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Serum folic acid levels in epilepsy patients before and after phenytoin therapy

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Abstract

Background & Objectives: Low serum folate level is often reported as an adverse drug sequela of long term phenytoin usage seen with prolonged duration of phenytoin therapy. There is no previous study to prospectively track the serum folate level with usage of phenytoin, which is the objective of this study. *Methods:* Twenty-five patients between the ages of 18-50 years diagnosed to have epilepsy and planning to start phenytoin were recruited in this study. Assessment of serum folic acid was done by chemiluminiscent method prior to the start of phenytoin and after 6 months of treatment. The serum folate level of 10 age and sex matched healthy control was also taken. *Results:* The average serum folate level was 7.48 $\pm$ 2.04 ng/mL prior to the start of phenytoin therapy, which fell to 3.9 $\pm$ 1.95 ng/mL after 6-month of phenytoin therapy (p-value <0.001). The average serum folate level for the age and sex matched 10 control samples was 14.46 $\pm$ 2.81 ng/mL.
Conclusion: A significant fall of serum folic acid levels is seen in epilepsy patients after 6 months treatment with phenytoin.

INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by recurrent seizures of cerebral origin, with episodes of sensory, motor or autonomic manifestation with or without loss of consciousness.[1] A recent meta-analysis of published and unpublished studies puts an overall prevalence rate of epilepsy in India at 5.59 per 1,000 populations.[2] Despite the tremendous advances in the management of epilepsy, phenytoin remains the drug of choice particularly in many developing countries.[3,4] However, long term administration of phenytoin has a number of adverse effects[5,6], one of which is lower serum folic acid levels.[7] While the reason for this remain obscure, several mechanisms have been proposed. One hypothesis is the increased pH of the small intestine, inhibiting the intestinal conjugase activity and impairing the intestinal absorption of folate. Other hypotheses include direct competition between folate and phenytoin for uptake sites, inhibition of folate inter-converting enzymes by phenytoin, increased catabolism of folate by induction of folate catabolic enzymes, inhibition of central appetite centers by phenytoin decreasing food intake, thereby leading to decreased tissue folate concentrations.[8-11] However there are no reported studies which estimate the serum folate levels before the start and following usage of phenytoin. We conducted this study to track the levels of serum folate before and after prolonged administration of phenytoin.

METHODS

The study subjects consisted of epilepsy patients who consulted the Department of Neurology, Victoria Hospital, Bangalore during the period of Jan 2009 to Dec 2009. The inclusion criteria were: (1) Age 18-50 years; (2) Patients who were being started phenytoin. The exclusion criteria were: (1) Patients with other systemic diseases; (2) Patients on any type of pharmacologic therapy including multi-vitamins or, folate supplements for treatment of megaloblastic anaemia or, who were on folate antagonists; (3) Patients who may have increased requirements of folic acid, such as those who were pregnant or expected to conceive. We also estimated the serum folate level of 10 control patients. The controls were age and sex matched to the patients and were healthy. Ethical clearance was obtained by the ethical committee of the institution and the Bangalore Medical College

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Table 1: Serum folate levels before and after phenytoin therapy in ng/ml

Folate Level	Mean	Std dev	Min	Max	Range
Overall	7.48	2.04	3.87	10.9	7.03
Male (Before)	7.16	2.19	3.87	10.9	7.03
Female (Before)	8.49	1.06	6.62	9.39	2.77
Overall	3.90	1.95	2.02	8.71	6.69
Male (After)	3.63	2.01	2.02	8.71	6.69
Female (After)	4.77	1.58	2.46	7.43	4.97

and Research Institute and Associated Hospitals. Written informed consents were obtained before the study.

The study patients were subjected to detailed history and thorough clinical examination using a specially prepared proforma. Routine hematological examination, with determination of the serum folic acid level was done. None of the patients and controls was on folate or, any other nutritional supplements during the duration of the study. The serum folic acid level was repeated at the end of 6 months of phenytoin therapy. The serum folic acid level was obtained before the morning dose of phenytoin.

Assessment of serum folic acid level was done by chemiluminiscent method using Immulite kit prior to the start of phenytoin therapy. For this, following an overnight fasting period, 5 ml of venous blood was taken from patients from the antecubital vein using a sterile disposable syringe in the sitting position between 8 A.M. and 10 A.M. Serum was immediately separated by ultracentrifugation. The supernatant was discarded and the rest of the sample was stored at -20 degrees Celsius. The Flurometer was set at 370 nm excitation with emission monitored at 470 nm. Flow rate was adjusted as 1.3 ml/min.

The statistical analysis was done using paired t-test.

RESULTS

The study consisted of 25 patients, 19 male (76%) and 6 female (24%), with 10 controls. The mean age of the study patients was 30.1 years (range 18-50 years). The mean age of the male patients was 30.3 years (range 18-50 years), and mean for the females was 29.5 years (range 20-36 years).

The average serum folate level was 7.48 $\pm$ 2.04 ng/mL prior to the start of phenytoin therapy with an average serum folate level of 7.16 $\pm$ 2.19 ng/mL for the male and 8.49 $\pm$ 1.06 ng/mL for the female patients. After 6 months of phenytoin therapy, the average serum folate level was 3.9 $\pm$ 1.95 ng/mL, 3.63 $\pm$ 2.01 ng/mL for the male and 4.77 $\pm$ 1.58 ng/mL for the females. The average serum folate level for 10 control subjects was 14.46 $\pm$ 2.81 ng /mL. (Table 1) The reduction in mean serum folate levels before and after 6 months of phenytoin treatment was statistically significant with a *p*-value of <0.001. (Table 2)

DISCUSSION

In our study, assessment of serum folate level was done by chemiluminiscent method using Immulite kit prior to the start of and after 6 months of phenytoin therapy. A number of methods can be used to quantify folate, including microbiologic methods, assays using folate-binding protein, and various chromatographic techniques.[12-16] Most current commercial assays use folate-binding protein with chemiluminescence detection.[17]Other significant methods used to assess serum folate that have been described earlier in the literature included the immunoassay method and the less reliable and a relatively less sensitive assay of serum folate levels using *Lactobacillus casei* as

Table 2: Comparison of mean serum folate levels in ng/ml in the test group before and after 6 months of phenytoin therapy

Group	n	Mean	Std dev	Mean difference	t	P-Value
Before treatment	25	7.48	2.04	3.574	10.242	<0.001
After treatment	25	3.90	1.95			

the test organism.

Average range of serum folate levels in normal controls as standardized by few studies has been found to be 3-17 ng/mL. Normal range of serum folate in healthy adults as estimated by the *Lactobacillus casei* method, on the other hand, has been standardized to be 2.5-15 ng/mL.[8-11] The wide variations found in the mean and average range of serum folate levels in different age groups and genders from the previous studies likely reflected the differences among the study samples in terms of age and health status. It could also be contributed by the differences in the procedures used in serum folate assessment which were characterized by marked differences in their sensitivities.

There is substantial analytical variation however between the different assay methods. Several studies have reported different results between microbiological method and radioassays.[18-20] Inter-laboratory comparisons of serum and red cell folate concentrations using 4 different assay methodologies showed considerable variation between the different methods (overall coefficient of variation 18-41%) and between different laboratories using a similar assay kit/protocol.[21] Gunter *et al*. also showed large intra- and inter-method variations among 20 participating laboratories using 7 different types of assays analysing a common series of serum and whole blood pools. The greatest variations were found at low and high concentrations of folate.[22]

The agreement between the different assays is also dependent on the composition of the blood sample used for folate measurement and the genotype of the individual. It has been demonstrated that the radioassay under-recovers 5-MTHF but correctly recovers folic acid[23]; differential recovery was also observed for other reduced folate forms.

It has been shown that the patients with epilepsy have lower serum folate levels than the general population, even without any treatment.[24] This suggests that there is a direct correlation between serum folate levels and epilepsy, independent of anti-epileptic drugs. The postulations include poor socioeconomic status associated with epilepsy as well as poor nutrition leading to low serum folate levels obtained in this set of patients. The difference in the methods used in the estimation may also contribute to this difference. Our study by tracking the folate in epilepsy patient before and after starting phenytoin, demonstrate that the drop in folate level is indeed due to the use of phenytoin.

In view of the result of our study, folic acid supplement should be administered to patients who are on long term phenytoin therapy. On the other hand, folic acid use has been blamed for a decrease in the serum concentration of phenytoin resulting in increased seizures.[25-8] Serum folate has even been proposed to have direct excitatory effects on the nervous system.[29-30] Thus, the use of folic acid as an adjuvant to phenytoin mandates further study to guide daily clinical practice.

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DISCLOSURE

Conflict of interest: None

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