

Serum vitamin B₁₂, folic acid, and homocysteine levels in children with febrile seizure

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Received: 14 January 2015, Revised: 19 February 2015, Accepted: 20 March 2015

SUMMARY: Özkale Y, Erol İ, Kılıçarslan B, Özkale M, Saygı S, Sarıtürk Ç, Sezgin N. Serum vitamin B₁₂, folic acid, and homocysteine levels in children with febrile seizure. *Turk J Pediatr* 2015; 57: 345-352.

The aim of this study is to investigate the associations between febrile seizure and serum levels of vitamin B₁₂, folic acid, and homocysteine. One hundred and four children who presented with febrile seizure and 75 controls who presented with febrile illness unaccompanied by seizure were enrolled into the study. Mean levels of vitamin B₁₂, folic acid and homocysteine were compared between two groups. Mean vitamin B₁₂ level in the febrile seizure group was significantly lower than the control group. The febrile seizure patients with 3 or more had significantly lower serum folic acid than the subgroups with two or one episode only. Serum concentrations of folic acid were significantly lower in the febrile seizure subgroup with body temperature 37.5-39.0°C at time of convulsion. Low serum vitamin B₁₂ may reduce a child's threshold for seizure and may be a risk factor for febrile seizure. Low serum folic acid level may be predisposed to recurrent febrile seizure.

Key words: febrile seizure, vitamin B₁₂, folic acid, homocysteine.

Febrile seizure (FS) is one of the most common problems in pediatric practice. The International League Against Epilepsy (ILAE) defines FS as seizures that i) occur in children older than 1 month (typically 3 months to 6 years of age), ii) are associated with febrile illness unrelated to infection of the central nervous system, iii) are not associated with previous neonatal or unprovoked seizure, and iv) do not meet the criteria for other acute symptomatic seizures.^{1,2} Febrile seizure is classified as "simple" if the seizures are brief (15 minutes or less) with no lateralizing features, and as "complex" if the seizures are longer than 15 minutes, have focal features, or recur within 24 hours.³⁻⁵ Febrile status epilepticus (FSE) is defined as a seizure episode 30 minutes or longer in which the patient does not fully regain consciousness between seizures.¹ Febrile seizure is the most common brain-related disease in children, yet its precise etiopathogenesis is unknown. Genetic and environmental factors, such as micronutrient deficiency and immunologic reactions, are thought to be involved.

Several studies have examined the potential roles that neurotransmitters and changes in trace element contents of biological fluids play in the pathogenesis of FS.⁶⁻⁹ Some investigations have revealed significantly lower serum zinc levels in patients with FS compared to febrile children without seizure.^{7,10,11} Other authors have observed significantly higher rates of iron deficiency anemia in children with FS than in febrile children without seizure.^{6,12-14} Some small studies have indicated that low levels of vitamin B₁₂ may be a factor in provoking seizure, but it is still unclear whether vitamin B₁₂, folic acid, and/or homocysteine play such a role in FS.^{15,16} Our aim in this study was to assess for associations between FS and serum levels of vitamin B₁₂, folic acid, and homocysteine.

Our study is approved by Baskent University Institutional Review Board and Ethics Committee. Written informed consent was obtained from the parents of all participants. All febrile children who were admitted to Baskent University Adana Hospital between

October 2012 and January 2013 with or without seizures were eligible to participate. The exclusion criteria were as follows: infection of the central nervous system or any confirmed neurological illness; developmental delay; history of documented vitamin B₁₂ deficiency anemia; regular blood transfusion or regular therapeutic doses of vitamin B₁₂ supplements; chronic metabolic disease; cardiac disease; kidney disease; malabsorption syndrome; current prescription for anticonvulsant therapy. Based on these criteria, 179 patients were enrolled: 104 febrile children with seizure (the FS group) and 75 febrile children without seizure who had similar age distribution (the control group).

All 104 patients in the FS group underwent a comprehensive clinical evaluation. Each of these children underwent a diagnostic workup with clinical and laboratory techniques recommended by ILAE, and electroencephalography (EEG) and computed tomography and magnetic resonance imaging studies of the brain were performed when clinically indicated. For febrile seizure patients, frequency of febrile seizure episodes previous to the study was obtained from the parents. Type of FS at presentation, history of FS or epilepsy, findings regarding any underlying illness and frequency of FS episodes were recorded. Axillary body temperature which was measured by parents at home or nurses at the emergency department at time of convulsion was also recorded for FS patients.

For the 75 control children, we recorded axillary body temperature at presentation and underlying illness at presentation.

Testing

For all 179 participants, a single fasting blood sample was drawn from the antecubital vein within 24 hours after admission. Fasting time ranged from 4 hours to overnight. Laboratory testing included complete blood count and serum levels of vitamin B₁₂, folic acid, and homocysteine. The serum testing was done via chemoluminescent microparticle immunoassay using a commercial kit (Abbott Laboratories, Abbott Park, IL, USA) and an Abbott Architect I2000 system (Abbott Inc, IL, USA). Patients with serum vitamin B₁₂ below normal range (defined below) also underwent urine assay for methylmalonic acid. Urinary organic acid analysis was performed by capillary gas

chromatography-mass spectrometry.

Anemia was defined as hemoglobin (Hb) below normal range for age (<11 g/dl for ages 6 months through 4 years; <11.5 g/dl for ages 5-7 years). The age-based normal ranges for mean corpuscular volume (MCV) were 70-76 f/L for ages 6 months to 2 years, 73-75 f/L for ages 2-4 years, and 75-95 f/L for ages 5-7 years. The normal ranges for the biochemistry parameters were as follows: serum vitamin B₁₂ 180-1165 pg/ml, serum folic acid 3-17 ng/ml, serum homocysteine 5-15 μmol/l, and urinary methylmalonic acid 0-5 μmol/mmol creatinine.

Mean of Hb, MCV, and serum vitamin B₁₂, folic acid, and homocysteine were compared between 2 groups. Findings for serum vitamin B₁₂, folic acid, and homocysteine were also compared with the FS patients categorized according to type of FS at presentation, frequency of FS episodes experienced, and axillary body temperature at time of convulsion.

Statistical analyses

Statistical analysis was performed using the statistical package SPSS software (Version 17.0, SPSS Inc., Chicago, IL, USA). If continuous variables were normal, they were described as the mean ± standard deviation ($p > 0.05$ in Kolmogorov-Smirnov test or Shapiro-Wilk ($n < 30$)), and if the continuous variables were not normal, they were described as the median. The continuous variables were compared by the use of Student t-test or Mann-Whitney U test depending on parametric or non-parametric values; respectively. The categorical variables

Table I. Mean Results for Hemoglobin Level and Mean Corpuscular Volume, and Proportions of Patients with Macrocytosis and Anemia, Respectively, in the Two Study Groups.

| | FS group (n=104) | Control group (n=75) | p |
|-----------------------|---------------------|----------------------------|------|
| Hb (g/dl) | 11.5±1.0 | 11.2±1.0 | 0.16 |
| MCV (f/L) | 75.3±6.0 | 75.0±5.5 | 0.71 |
| Macrocytosis n (%) | 1 (1.0%) | 0 (0.0%) | |
| Anemia n (%) | 31 (29.8%) | 24 (32.0%) | 0.75 |

FS, febrile seizure; Hb, hemoglobin; MCV, mean corpuscular volume

Table II. Results (Mean ± Standard Deviation) for Serum Levels of Vitamin B₁₂, Folic Acid, and Homocysteine in the Two Study Groups, and Proportions of Each Group with Low and Normal Levels*.

| Parameter (normal range) | FS group (n=104) | Control group (n=75) | <i>p</i> | Result category | FS group n (%) | Control group n (%) | <i>p</i> |
|---|---------------------|-------------------------|----------|--------------------|-------------------|---------------------------|----------|
| Vitamin B ₁₂ (180-1165 pg/ ml) | 453.2±206.0 | 521.9±246.6 | 0.04** | Low | 7 (6.7) | 1 (1.3) | 0.08 |
| | | | | Norm | 97 (93.3) | 74 (98.7) | |
| Folic acid (3-17 ng/ml) | 11.8±4.0 | 12.0±4.4 | 0.75 | Low | 1 (1.0) | 1 (1.3) | 0.81 |
| | | | | Norm | 103 (99.0) | 74 (98.7) | |
| Homocysteine (5-15 μmol/L) | 8.0±4.1 | 9.1±4.3 | 0.09 | High | 6 (5.8) | 5 (6.7) | 0.80 |
| | | | | Norm | 98 (94.2) | 70 (93.3) | |

FS, febrile seizure; Norm, normal

*Normal ranges: vitamin B₁₂ 180-1165 pg/ml; folic acid: 3-17 ng/ml; homocysteine: 5-15 μmol/L

** Statistically significant

between the groups were analyzed by using the Chi square test or Fisher's Exact Test.

Comparisons between "Number of febrile seizure episodes" were applied using Kruskal Wallis test for the data non-normally distributed. Since analysis of variance was significant, comparisons were applied using the Mann-Whitney U test. A multiple logistic regression analysis was used to know associations between FS and other measurements, with diagnosis (FS and Control group) as dependent variable. Values of $p < 0.05$ were considered statistically significant.

Results

The FS group consists of 47 boys and 57 girls of mean age 2.2 ± 1.1 years (range, 0.5–5.5 years). The control group consists of 38 boys and 37 girls of mean age 2.1 ± 1.3 years (range, 0.5-6 years). There were no significant differences between the two groups with respect to age, weight or sex ($p > 0.05$).

The most common cause of fever in the FS group, was acute tonsillitis (44/104), followed by lower respiratory tract infections (13/104), acute otitis media (AOM) (11/104), acute gastroenteritis (AGE), (11/104), and the other causes (22/104). In the control group the most common cause was acute tonsillitis (25/75) followed by AOM (14/75), AGE (10/75), lower respiratory infections (10/75), urinary tract infections (6/75), acute sinusitis (4/75) and 6 (6/75) had other reason. Twenty-one of 104 children in FS group and 14 of 75

children in control group were hospitalized and the frequency of difference was insignificant ($P > 0.05$). Eighteen of 104 children in FS group were admitted to emergency department during convulsion. These patients were received intravenous midazolam treatment.

Of the 104 FS patients, 91 (87.5%) were diagnosed with simple FS, 12 (11.5%) with complex FS, and 1 (1.0%) with febrile status epilepticus. Forty-nine (47.1%) of the FS patients had a history of recurrent FS. Of these 49 children, 27 (55.1%) had experienced 2 FS attacks and 22 (44.9%) had experienced 3 or more FS attacks prior to the study. Sixty-two of the FS patients (60.0% of total) had recurrent seizure or complex FS or febrile status epilepticus, and these patients were evaluated with EEG. Only 2 (3.2%) of these individuals had EEG abnormality consistent with epileptiform discharges whose vit B₁₂ levels were normal. The remaining 60 EEG studies were normal. Twenty-five children in the FS group (24%) had a family history of FS.

Table I summarizes results for Hb and MCV in the FS and control groups. There were no significant differences between the group means for Hb or MCV ($p > 0.05$). Thirty-one (29.8%) of the FS patients and 24 (32.0%) of the controls were anemic. One (1.0%) of the FS patients and none of the controls exhibited macrocytosis.

Table II lists the serum findings for vitamin B₁₂, folic acid, and homocysteine in the two study groups. The proportions of FS patients

Table IV. Results (Mean \pm Standard Deviation) For Serum Levels of Vitamin B₁₂, Folic Acid, and Homocysteine with the Febrile Seizure Patients Categorized According to Type of FS.

| Parameter (normal range) | Simple FS (n=91) | Complex FS (n=12) | FSE (n=1) | <i>p</i> |
|---|---------------------|----------------------|-----------------|----------|
| Vitamin B ₁₂ (180-1165 pg/ml) | 454.7 \pm 205.3 | 462.5 \pm 215.6 | 197.0 \pm 0.0 | 0.33 |
| Folic acid (3-17 ng/ml) | 12.0 \pm 3.9 | 10.4 \pm 4.6 | 7.5 \pm 0.0 | 0.22 |
| Homocysteine (5-15 μ mol/L) | 7.9 \pm 4.1 | 8.6 \pm 3.7 | 13.6 \pm 0.0 | 0.17 |

FS, febrile seizure; FSE, febrile status epilepticus

and controls with vitamin B₁₂ deficiency were not significantly different ($p > 0.05$); however, the mean vitamin B₁₂ level in the FS group was significantly lower than that in the control group ($p = 0.049$). Of the 7 FS patients with vitamin B₁₂ deficiency, 5 had normal homocysteine levels and 2 were hyperhomocysteinemic. These same 2 patients also had increased methylmalonic acid excretion and were the only vitamin B₁₂-deficient patients with this abnormality. A multiple logistic regression analysis also showed that low level of vitamin B₁₂ is an independent risk factor for FS (Table III).

There was no significant difference between the mean folic acid levels in the FS and control groups ($p > 0.05$), or between the proportions of FS and control children with folic acid deficiency ($p > 0.05$). In both groups, the mean serum homocysteine level was higher in the subgroup with low folic acid level. There was no significant difference between the mean homocysteine levels in the FS and control groups ($p > 0.05$), or between the proportions of FS and control children with hyperhomocysteinemia ($p > 0.05$).

Within the FS group, the mean serum vitamin B₁₂ levels in the subgroups with simple FS, complex FS, and febrile status epilepticus were not statistically different, and the same was observed for folic acid and homocysteine ($p > 0.05$ for all) (Table IV). A multiple logistic regression analysis also showed that level of folic acid and homocysteine are not an independent risk factor for FS.

When the serum findings for the FS group subgroups with 1 FS group episode only, 2 previous FS group attacks, and 3 or more

previous FS group attacks were compared, the only significant difference identified was among the three subgroups mean folic acid levels ($p = 0.04$). There was also significant difference between the mean ages of subgroups, according to number of seizure ($p = 0.03$) (Table V).

When the serum findings for the FS subgroups with different severity of fever were compared, mean folic acid level was significantly lower in the patients with lower body temperature (37.5-39.0°C) at time of convulsion than in those with higher temperature (39.1-40.0°C) at time of convulsion ($p = 0.04$) (Table VI).

Discussion

Our main finding was that the children with FS group had a significantly lower mean serum vitamin B₁₂ level than the febrile children without seizure. No such difference was observed with respect to serum folic acid or homocysteine; however, folic acid levels did differ significantly among certain subgroups of FS patients. Children with FS group who had 3 or more febrile seizure group attacks prior to the study had significantly lower mean serum folic acid than those who had two or one FS episode only. As well, FS group patients with lower body temperature at time of seizure (37.5-39.0°C) had significantly lower mean serum folic acid than their counterparts with higher temperature at time of seizure (39.1-40°C).

Vitamin B₁₂ is a water-soluble vitamin that is essential for growth and development in humans, and that must be supplied by diet. Animal products, such as meat and dairy foods, are the only dietary sources of vitamin B₁₂.¹⁷ Children develop vitamin B₁₂ deficiency due to

Table V. Results (Mean ± Standard Deviation) For Serum Levels of Vitamin B₁₂, Folic Acid, and Homocysteine with the Febrile Seizure Patients Categorized According to Number of Febrile Seizure Attacks Experienced.

| Parameter (normal range) | No. of febrile seizure episodes | | | p |
|---|---------------------------------|-------------|---------------------|-------|
| | 1 (n=55) | 2 (n=27) | 3 or more (n=22) | |
| Vitamin B ₁₂ (180-1165 pg/ml) | 463.3±218.4 | 462.7±173.0 | 416.0±215.8 | 0.64 |
| Folic acid (3-17 ng/ml) | 12.7±4.4 | 11.0±3.4 | 10.5±3.1 | 0.04* |
| Homocysteine (5-15 μmol/L) | 8.1±4.9 | 7.9±2.4 | 8.0±3.7 | 0.99 |
| Age in years (mean range month) | 1,93±1.02 | 2.47±1,12 | 2.87±1,40 | 0.03* |

* Statistically significant

decreased B₁₂ intake, abnormal absorption of B₁₂, or inborn errors of vitamin B₁₂ transport and metabolism.¹⁸ In clinical practice, serum total vitamin B₁₂ level is the first-line test for detecting deficiency, as this test is widely available and inexpensive.¹⁹

Vitamin B₁₂ is a cofactor for two important metabolic reactions: methylation of homocysteine to methionine, and conversion of methylmalonyl coenzyme A (CoA) to succinyl-CoA. As these precursors accumulate in humans who are deficient in vitamin B₁₂, measurements of serum homocysteine and MMA are useful for diagnosing this condition.¹⁷⁻²⁰ Possible deficiency of vitamin B₁₂ was our basis for comparing serum vitamin B₁₂ and serum homocysteine levels, respectively, in febrile children with and without seizure.

Folic acid is present in fresh green vegetables, liver, yeast, and some fruits. This nutrient is essential for DNA replication and for reconversion of homocysteine to methionine. As a major source of methyl groups, folic acid is also involved in epigenetic methylation reactions.^{21,22} The functional connection between folic acid and homocysteine was our basis for investigating serum folic acid status in febrile children with and without seizure.

Vitamin B₁₂ deficiency is known to be a causal factor in a variety of diseases, and a risk factor for others. Epilepsy and EEG abnormalities are manifestations of vitamin B₁₂ deficiency in pediatric and adult patients. Only a few reports have documented epileptic seizures

in children with vitamin B₁₂ deficiency.^{23,24} Erol et al²³ reported the case of a 10-month-old girl diagnosed with West syndrome who had vitamin B₁₂ deficiency but did not have macrocytic anemia. Biancheri et al²⁴ reported epilepsy in 9 children with vitamin B₁₂ deficiency, and documented EEG abnormalities in all these cases. Osifo et al¹⁵ investigated folic acid and vitamin B₁₂ levels in the serum and cerebrospinal fluid of 40 febrile pediatric patients and in healthy control children. Eighteen of the febrile children were in a state of FS when samples were taken and 22 were not seizing. The authors observed significantly lower serum vitamin B₁₂ levels in the 40 febrile children than in the controls. The 18 children with FS had significantly lower mean serum vitamin B₁₂ than the non-seizing febrile children and the controls. In addition, Osifo et al¹⁵ observed no difference in cerebrospinal fluid vitamin B₁₂ levels between their FS group and non-seizing group. According with these findings, our pediatric FS patients had significantly lower mean serum vitamin B₁₂ than our febrile controls without seizure. This supports the hypothesis that low serum vitamin B₁₂ level may trigger convulsions in febrile children.

The exact mechanism for vitamin B₁₂-related epileptogenesis has not been established. In individuals with vitamin B₁₂ deficiency, methylmalonyl-CoA accumulates and is used in the synthesis of fatty acids instead of acetyl-CoA. This results in unstable myelin that degrades more easily and negatively affects the

Table VI. Results (Mean \pm Standard Deviation) for Serum Levels of Vitamin B₁₂, Folic Acid, and Homocysteine with the Febrile Seizure Patients Categorized According to Severity of Fever.

| Parameter (normal range) | FS subgroup | | p |
|---|------------------------------------|------------------------------------|-------|
| | Group 1 (37.5-39.0°C) (n=58) | Group 2 (39.1-40.0°C) (n=34) | |
| Vitamin B ₁₂ (180-1165 pg/ml) | 451.2 \pm 212.5 | 441.2 \pm 165.5 | 0.81 |
| Folic acid (3-17 ng/ml) | 11.2 \pm 4.0 | 12.9 \pm 3.9 | 0.04* |
| Homocysteine (5-15 μ mol/L) | 8.0 \pm 4.6 | 7.8 \pm 3.0 | 0.88 |

brain development and cognitive performance of growing children.²⁵ We observed increased MMA excretion in only 2 of our 7 FS pediatric patients with low serum vitamin B₁₂. We did not compare mean urinary MMA concentrations in febrile children with and without seizure, and, therefore, cannot comment on a possible role of MMA in the FS. Further studies are needed to elucidate whether or how MMA is involved in this process in patients with low vitamin B₁₂.

Homocysteine, a sulfur-containing amino acid, and its metabolic product homocysteic acid have been proven to induce seizures in immature and adult rats, with some age-dependent differences in seizure pattern (i.e., longer seizures representing epileptic status in immature rats, and shorter seizures in adult rats).^{24,26,27} The mechanism of action is still unknown, but experiments with immature rats have revealed that N-methyl-D-aspartate (NMDA) receptors and non-NMDA receptor antagonists prevent seizures from being induced by homocysteine and homocysteic acid.²⁸⁻³⁰ On the other hand elevated serum homocysteine may induce neurological dysfunction via oxidative stress caused by enhanced production of reactive oxygen and oxidative deactivation of nitric oxide and lipid peroxidation.³¹⁻³³ In our study, we observed no significant difference in mean serum homocysteine levels between our FS group and control groups. We also found no statistical difference between the proportions of patients with hyperhomocysteinemia in these two groups. Our findings suggest that hyperhomocysteinemia does not affect

epileptogenesis in patients with FS.

The CD320 gene knockout mouse provides a model to study the consequences of vitamin B₁₂ deficiency in the central nervous system³⁴. The nutrient deficiency in the brain is amply demonstrated by alterations in the concentrations of metabolites such as increased homocysteine and MMA and the decreased S-adenosylmethionine (SAM)/S-adenosylhomocysteine (SAH) ratio. The altered SAM/SAH ratio suggests disruption of vitamin B₁₂/folic acid pathways that could affect methylation status. This is further supported by the global hypomethylation of DNA observed in the brains of these mice.³⁵ According to this observation, it can be speculated that cerebrospinal fluid levels of vitamin B₁₂ may be lower than serum vitamin B₁₂ in patients with vitamin B₁₂ deficiency. Therefore toxic intermediates, which may trigger seizure, can be very high in cerebrospinal fluid of these patients. However, vitamin B₁₂, homocysteine and MMA levels in cerebrospinal fluid were not studied in our study. So further studies are needed to elucidate whether, cerebrospinal fluid vitamin B₁₂, homocysteine and MMA levels are involved in triggering seizure in patients with low vitamin B₁₂.

Osifo et al¹⁵ detected higher serum folic acid in their 40 febrile pediatric patients with and without seizure than in their healthy controls; however, the FS subgroup had the highest mean folic acid level. The same study revealed no significant difference between mean cerebrospinal fluid folic acid levels in their FS group and non-seizing febrile children

groups.¹⁵ Another study by Osifo et al¹⁶ investigated the relationship between serum folic acid level and occurrence of convulsion in 32 febrile children aged 8 months to 5 years. The authors observed significantly higher folic acid levels in the serum and red cells of the children with FS than in those without seizure. They also found that children with FSE had significantly higher red cell folic acid levels than children with convulsions of less than 30 minutes duration.¹⁵ Osifo et al¹⁵ suggested that accumulation of folic acid in serum and red cells may be causally related to development of the convulsing state in febrile children. Epilepsy due to an inborn error of folic acid metabolism has been previously reported.³⁶ Folic acid as methyl donor plays an important role in cerebral mitochondrial function and nucleic acid synthesis.³⁷ Moreover, a decrease of brain oxygenation has been linked to folic acid deficiency, which may influence seizure activity.³⁸

In contrast to these results, we detected no statistical difference in mean folic acid level between our FS group and control group. Reasons for this discrepancy may include small patient numbers in previous studies, and different methods and time points for measuring serum folic acid concentration. In both our study groups (FS and non-seizing febrile controls), we found that children with low serum folic acid had higher mean serum homocysteine than their counterparts with high serum folic acid. We also observed that FS patients with more than 3 FS episodes prior to the study had significantly lower serum folic acid than those with two or only one FS episode. On the other hand patients with more than 3 FS episodes were older than those with two or only one FS episode. Furthermore, our FS patients with lower body temperature (37.5-39.0°C) at time of convulsion had significantly lower serum folic acid than those with more severe fever (39.1-40.0°C) at time of convulsion. Based on these findings, we suggest that low serum folic acid level may promote recurrence of FS and may trigger seizures in children with FS who have body temperature 37.5-39.0°C at time of convulsion. These results indicate that low serum folic acid and age of the patients may be associated with FS. Therefore they do not identify the causal relationship with

folic acid in this condition. Future studies of serum folic acid levels in children with FS may provide greater understanding of the various mechanisms underlying in FS.

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.³⁹ We observed no significant differences between our FS group and febrile control children with respect to mean Hb or mean MCV. 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.³⁹ Only 1 of our 104 pediatric FS patients had macrocytic anemia.

Conclusion

To the best of our knowledge, this is only the third study in the English-language medical literature to report serum vitamin B₁₂ and folic acid levels in febrile children with and without seizure. It is the first report of serum homocysteine levels in these patient groups. Our results suggest that low serum vitamin B₁₂ may reduce a child's threshold for seizure and may be a risk factor for FS. The findings also suggest that children with low serum folic acid have greater likelihood of recurrent FS, and that low serum folic acid may trigger FS in febrile children who have body temperature 37.5-39.0°C at time of convulsion. Further large-scale studies are warranted to explain the roles that vitamin B₁₂, folic acid, and homocysteine play in FS.

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