; Vit B12.

Epilepsy is a chronic, non communicable condition of the brain which affects people of all ages. Around 50 million people in the world have epilepsy, thus making it one of the most common neurological diseases globally[1]. In India, there are greater than 12 million people with epilepsy, and contributes to nearly one-sixth of the global burden[2]. Over 60% epilepsy starts in childhood, hence, it is of major importance to paediatricians[3].

Epilepsy is caused by recurrent seizures, which involves brief episodes of involuntary movement of body (partial) parts or the entire body (generalized), sometimes with loss of consciousness and involuntary control of bowel or bladder function[4]. Epilepsy can also increase the risk of physical injuries like fractures and cause psychological derangements like anxiety, depression, etc.

Epilepsy affects majority of children and is one among the most frequent chronic neurologic condition in children. The mortality rate is 2–4 times higher in people affected by epilepsy than the rest of the population, and even higher in children i.e., upto 5–10 times[5]. The onset of epilepsy in children is attributed to certain risk factors like maternal alcohol abuse, smoking in pregnancy, premature birth, positive family history, infections, etc. A multicentre approach has been implemented for the early diagnosis of epilepsy in children. Educating the parents about the first signs, EEG, are a few among them. Early diagnosis and treatment have made it possible to decrease the mortality and morbidity among the children.

Children with epilepsy need to take antiepileptic drugs (AED's) for a minimum of 2 to 3 years. Phenobarbitone (PB) and Phenytoin (PHT) were the mainstay of treatment. With the advent of newer AED's many children, along with the conventional AED's, are on AEDs like Carbamazepine (CBZ), Sodium Valproate (SV) or Levetiracetam (LEV). There are studies that have proven that long term Phenytoin therapy (PHT) can cause Vitamin B12 and Folic acid deficiency in adults. Very scarce data is available on the effect of newer AED's like CBZ, SV and LEV on levels of Vitamin B12 and Folic acid in children. In addition, children on long term treatment are at risk of developing Folate and Vitamin B12 deficiency, which in turn can cause anaemia to a significant level[6]. The former studies showed that the bioavailability and metabolism of Folate and Vitamin B12 levels were altered in adults who were on long term treatment especially on PHT[7]. The manifestation caused by these two deficiencies can result in megaloblastic anaemia and cognitive impairment. Despite the use of newer AED's, conventional AED's remain mainstay in the treatment of epilepsy due to broad spectrum of activity and cost[8]. Hence, the present study aims at looking into the need for supplementation of Folic acid and Vitamin B12 in children prescribed with long term treatment with CBZ, SV and LEV monotherapy.

In 2019, global anaemia prevalence was 39.8% in children aged 6-59 months[9]. Anaemia is a one of the global public health problem affecting 1.62 billion (24.8%) people worldwide. It is seen at all stages of the life and more prevalent in pre-school aged children who are under five years of age. There are several factors that contribute to the occurrence of anaemia and approximately half (43%) of the anaemia in childhood is because of iron deficiency. While other causes of anaemia cab be due to Folate and Vitamin B12 deficiency[10]. The prevalence of Folate and Vitamin B12 deficiency among North Indian children aged 6-59 months is 10.9% and 22.3% respectively[11]. A study conducted by Sundarakumar et al., concluded that the prevalence of Vitamin B12 deficiency who are lesser 200 pg/ml, among rural subjects was 42.3% and Folic acid deficiency (<3 ng/ml) was 11.1%[12].

Since the parents of epileptic children are concerned more about the seizures, while anaemia being a major problem is neglected and leads to harmful consequences and derangements in children. The children may be anaemic before the onset of seizure or the antiepileptic drugs may aggravate the existing anaemia. In both scenarios, it is necessary to take adequate measures and supplement the children with Vitamin B12 and Folic acid. Therefore, this study, can throw light if administration of these AED's can lead to deficiency of Vitamin B12 and Folic acid either clinically or in subclinical states. So remedial measures can be adopted to prevent these.

A study conducted by Gorjipour et al.[13], they found that treatment with carbamazepine was associated with decrease levels of serum Folate and SV was associated with decrease levels of Vitamin B12. Similarly in another study conducted by Hong-Li Huang et al.[14], it was established that the levels of Folate and Vitamin B12 decreased after the administration of AEDs and the differences were statistically significant. In a study conducted by Sharma et al.[15], they noticed a highly significant reduction in Folate and Vitamin B12 levels, after the treatment with CBZ monotherapy in epileptic children, as compared to before therapy. Hence, keeping these in mind, we aimed to assess the

serum levels of Vitamin B12 and Folic acid among children with epilepsy receiving antiepileptic drugs like Carbamazepine, Sodium Valproate and Levetiracetam monotherapy for >1 year.

MATERIALS

In this prospective conventional study, a total of 77 children with epilepsy (<18 years), on monotherapy with SV, CBZ, LEV, for minimum one year, were enrolled after obtaining consent from the parent/guardian. Children with co morbid conditions like chronic kidney disease, chronic liver disease, those children who were on multiple AED's, and children receiving treatment for <1year were excluded from the study.

Methods of data collection

Approval was taken from Institutional ethics committee. An informed consent was obtained from the parent/guardian. The patients were selected under strict inclusion and exclusion criteria. Demographic data like name, age, sex, address was collected. Disease characteristics like the type of epilepsy, age of onset and duration, time since last attack, frequency, and family history were recorded. Treatment details like the drug taken, duration of treatment, frequency and adherence to the treatment, alternate system of medication were noted and entered in the Case Record Form (CRF). 2ml of blood was collected for estimation of Vitamin B12, Folic acid by Elisa technique collected samples were stored -80C until analysis. Batch analysis was done on sufficient sample collection.

RESULTS

Out of the 77 patients, who were selected for the study, 46 were males and 31 were females. Majority of patients were in 0 to 5 years of age (43%), followed by 6 to 10 years (34%) and the rest were between 11-18 yrs. According to Kuppuswamy scale, we analyzed the socioeconomic status and found that 53% of the patients belonged to lower-middleclass family. 64.9% of children were diagnosed with Generalised tonic clonic seizures (GTCS), 24.6% with partial seizures, while others included focal, neonatal and absence seizures. With regards to the AED treatment, 40 patients were being treated with SV, 12 patients with CBZ and 25 patients with LEV.

On analysing the status of Vit B12 and Folic acid levels, Vitamin B12 deficiency was noted in patients treated with SV, CBZ, and LEV were 50%, 41.6%, 68%, respectively. Folic acid deficiency, in patients treated with SV, CBZ, LEV, were, 35%, 8.3%, 44% respectively. The deficiency of Vit B12 and Folic acid among these children was irrespective of the duration of treatment. Low Haemoglobin levels was found in 22% of the patients, irrespective of the drug and duration of treatment. Clinical signs like pallor, nail changes were observed in the patients who had low levels of Haemoglobin.

Chi-square test for Association [Table 1,2,3] and Proportion test were carried out for all possible combinations and only significant results are reported below and other results are mentioned later. The tests were carried out using online calculators available in Statistics Kingdom[16]. Chi-square of independence is used for categorical variables to assess the degree of association between two variables. This test helps to compare the sample information i.e the observed data, with the values expected if the two variables were indeed independent[17].

The Chi-square value at 2 degrees of freedom is 6.2009 with p value 0.0450, hence significant at 5% level of significance. Therefore, there is a significant association with the duration of treatment and the levels of Vitamin B12 [Table 1], and also duration of treatment with the levels of Folic acid had 2 degrees of freedom which was 7.72 with p value 0.02107, hence significant at 5% level of significance. Therefore, there is significant association between the duration of treatment and the levels of Folic acid [Table 2]. On analyzing the association between age and the levels of Folic acid the Chi-square value at 2 degrees of freedom is 6.0479 with p value 0.04861, hence significant at 5% level of significance. Thus, there is significant association between age of patient and the levels of Folic acid, found after treatment with any one of the three AED's [Table 3].

We analyzed the data using Z-test for two proportions. A z-test for two population proportions is a hypothesis that makes a claim about the population proportions P1 and P2. The null hypothesis is a statement, which is about the population proportion parameter which

indicates no effect, and the alternative hypothesis is the complementary hypothesis to the null hypothesis[18]. Proportion of patients developing Folic acid deficiency after treatment with any one of the three AED's, irrespective of the duration of treatment, in the age group 5-10 years, is significantly greater than the same proportion in the age group 1-5 years (p value 0.0148203, H_0: the two proportions are same, H_1: Proportion 1 >Proportion 2, sample proportion1 =13/25=0.52, sample proportion 2 = 6/29=0.206). Proportion of patients developing Vitamin B12 or Folic acid deficiency after treatment with any one of the three AED's, irrespective of age, for 1-2 years duration, is significantly greater than the same proportion after treatment with any of the AEDs for 2-3 years (p value 0.0474215, H_0: the two proportions are same, H_1: Proportion 1 >Proportion 2, sample proportion 1 =5/17=0.29, sample proportion 2=7/10=0.7).

Proportion of patients developing Vitamin B12 or Folic acid deficiency after treatment with CBZ, irrespective of duration of treatment and age, is significantly less than the same proportion after treatment with LEV. (p value 0.0403117, H_0: the two proportions are same, H_1: Proportion 1 < Proportion 2, sample proportion 1 =1/8=0.125, sample proportion2 =9/15=0.6). Proportion of patients developing Folic acid deficiency after treatment with LEV, irrespective of the duration of treatment, was significantly greater than the proportion after treatment with CBZ. (p value 0.0346644, H_0: the two proportions are same, H_1: Proportion 1 > Proportion 2, sample proportion 1=11/25=0.44, sample proportion 2 =1/12=0.08).

Table 1. Chi-square tests for Association between treatment duration and Vit B12 levels

Treatment duration	Vitamin B12 levels	
	Normal	Deficiency
1-2 years	16	20
2-3 years	10	18
>3 years	10	3

Table 2. Chi-square tests for Association between duration of treatment and Folic acid levels

Treatment duration	Folic acid levels	
	Normal	Deficiency
1-2 years	28	8
2-3 years	13	15
>3 years	10	3

Table 3. Co-relation between patients age and folic acid levels

Age of patient	Folic acid levels	
	Normal	Deficiency
1-5 years	23	6
5-10 years	12	13
10-18 years	16	7

Reference range for Vitamin B12- 180-914 pg/ml and Folic acid-1.6-19.5 ng/dl

Table 4. Cluster analysis

Variable	Cluster1	Cluster 2	Cluster 3	p-value
Size	21	21	35	—
Age	7.7	13	3	<0.001
Gender Male (%)	66.7	47.6	63	0.3979
Region Urban (%)	14.3	19	8.6	0.5174
Duration (years)	2.8	2.8	1.8	0.0001
Vitamin B12 Normal (%)	62	33.3	40	0.1405
Folic acid Normal (%)	52.4	66.7	74.3	0.2444
Generic name (%)				
SV	61.9	38.1	54.3	
LEV	23.8	38.1	34.3	0.5152
CBZ	14.3	23.8	11.4	

Cluster Analysis was done and R analysis version 4.1.2 that is used to perform cluster analysis. A scree plot that is drawn to estimate the likely clusters number within the studied population. And from the scree plot, the chosen number of clusters is three and the size of the clusters is 21, 21 and 35 respectively. In k-means cluster analysis this estimate was pre specified and was used as the principal clustering technique. K- is a clustering method used by taking the centroids as a seeds of cluster that is obtained. To compare the differences between clusters, analysis of variance is done using Kruskal-Wallis test for continuous variables and chi-square test for categorical variables. The clusters differ significantly with respect to age and duration and are not significant with respect to any other variables.

Cluster 1 and cluster 2 are of the same size and cluster 3 is the largest among the three clusters. Minimum age group persons belong to cluster 3 (average 3.0 years) and maximum average age group (13.0 years) patients are from cluster 2. Highest normal vitamin B12 levels (62.0%) and highest usage of drug SV (61.9%) is from cluster 1. Cluster 3 has the highest normal folic acid levels (74.3%) and cluster 1 has lowest normal folic acid levels.[Table 4]

DISCUSSION

A study conducted by Verotti et al. [19], it was found that, after one year of therapy with SV and CBZ, there was a significant decrease in the serum Folic acid levels, while the levels of serum Vitamin B12 did not show any significant reduction. This study also included the patients as well as healthy subjects as controls.

Contrary to this study, our study did not find any significant decrease in the levels of Folic acid in patients treated with SV or CBZ monotherapy. Similar to this study, our study is also in accordance with the fact that there is no significant decrease in serum Vitamin B12 in patients treated with SV or CBZ.

In a study conducted by Eldeen et al.[20], it was observed that the mean serum levels of Folic acid had non-significant difference between epileptic patients and controls, while the mean level of Vitamin B12 was significantly higher in epileptic patients compared to that of controls. The mean serum levels of Folic acid and Vitamin B12 had comparable results with non-AED users, AED users and controls. There was a negative correlation between the duration of treatment and Folic acid levels.

In a study conducted by Aslan et al.[21] et al. aimed to find the relation between the drugs carbamazepine, Oxcarbazepine and valproate monotherapy with homocystiene, serum Vitamin B12, erythrocyte Folic acid levels and Folic acid and found that 75% of the patients had low serum Vitamin B12 levels with CBZ monotherapy. The study concluded that long term administration of AED's may result in subnormal levels of serum Vitamin B12 but added that there was no significant correlation between Vitamin B12 and Folic acid and the drugs.

Our study found that there was no significant decrease in the levels of serum Vitamin B12 and Folic acid in patients with SV, CBZ and LEV monotherapy. We also found an association between the treatment duration and normal-deficient levels of Folic acid, irrespective of the drug (p value 0.02107).

In a study conducted by Mahdevi et al.[22], the objective was to find out the effect of Levetiracetam and homocystiene levels, serum Vitamin B12 and Folic acid. The subjects included patients with epilepsy on LEV monotherapy in the age group 18-60 for 6 months and controls. They concluded that there was no significant effect of LEV on homocystiene levels, serum Vitamin B12 and Folic acid.

Our study found similar results, out of all the patients treated with LEV, 68% developed serum Vitamin B12 deficiency while 44% developed serum Folic acid deficiency, although we aimed at studying similar variables on children (<18 years). We also found that proportion of patients treated with LEV developing Vitamin B12 or Folic acid deficiency, irrespective of age and duration of treatment was greater than the same proportion treated with CBZ. There are not many studies of LEV monotherapy on children and it is desirable to have more studies similar to ours.

In a study carried out by Linnebank et al.[23], it was found that patients receiving carbamazepine, gabapentin, Oxcarbazepine, Phenytoin, primidone, or valproate were associated with lower mean serum Folate levels. Vitamin B12 levels were

higher in patients who received valproate compared with the untreated patients, entire group of patients, and among healthy controls. They also concluded that the Vitamin B12 and serum Folic acid levels showed normal values in 95% of the patients after supplementation for 3 months.

The following associations were not found to be statistically significant and more data need to be collected and comprehensively studied. The drugs and serum Vitamin B12 and Folic acid deficiency. Duration of intake of each drug and the normal-deficient values of serum Vitamin B12 and Folic acid. Age and levels of serum Vitamin B12. The gender and levels of serum Vitamin B12 and Folic acid. The drugs and Haemoglobin levels. The treatment duration and Haemoglobin levels.

CONCLUSION

From this study, we conclude that, there is significant association between duration of treatment and levels of serum Vitamin B12 and Folic acid. There is significant association between age and levels of Folic acid. Number of patients developing Folic acid deficiency after receiving any one of the three AED's, irrespective of the duration of treatment, in the age group 5-10 years, are greater than the number in the age group 1-5 years. Number of patients developing Vitamin B12 or Folic acid deficiency after receiving CBZ, irrespective of duration of treatment and age, is less than the number of patient receiving LEV. Number of patients developing serum Folic acid deficiency after receiving LEV, irrespective of the duration of treatment, is greater than the number of patients receiving CBZ. Hence, it is very important to assess the serum Vitamin B12 and Folic acid levels of any child receiving AED's, irrespective of the drug. Clinical signs and Hemoglobin assessment aid in the diagnosis of anemia. Based on the results, appropriate supplementation should be provided to prevent AED induced anemia.

Limitations of the study

Since very little research exists in the literature on each AED, altering the metabolism of Vitamin B12 and Folic acid in children, there is a need for more studies on this topic, so that, if proven, supplementation can be started in due time before the manifestations set in. In resource poor settings it is better to supplement Vitamin B12 and Folic acid to all the children receiving AEDs to socioeconomic and technological constraints to carry out the appropriate laboratory tests. The sample size of our study was 77 children receiving AED. More reliable results can be obtained if such a study is conducted in a larger population.

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Conflict of Interest

There is no conflict of interest.

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