International Journal of Contemporary Pediatrics
Deopa B et al. Int J Contemp Pediatr. 2020 Jul;7(7):1554-1557
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20202615

Original Research Article

An observational study to determine the status of serum and RBC folate in drug resistant epileptic children at a tertiary care centre in Western Rajasthan, India

Bindu Deopa*, Bhawna Chaudhary, Ashish Gupta

Department of Pediatrics, GMC Haldwani, Uttarakhand, India

Received: 03 May 2020
Accepted: 27 May 2020

***Correspondence:**
Dr. Bindu Deopa,
E-mail: bindu.deopa@gmail.com

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ABSTRACT

Background: Drug resistant epilepsies constitute about 10-20% of childhood epilepsies and treated with higher doses and multiple AEDs. AEDs increases folate metabolism by enzyme induction thus causes deficiency of folic acid. Objective was to evaluate the effect on serum and RBC folate in children having drug resistant epilepsy.
Methods: In a prospective observational study 83 drug resistant epileptic children of age 6months to 180 months fulfilled the inclusion criteria enrolled in study, from Oct 2014 to Nov 2016 for a period of two years. Serum and RBC folate levels were done in these children. Epileptic children already receiving folic acid supplementation/treatment were excluded from the study. Children with serum folate level <5ng/ml and RBC folate <280ng/ml was considered as folate deficiency.
Results: Total 83 children had drug resistant epilepsy (defined by ILAE). Mean age of children with drug resistant epilepsy was 71.39±49.76 months. 71.08 % were male and 28.91% were female. Mean serum folate in these children was 7.75±2.77 ng/ml and RBC folate 381.63±164.54 ng/ml which was significantly lower as compared to healthy children or epileptic not receiving AEDs. 14.45 % children in drug resistant epilepsy had serum folate <5ng/ml while 20.89% were found to be RBC folate deficient (RBC level <280ng/ml).
Conclusions: Antiepileptic drugs are associated with lower blood folate status which deteriorates further with increasing number and doses of AEDs in drug resistant patients. Therefore blood folate monitoring should be done in all children on AEDs on regular intervals and should be considered in the etiologic differentials of drug resistant epilepsy.

Keywords: Drug resistant epilepsy, Folic acid, Refractory seizure

INTRODUCTION

Epilepsy is a chronic noncommunicable disorder of brain and can affects the people of all age group. Approximately 50 million people currently live with epilepsy worldwide and it accounts for 0.75% of global burden of disease.[1] Drug resistant epilepsies constitute about 10-20% of childhood epilepsies. In infancy and early childhood, epileptic encephalopathies such as Lennox-Gastaut syndrome (LGS), West's syndrome, Dravet syndrome, etc. tend to be drug resistant to treatment.[2] Anti-epileptic drugs (AEDs) are the main form of treatment for people with epilepsy. Folate deficiency occurring after anticonvulsant therapy might result from increased metabolic requirements for folate by the liver as a result of increased activity of drug-metabolizing enzymes.[3] Folic acid is required for the synthesis of S-adenosylhomocysteine, which is extremely important for the further biosynthesis of brain neurotransmitters (serotonin and dopamine) and

phospholipids. It's deficiency can precipitate to low homocysteine levels which having epileptogenic potential.[4] Therefore our research question is whether drug resistant epilepsy associated with low serum and RBC folate levels. And our hypothesis is drug resistant epileptic children have low folate level. Objectives of the study to find out the serum and RBC folate level in drug resistant epileptic children.

METHODS

The current study was a prospective observational study conducted at the Pediatric Neurology Clinic, Department of Pediatrics, Dr S N Medical College, Jodhpur from Oct 2014 to Nov 2016 for a period of two years. Children aged 6 month to 180 months with drug resistant epilepsy (Case definition as per ILAE and outlined below) were enrolled in the current study.

The diagnosis of all these patients was reviewed and confirmed by a trained Paediatric Neurologist. Epileptic children already receiving folate supplementation/treatment were excluded from the study. Children with serum folate level <5ng/ml and RBC folate <280ng/ml was considered as folate deficiency.[5]

Case definitions

Epilepsy- Occurrence of at least 1 unprovoked epileptic seizure with either a second such seizure or enough EEG and clinical information to convincingly demonstrate an enduring predisposition to develop recurrences.[6]

Drug resistant epilepsy-Defined as failure of adequate trials of two tolerated and appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom (ILAE).[7]

Detailed clinic-epidemiologic information of all the patients, including history of frequency, duration and type of seizures, details of antiepileptic therapy were recorded in a predesigned proforma. Serum folate and RBC folate levels were performed at the time of enrolment in drug resistant epileptic children. Epileptic children not on AEDs and healthy children were taken as control.

Consent and ethical approval

The present study was approved by the scientific and ethics committees of our institution. All parents had signed an informed consent form to participate in the study.

Laboratory method

Folate assay (serum) was performed on immunoassay analyzer (SIEMENS ADVIA CENTAUR CP) with chemiluminescence competitive assay. Coefficient of variation for between days impression for both analytes was below 6%. External quality control was carried out with the EQAS immunoassay programme from Bio-Rad USA. The same method was used for RBC folate levels after preparation of a haemolysate. 100µL of EDTA anticoagulant blood was collected into an EDTA vacunator tube and treated with 2000 µL freshly prepared 0.2% ascorbate solution (200mg, ascorbic acid /100ml AD). The resulting hemolysate was assayed with the following formula: RBC folate (µg/L)=hemolysate folate × 2100 / hematocrit (%).

Outcome measures

- To find out the serum and RBC folate status in drug resistant epileptic children.
- Morphology of RBC on peripheral blood film.

Statistical analysis

The data were analysed with the help of Microsoft Excel 2007 and window SPSS version 23. Numerical data were expressed as mean±SD. Median and categorical data were expressed as percentage.

ANOVA test used to evaluate the difference in quantitative variables. The appropriate p value was calculated and difference between the two values was considered to be significant if p value was <0.05.

RESULTS

Total 83 children had drug resistant epilepsy (defined by ILAE). Mean age of children with drug resistant epilepsy was 71.39±49.76 months. 71.08 % were male and 28.91% were female, 83.13% (69) of children in study group had generalised seizures, while 16.87% (14) had focal seizures.

Mean duration of use of antiepileptic was 19.80±18.56 months. Mean serum folate in these children was 7.75±2.77 ng/ml and RBC folate 381.63±164.54 ng/ml. Mean serum and RBC folate level was significantly lower in children with drug resistant epilepsy as compared to age and gender matched epileptic not receiving AEDs and healthy controls (Table 1).

About 14.45 % children in drug resistant epilepsy had serum folate <5ng/ml while 20.89% were found to be RBC folate deficient (RBC level <280ng/ml). On peripheral blood film examination macrocytosis was seen in 3.61% children having Serum folate levels less than 3ng/ml and RBC folate levels less than 240ng/ml. Mean haemoglobin level was significantly lower in children with drug resistant epilepsy as compared to control group (p value <0.05), while MCV was significantly higher in study group as compared to control group (p value <0.05) (Table 2).

Table 1: Comparison of mean serum and RBC folate level among various groups.

Group (n)	Serum folate ng/ml (mean±SD)	RBC folate ng/ml (mean±SD)
Drug resistant epilepsy (83)	7.75±2.77	381.63±164.54
Healthy (46)	12.62±4.88	500.78±150.42
Epileptic not on AEDs (31)	11.43±4.27	472±168.72

p value, serum folate of drug resistant vs healthy control <0.0001, drug resistant vs children not receiving AEDs <0.0001.
RBC folate: drug resistant vs healthy control 0.034, drug resistant vs epileptic not on AEDs 0.045

Table 2: Comparison of various hematologic and biochemical parameters between the study and control group.

Biochemical parameters	Study group	Control group	p value
	Total (n=83) Mean±SD	Total (n=77) Mean±SD	
Haemoglobin (g/dl)	10.57±1.71	11.17±1.62	0.003
Platelets (lacs)	274385.71±117104	302250±129979	0.06
MCV(FL)	81.74±5.69	73.27±7.29	0.013

DISCUSSION

Most of the anticonvulsant drugs are powerful enzyme-inducing agents and lead to folate deficiency on a long term use. In this study, mean serum folate level of children receiving AEDs was significantly lower compared to control group. This is supported by studies done by Ufuk Sener et al, Karabiber et al.[8,9] However in our study mean folate level was higher compared to world literature. In most of the other studies had a small sample size (<40 in each group) and monotherapy patients were taken, therefore the observations may not be very representative. Also in our study only drug resistant epileptic patients were taken who already receiving combination of older and newer AED that could be a cause of higher mean folate level in current study as newer drugs having less effect on folic acid metabolism.

Folic acid acts as a carbon carrier in the formation of heme (iron containing protein in hemoglobin) and is necessary for the formation of red blood cells. Folic acid is required for DNA synthesis and cell growth and is important for red blood cell formation.[10] Out of 83 drug resistant epilepsy patient 3 had Macrocytic anemia in current study. These observations are supported by a WHO report which mentioned that folate concentrations in serum <3ng/ml and red blood cell folate level <100 ng/ml in all age groups was associated with macrocytic anaemia.[11] Similar observation was seen by B Toprak et al, and Linnebank et al, who reported that macrocytic anemia occurs when serum folate levels below the reference range (<3ng/ml) are associated with higher mean corpuscular volume (MCV) in there study group.[12,13]

As per our current study, it was recommend that there should be regular monitoring of serum folate levels in children receiving long term AEDs. Macrocytosis usually appears in Peripheral blood smear after folate level in serum reduces to <3 ng/ml and therefore blood folate levels are more reliable.

CONCLUSION

Antiepileptic drugs are associated with lower blood folate status which deteriorates further with increasing number and doses of AEDs in drug resistant epileptic patients. Therefore blood folate monitoring should be done in all children on AEDs on regular intervals and should be considered in the etiologic differentials of drug resistant epilepsy.

ACKNOWLEDGEMENTS

Authors would like to thank Dr Manish Parakh professor Dr. SNMC Jodhpur for esteemed support and guidance.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Deopa B, Chaudhary B, Gupta A. An observational study to determine the status of serum and RBC folate in drug resistant epileptic children at a tertiary care centre in Western Rajasthan, India. Int J Contemp Pediatr 2020;7:1554-7.