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Original article:

Role of vitamin B12 and folic acid deficiency as risk factors in pediatric patients of febrile seizures in a tertiary care centre

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Abstract

Objective- To study the association of Vitamin B12 and folic acid deficiency as risk factors in Febrile seizures.

Material and Methods-It was a Hospital based analytical cross-sectional study conducted in the Department of Pediatrics of SGT Hospital. Sample size comprised of two groups each comprising of 35 children each. Children with Febrile seizures (FS) in Group A/ Study Group and with febrile illnesses without seizures in Group B/ Control Group. All children in the age group of 6 months-60 months admitted to the department of Pediatrics in SGT Hospital with seizures and fever (temperature >38^{o}C or >100.4^{o}F) who were without neurologic abnormality by examination or by developmental history and other febrile illnesses were included as cases by consecutive sampling. Age and sex-matched children admitted with fever without CNS infection were included as controls. Children were enrolled in the study after taking informed written consent from the parents. Ethical clearance was also taken from institutional ethical committee.

Results- Mean serum B12 in study group was 222 pg/ml $\pm$ 86.7 while that in controls was 305 pg/ml $\pm$ 80.7 ($p< 0.001$). Mean serum folic acid in study group was 13$\pm$5.86 ng/ml while that in controls was11.7$\pm$4.41 ng/ml. With a 95% confidence interval odds ratio was found to be >1 which signifies that the children with B12 deficiency are more likely to develop FS than the children who do not have vitamin B12 deficiency.

Conclusion-The study suggests that vitamin B12 deficiency may be a risk factor for febrile seizures whereas Folic acid deficiency was not found to be of any significant value in patients with FS. Screening for vitamin B12 deficiency should be considered in children with FS as we may be able to reduce the incidence of febrile seizures by Vitamin B12 supplementation.

Keywords- Febrile seizures, serum Vitamin B12, serum Folic acid

Introduction

Febrile seizures (FS) are the most common seizure disorder during childhood.[1]They are age-dependent and occur in 2 to 5% of paediatric population younger than age 5 years with highest incidence in the second year of life[2]. They are commonest brain-related disease in children, yet its exact etiopathogenesis is unknown. Genetic and environmental factors, like micronutrient deficiency and immunologic reactions, are thought to be involved.[3]The prognosis of FS has been found to be favorable and they are not related to any brain damage and epilepsy but

develop in only a small number of paediatric population who previously had FS.[1-5]Vitamin B12 is a water-soluble vitamin that is essential for growth and development inhumans, and that must be supplied by diet. Neurological symptoms of vitamin B12 deficiency are heterogeneous and includes irritability, lethargy, apathy, regression of neuromotor development, and convulsions.[6,7]Developmental mechanisms of neurological symptoms are not fully known in vitamin B12 deficiency. But, vitamin B12 plays a role as a cofactor in homocysteine remethylation and methyl malonyl CoA degradation. It is thought that methionine synthesis is interrupted and guanidinoacetate accumulation leads to neurotoxicity in vitamin B12 deficiency. Homocysteine and methylmalonic acid levels increase in vitamin B12 deficiency. These parameters are playing a role in demyelination, axonal degeneration, and neuronal death.[8]Folic acid is essential for DNA replication and reconversion of homocysteine to methionine.As a major source of methyl groups, folic acid is also involved in epigenetic methylation reactions.[9] Moreover, a decrease of brain oxygenation has been linked to folic acid deficiency, which may influence seizure activity.[10] Deficiency appears to start very soon after birth, with poor maternal status, low concentrations of the vitamin in breastmilk, and a low intake of animal productsas likely risk factors.[8,9]Our aim in this study is to assess for an association between vitamin B12 and folic acid deficiency as risk factors for FS. Research regarding the association of vitamin B12, Folic acid deficiency, and seizures has shown scant literature with conflicting results, with most comparing only the iron status in children with FS.

Material and Methods

It was a Hospital based analytical crossectional study conducted in the Department of Paediatrics of SGT Hospital, Gurgaon for 18 months. Sample size comprised of Two groups each comprising a minimum of 35 children each. Children with FS in Group A/ Study Group and with febrile illnesses without seizures in Group B/ Control Group.All children in the age group of 6 months-60 months admitted to the department of Paediatrics in SGT Hospital Gurgaon with seizures and fever (temperature >38^{o}C or >100.4^{o}F) who were without neurologic abnormality by examination or by developmental history and other febrile illnesses were included as cases by consecutive sampling. Exclusion criteria included children with CNS infections, seizure disorders due to other causes, confirmed neurological illness, head Injury, developmental delay and cerebral palsy, children on iron, B12, and folic acid therapy, history of blood transfusion in last 6 months, haemolytic anaemia, chronic metabolic disease, cardiac disease, kidney disease, malabsorption syndrome, hypoxic-ischemic encephalopathy, anticonvulsant therapy, metabolic causes of seizures. Age and sex-matched children admitted with fever without CNS infection were included as controls. Children were enrolled in the study only after taking informed written consent from the parents and ethical clearance from institutional ethical committee.

The diagnostic threshold for anemia was Hb <11 gm.dl. Normal ranges of the following parameters were considered.[11,24]

1. MCV – 76-104 fl
2. MCH – 27-32 pg
3. Serum B12 - 200 - 500 pg/ml
4. Serum Folic acid - 5 - 20 ng/ml

Under strict aseptic precautions, single fasting blood sample was collected from all participants at the time of admission within 24 hrs of the onset of the seizure. A total of 5ml of blood was taken in 2 vials, 2ml in EDTA(Purple) vial, and 3ml in Plain(Red) vial. Blood samples in EDTA vial for measurement of Hemoglobin (Hb), Haematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hb (MCH), Mean Corpuscular Hb Concentration (MCHC), Peripheral Smear, and in Plain vial for Serum B12, Serum Folic acid, Serum Calcium, Serum Potassium, Serum Sodium, and Blood sugar. Collected samples were centrifuged at 2000-2500 rpm for 10 min and serum separated immediately and stored at -20^{o} Celsius in the deep freezer for estimation of vitamin B12 and Folic Acid. The Serum vitamin B12 and folic acid testing were done via Chemo luminescent immunoassay (CLIA) using commercial kits on Maglumi 800 Analyzer. Reference Range of serum B12 that was followed was 200-500 pg/ml and that for folate was 5.21-20 ng/ml. [24] A complete hemogram was obtained using the SYSMEX KX-cell counter. Peripheral smear for blood picture was stained using Leishmann's stain and was examined with a lab microscope by oil immersion field.

Data was entered in Microsoft excel spreadsheet and descriptive statistics were analysed with SPSS (Statistical Package for Social Sciences) version 21.0 software. Continuous variables were presented as mean $\pm$ SD and categorical variables were presented as frequencies and percentages (%).The Pearson's chi- square test or Fisher's exact test was used to determine the relationship between the two qualitative categorical variables. Unpaired t test was used for quantitative variables. p value less than 0.05 was considered statistically significant.

Results

Thirty-five cases of FS and thirty-five controls were analyzed in this study. All the patients in study group had generalized tonic-clonic type of seizures.

Out of 35 cases in study group, 26 (74.3%) had no family history of FS as compared to 33 (94.3%) controls, and 9 (25.7%) cases had a family history of FS as compared to only 2 (5.7%) controls and p-value being significant. In study group, 27 (77.1%) had a FS once and 8 (22.9%) had FS twice, 24 (68.6%) had duration of seizure <5 minutes, 6 (17.1%) had seizure for 5 – 10 minutes, and 5 (14.3%) had seizure for 10 – 15 minutes. In this study, 12 out of 15 females (80%) as compared to 12 out of 20 males (60%) had a seizure of <5 minutes duration, 2 out of 15 females (13.3%) as compared to 4 out of 20 males (20%) had seizure lasting for 5 – 10 minutes and only 1/15 female (6.7%) as compared to 4/20 males (20%) had seizure lasting 10 – 15 minutes. 4/35 cases (11.4%) had temperature between 100-100.9^{0}C, 16/35 cases (45.7%) had temperature between 101-101.9^{0}C, 13/35 cases (37.1%) had temperature between 102-102.9^{0}C and 2/35 cases (5.7%) had temperature between 103-103.9^{0}C at the time of seizure. Mean temperature in study group was 102^{0}C $\pm$ 0.743. In this study, 25 out of 35 cases (71.4%) had fever due to URTI (upper respiratory tract infection), 9(25.7%) had fever following AGE (acute gastroenteritis) whereas 1 (2.9%) had fever following vaccination. Upper respiratory tract infection was the predominant cause of fever in cases 25/35 (71.4%) as well as controls 19/35 (54.3%) followed by acute gastroenteritis in cases 9/35 (25.7%) and acute gastroenteritis and lower respiratory tract infections in controls and 1 case (2.9%) had fever following vaccination which was not a causative factor in controls.In this study, all the cases had uprolling of eyes associated with the seizure but only 6 (17.1%) cases also had a deviation of angle of mouth with the seizure. With a 95% confidence

interval odds ratio was found to be >1 which signifies that the children with B12 deficiency are more likely to develop FS than the children who do not have vitamin B12 deficiency.

Table 1. Demographic data of cases and controls

	Study group (n=35)	**Control group(n=35)**	**p value**
Age group (in months)			
6-12			
13-24	7(20.0)	9(25.7)	0.694
25-36	14(40.0)	10(28.6)	
37-48	6(17.1)	10(28.6)	
49-60	5(14.3)	4(11.4)	
Sex	3(8.6)	2(5.7)	0.339
Female			
Male			
Socioeconomic status	15(42.9)	19(54.3)	
	20(57.1)	16(45.7)	
Upper			0.848
Upper middle			
Lower middle	0	0	
Upper lower	7(20)	10(28.6)	
Lower	16(45.7)	14(40)	
	8(22.9)	8(22.9)	
	4(11.4)	3(8.6)	

Table 2- Characteristics of Febrile episodes of the cases and controls

	Study group (n=35)	**Control group (n=35)**	**p value**
Family History			
No	26(74.3)	33(94.3)	0.022
Yes	9(25.7)	2(5.7)	
Temperature			
100-100.9	4(11.4)	4(11.4)	
101-101.9	16(45.7)	16(45.7)	0.841
102-102.9	13(37.1)	11(31.4)	
103-103.9	2(5.7)	4(11.4)	
Etiology			

URTI	25(71.4)	19(54.3)	
AGE	9(25.7)	8(22.9)	0.020
Vaccination	1(2.9)	0(0)	
LRTI	0(0)	8(22.9)	
Duration			
<5 minutes	24(68.6)		
5-10 minutes	6(17.1)		<0.001
10-15 minutes	5(14.3)		
Day			
Day 1	20(57.1)		
Day 2	13(37.1)		<0.001
Day 3	2(5.7)		

Table 3-Hematological and biochemical investigations of cases and control

	Study group (n=35)	Control group(n=35)	**p value**
Hemoglobin (gm/dL)			
7-8.5	16(45.7)	1(2.9)	
8.6-10	7(20.0)	11(31.4)	< .001
10.1-11.5	7(20.0)	10(28.6)	
11.6-13	5(14.3)	13(37.1)	
Peripheral blood film			
Macrocytic hyperchromic	15(42.9)	3(8.6)	
Normocytic normochromic	14(40.0)	28(80)	0 .001
Microcytic hypochromic	6(17.1)	4(11.4)	
Serum B12(pg/ml)			
100-200	17(48.6)	4(11.4)	
201-300	12(34.3)	10(28.6)	< .001
301-400	4(11.4)	16(45.7)	
401-500	2(5.7)	5(14.3)	
Serum Folic Acid(ng/ml)			
<5			

5.1-10	2(5.7)	0(0)	
10.1-15	10(28.6)	14(40)	
15.1-20	11(31.4)	12(34.3)	0.487
>20	8(22.9)	7(20)	
MCV±SD	4(11.4)	2(5.7)	
MCH±SD	85.6±14	75.8±7.14	<0.001
	29.6±7.42	24.9±3.34	

With a 95% confidence interval odds ratio was found to be <1 which signifies that the children with Folic acid deficiency are less likely to develop FS than the children who do not have Folic acid deficiency.

Table 4-Odds ratio of Vitamin B12 and folic acid

		95% Confidence Intervals of Vitamin B12	
	Value	**Lower**	**Upper**
Odds ratio	7.32	2.13	25.2
Relative risk	2.20	1.45	3.36

		95% Confidence Intervals of Folic acid	
	Value	**Lower**	**Upper**
Odds ratio	0.189	0.00874	4.08
Relative risk	0.485	0.380	0.620

Discussion

A febrile seizure is one of the most common benign neurological conditions of childhood.[1] Two to 5% of children under the age of 5 years experience febrile seizures.[2] Although most FS are benign, one-third of them are complex with prolonged duration (>15min.) and are associated with a risk of subsequent temporal lobe epilepsy.[12] Although several susceptibility genes associated with fever-induced convulsions have been identified, the precise path physiologic mechanisms that trigger a FS is unclear.[13]

The shorter the duration of recognized fever, the higher the chances of recurrence. In our study, 57.1% had a seizure on day 1 of fever, 37.1% had a seizure on day 2 of fever and 5.7% had a seizure on day 3 of fever. It is seen that in infants in whom FS occur at the onset of fever, they have the highest risk of recurrence and thus, have implications for prophylactic strategies that rely on giving medications at the onset of febrile. Millichap *et al* have postulated a convulsive threshold beyond which the seizure is precipitated.[14] Berg *et al* found 44% of infants had experienced less than 1hr of fever at the time of their FS, only 13% had fever more than 24 hours duration. In the present study

57.1% of the cases presented within 24 hours from the onset of fever which was almost similar to the above-quoted studies.[15]

Upper respiratory tract infection is the commonest trigger of FS in the present group of children. This is in keeping with Azhar S Daoud *et al* from Jordan who reported URTI is a commonest triggering factor, diagnosed in 53% of the cases, which is comparable to present study.[16]Farwell *et al* had shown that in up to one-third of the cases of FS, the cause of fever is unknown.[17]However, the etiology of a fever varies from country to country due to different infection profile. Diphtheria, whole-cell pertussis and tetanus toxoid vaccine, and measles, mumps, rubella vaccine has been reported to be associated with a transiently increased risk of a FS on the day of vaccination and 8-14 days after vaccination respectively as shown by Offringa*et al* and Millichap *et al*.[14,23] According to Hertz and Nelson vaccination constitute only 2.2% of the FS.[18] One (2.9%) case had fever following vaccination in the present study.

Vitamin B12 deficiency can potentially cause severe and irreversible damage, especially to the brain and nervous system. At levels only slightly lower than normal, a range of symptoms such as fatigue, depression, and poor memory may be experienced.[8]Megaloblastic anemia (pernicious anemia) which results from inhibition of DNA synthesis in red blood cell production is often due to deficiency of vitamin B12 and/or folic acid. One study had indicated that low levels of vitamin B12 may be a factor in provoking seizures, but it was unclear whether vitamin B12, folic acid, and/or homocysteine played such a role in the FS.[19]In our study, 42.9% of cases had a macrocytic hyper chromic picture, 40%had a normocytic norm chromic picture, 17.1% had a microcytic hypo chromic picture on peripheral blood film. This suggests that the macrocytic hyper chromic picture was more in the study group as compared to the control group.

Our study also showed that 48.6% cases as compared to 11.4% controls were deficient in vitamin B12. On the other hand, only 5.7% of cases had Folic acid deficiency, 80.9% of cases had normal levels of serum Folic acid, and 11.4% had a high level of serum Folic acid in contrast to none of the children in the control group having Folic acid deficiency. Out of total 70 children in the study, 30% had vitamin B12 deficiency and 2.8% had Folic acid deficiency.One study found that folic acid and vitamin B12 levels in the serum and cerebrospinal fluid of 40 febrile paediatric patients and healthy control children.The authors observed significantly lower serum vitamin B12 levels in the 40 febrile children than in the controls. Besides, they observed no difference in cerebrospinal fluid vitamin B12 levels between their FS group and non-seizure group. [20]

Initially, it was assumed that virtually all individuals with vitamin B12 deficiency would have macrocytic anemia. However, research has shown that 30% of such patients have Hb, hematocrit, and MCV all within reference ranges.[6,7,21]In the setting of vitamin B12 deficiency, macrocytic anemia develops as a result of disrupted DNA synthesis and resultant maturation disorder of the red cell nucleus, whereas the cytoplasm develops normally.[21]Recently another study found that the mean vitamin B12 level in the FS group was significantly lower than the control group. Low serum vitamin B12 may reduce a child's threshold for seizure and may be a risk factor for FS. They observed no significant differences between their FS group and febrile control children concerning mean Hb or mean MCV. Only 1 of their 104 paediatric FS patients had macrocytic anemia. They detected no statistical difference in mean folic acid level between the FS group and the control group.[22]Similar results were found in the present study.

However, there are some limitations of this study which we want to highlight before extrapolating our results for clinical use. FS usually occur at home and by the time patients are brought to the hospital, it is already too late to have the samples in the critical period of time (ictal or early postictal period). Since it was an observational study therefore it carries all the limitations of this type of study design. The study subjects were only from a single tertiary care center. Multicentric trials with a larger sample size are required to further confirm the etiological role and association of vitamin B12 and Folic acid deficiency with FS.

Conclusion and recommendations:

The results suggest that vitamin B12 deficiency may be a risk factor for febrile seizures whereas Folic acid deficiency was not found to be of any significant value in patients with FS.Fever can worsen the negative impact of anemia or vitamin B12 deficiency on the brain and a seizure can occur as a consequence. Alternatively, anemia can be associated with the severity of a febrile illness, and more severe cases could be more likely to get seizures.Screening for vitamin B12 deficiency should be considered in children with FS because if B12 deficiency in the febrile seizures prone age group can be prevented, we may be able to reduce the incidence of febrile seizures, thereby reducing unnecessary hospital admission and associated cost.

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