Micronutrient characteristic in recurrent seizure in medicine-controlled epileptic children with normal nutritional status

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Abstract

Background: Recurrent seizure in epileptic children is correlated with future motoric disorders, behavior problems, and intellectual disabilities. Various factors are thought to modulate the risk of recurrent seizure, including micronutrient status such as calcium, 25-dehydroxycholecalciferol (25-(OH)D), and serum iron presented as hemoglobin level.

Aim: To analyze correlation between micronutrient characteristics of epileptic children and recurrence of seizure.

Methods: This cross-sectional retrospective study was conducted in the pediatric clinic of Dr. Soetomo hospital from September to October 2019. Epileptic children with long-term anti-epileptic drugs (AED) for over 6 months and ages ranging 2-18 years were included. Recurrent and non-recurrent group were compared. Age, family history of seizure, and duration of AED administration were noted. Peripheral serum level of hemoglobin, calcium, and 25-(OH)D was measured. The median 25-(OH) D level of both groups were corelated with recurrent seizure by using Spearman test (95% confidence interval).

Results: Thirty children were enrolled. Recurrent seizure was occurred in 19 children. There was significant correlation on hemoglobin and calcium, and 25-(OH)D level with the recurrence of seizure ($p<0.05$). Among all observed micronutrients, 25-(OH)D has the strongest correlation ($r = 0.750$). There was no significant correlation between recurrent seizure and both family history and AED administration duration.

Conclusion: Micronutrients status is correlated with prevalence of recurrent seizure. Level of 25-(OH)D is strongly correlated, whereas level of hemoglobin, and calcium have weak correlation with recurrent seizure in epileptic children.

Keywords: Epilepsy, recurrent seizure, hemoglobin, calcium, vitamin D.

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INTRODUCTION

Epilepsy is a neurological condition where a person has a tendency to have seizures that start in the brain. The incidence of childhood epilepsy has been reported 0.5-1.0% of children younger than 16 years [(1)].

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It is estimated that approximately 50% of children and adolescents who have a first epileptic seizure will have at least one recurrence [2–5]. Recurrent seizure is defined as seizure that happen more than once. In the context of epilepsy, the definition of term "recurrent seizure" is hazy, since based on ILAE 2014 definition, epilepsy itself is a recurrent seizure occurring on defined timespan [6]. However, evidence showed that random epileptic seizure might happen occasionally within pharmacological intervention timespan [7]. Studies showed that despite of pharmacological and non-pharmacological efforts, there are about 30 to 40% chances of seizure being recurrent [8]. Because of this, ILAE proposed a definition that cover this recurrency as well as other refractory seizure condition as "failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom" [9].

In childhood, recurrent seizure after first unprovoked seizure may happen within half to first year [10], and often give diagnostic clue towards epilepsy than a simple febrile seizure. Recurrent seizure may cause future motoric disorder, behavior problem, intellectual disability, and socioeconomical burden. A study showed that economical loss due to recurrent seizure, status epilepticus, and their refractory form can reach between USD 10,000–100,000 per seizure episode [11]. Recurrent seizure in childhood is disabling, often tied to epilepsy, and may result to stigmatization for both parents and the child. Further when the children reach adulthood, they will face limited options in employment, as well as driving restriction, creating a huge burden and certain disabilities in adulthood [12].

Tied with chance of future diagnosis of epilepsy, scientists are prompted to predict this seizure recurrence. Several studies have found predictors for seizure recurrence, such as age at the first seizure, gender, family and perinatal history, seizure characteristics, electroencephalogram (EEG) results, anti-epileptic drugs (AED), vitamin D status, hemoglobin level, frequency of fever and infectious diseases [13–18]. Because of this, knowledge to recognize clinical features in patients with recurrence seizure is essential to establish proper diagnosis and treatment. Here in this paper, we aimed to assess the clinical micronutrient profile of recurrent seizure in Indonesian children.

METHODS

Study Design

This study is a cross-sectional study aimed to determine the correlation between micronutrient characteristic with the recurrence of seizure in epileptic children. The study was conducted from September to October 2019 in Dr. Soetomo General Hospital Pediatric Clinic. There were 2 groups, recurrent and non-recurrent seizure. In this study, recurrent seizure is defined as episode of one or more seizures under adequate antiepileptic drugs administration, while non-recurrent seizure is defined as the periods of no present seizure under adequate antiepileptic drugs administration. Patient characteristics, family history of seizure, and duration of AED administration were recorded in this study. This study was approved by the Ethics Committee Dr. Soetomo General Hospital, Surabaya, Indonesia with clearance No. 1498/KEPK/IX//2019.

Study Participants

Inclusion criteria for this study are all 2-18 years old epileptic children who have administered AED for more than 6 months. Also important for the inclusion criteria is nutritional status based on body weight and height percentile. The nutritional status measurement differs between ages, and along with its categorization, it is based on World Health Organization (WHO) classification of nutritional status of infants and children[19]. Informed consent was obtained from a legal representative of the patient and also serves as obligatory inclusion criteria. The patients were excluded if they were overweight, obese, wasted, severely wasted, suffering moderate malnutrition, or severe malnutrition based on the WHO criteria[19]. We also exclude patients who were having any of bone mineralization diseases, having bad compliance of AED, having genetic/inherited hematologic disease, and suffering from any severe neurological dysfunction. Patients with vitamin A or vitamin D supplementation were also excluded.

Serum Hemoglobin Measurement

In this study, hemoglobin was used as micronutrient parameter, since it may represent chronic iron deficiency and vitamin B12 deficiency due to the setting of inclusion

Table 1. Subject Characteristic

Characteristics	Recurrent Seizure (N=19)	Non-recurrent Seizure (N=11)
Age (year, median ± SD)	9.3 ± 3.93	9.2 ± 4.44
Duration of antiepileptic drugs (months, median ± SD)	12.0 ± 5.92	8.1 ± 2.73
Sex		
Male	13	5
Female	6	6
Place of residence		
Urban	7	7
Rural	4	4
Mother Employment		
Employed	9	3
Housewife	10	8
Mother education		
Elementary school graduate	0	0
Junior school graduate	6	2
Senior high school graduate	8	8
University graduate	5	1
History of seizure in the family		
Yes	4	1
No	15	10
First age of seizure (year, median ± SD)	4.7 ± 1.91	5.1 ± 1.86
Hemoglobin level (Z-score, median ± SD)	-1.5 ± 0.93	-0.8 ± 1.91
Serum calcium level (mg/dl, median ± SD)	9.0 ± 0.57	9.3 ± 0.31
Serum 25-(OH)D (ng/ml, median ± SD)	21.0 ± 7.79	39.2 ± 6.76
Anti-epileptic drug (AED) regimen		
Monotherapy		
Phenytoin	4	4
Valproic acid	3	7
Phenobarbital	1	0
Polytherapy		
Phenytoin + valproic acid	6	0
Phenobarbital + valproic acid	1	0
Phenytoin + phenobarbital + valproic acid	4	0

and exclusion criteria. Hemoglobin was measured by using routine medical hematology analysis. Sample of 3 mL venous blood was taken by using sterile syringe. Sample then stored into a purple BD Vacutainer® blood sampling tube containing ethylenediaminetetraacetic acid (EDTA). Sample then directly sent to the lab for analysis. Results then plotted into hemoglobin Z-score chart to obtain age-based hemoglobin status.

Serum Calcium Measurement

Serum calcium was measured by using routine medical electrolyte analysis. Similar to hemoglobin sample, 3 mL venous blood was taken by using sterile syringe. Sample then stored into a serum separating, yellow BD Vacutainer® blood sampling tube. Finally, sample then directly sent to the lab for analysis. Result is presented in mg/dL unit.

Serum 25-(OH)D Measurement

Serum 25-(OH)D level was measured by using enzyme-linked fluorescence assay (ELFA) methods with bioMérieux VIDAS® 25 OH Vitamin D Total reagent

Table 2. Correlation between Micronutrient Parameters and Seizure Recurrence in epileptic children

Characteristic	p*	r*
Hemoglobin level	0.016	-0.132
Serum calcium level	0.025	-0.137
Serum 25-hydroxycholecalciferol	0.000	-0.750

*Spearman correlation test, result is significant under p<0.05

N/A: Not available due non-significant result

(lot No. 190318-0). Sampling was done by collecting 100 μL of peripheral serum collected in BD Vacutainer® blood sampling tube. Department of Clinical Pathology, Universitas Airlangga supervised quality control. Vitamin D status is determined based on serum 25-(OH)D level according to the Endocrine Society, which are categorized as deficient (<20ng/ml), insufficient (20-29ng/ml), and normal (>29ng/ml) [20].

Data Analysis

Analysis was performed using Statistical Program for Social Science (SPSS) version 22.0. Prior to any data analysis, Kolmogorov-Smirnov test was done to all variables to examine the data distribution characteristics. Chi-square test was further used to analyze the difference between both groups. Spearman correlation strength test was used to measure correlation strength between groups. The result is considered statistically significant under p <0.05.

RESULTS

Participant Characteristic

The study was participated by 32 patients. Two patients were excluded because of technical difficulty on blood sampling and insufficient blood sample volume. There were 19 samples in recurrent seizure group and 11 samples in non-recurrent seizure group. Kolmogorov-Smirnov analysis showed that age and sex are distributed normally. However, we detected significant difference on serum hemoglobin level, serum calcium level, and serum 25-(OH)D (Chi-square, p<0.05). All of these results are summarized in table 1.

Data Analysis

In this study we measured the correlation between micronutrient parameters and recurrent epileptic seizure using Spearman correlation strength test. Serum hemoglobin level, serum calcium level, and serum 25-(OH)D showed significant correlation. All of the mentioned parameters showed negative correlation (r < 0), but only serum 25-(OH)D that showed strong negative correlation towards seizure recurrence (r = -0.750). These suggest that the lower complete results of data analysis are presented in table 2.

DISCUSSION

Low 25-(OH)D and its Correlation with Recurrent Seizure

Rationale of vitamin D as a factor influencing seizure in epilepsy can be traced back into its nature. In brain, vitamin D present as specific substance bound to Vitamin D-specific receptors and enzymes in neuron and glial cells. In genomic level, vitamin D3 is able to lower the expression of proconvulsant cytokines, such as IL-1β and TNF-α through binding with Vitamin D Receptor (VDR)[21]. The IL-1β is a potent proconvulsant cytokine, which known to phosphorylate the NR2B subunit of NMDA receptor[22] – an important glutamate receptor in the pathophysiology of seizure – as well as increasing neuronal excitability through increase of glutamate release and reduction of inhibitory effect of GABA-ergic CL- flux. [23] Vitamin D3 is also known to promotes the expression of the calcium binding proteins parvalbumin [24], which bound to excessive Ca2+ in the presynaptic terminal and preventing Ca2+-induced neurotransmitter release, and finally preventing seizure [25].

Vitamin D is a unique vitamin, since it is naturally rare in dietary source and often requires food processing to acquire the dietary-significant version [26]. The main production of vitamin D in human is exposure

of the skin to ultraviolet B of sunlight, converting the 7-dehydrocholesterol into provitamin D and finally into vitamin D3. This process further requires a pivotal role of liver to create 25-(OH)D, a precursor of biologically active and kidney metabolized 1,25-(OH)D [27]. Being a stable liver trans-product between biologically active vitamin D3 and its highly convertible precursors, 25-(OH)D serves as important clinical parameter for vitamin D3 status.

In tropical region such as Indonesia, abundance of biologically active vitamin D thanks to all year-round high-intensity ultraviolet B is logically expected. However, reality in this study showed that lower level of 25-(OH)D is exists and more prevalent in children with recurrent seizure. This because vitamin D level are influenced by several factors, including intake or production, metabolism, and degradation. While the participant population in this study is clearly not having apparent macronutrient intake problem due to inclusion criteria, micronutrient intake adequacy to combat AED side effects is still in question. The tropical region such as Indonesia has been correlated with higher provitamin-D conversion by higher intensity of sunlight ultraviolet B. However, it must be noted that even under high ultraviolet B exposure, cultural and dietary behavior may affect this natural provitamin D conversion significantly[28]. Study on 25-(OH)D level at 48 districts in Indonesia to determine the prevalence of vitamin D deficiency and insufficiency in the children population. Vitamin D insufficiency occurs in 38.76% of children while vitamin D deficiency occurs in 1.08% of children [29]. Our finding where nutritional status is normal in patients with recurrent seizure, but they have low 25-(OH)D suggest a clue on inadequate dietary vitamin D intake, as well as other possible explanation such as antiepileptic drug adverse effect.

Common antiepileptic drug adverse effect such as carbamazepine and lamotrigine are low serum vitamin D. A former study showed that vitamin D in its 25-(OH)D form, might be found lower in epileptic children due to antiepileptic drug administration [30]. Long-term administration of anti-epileptic drugs is known to activate pregnane-x receptor (PXR) which increase the enzyme activity of CYP24A1 and CYP3A4. The increase of CYP24A1 and CYP3A4 enzymes activity resulted as higher vitamin D catabolism into inactive substance, thereby reducing vitamin D level in the blood [31].

The correlation between vitamin D, its derivates, and recurrent seizure is yet to be explored. Many studies confirmed the correlation between epilepsy and lower level of circulating vitamin D, but data on whether vitamin D level is actually affecting the seizure recurrency is still scarce. In this study, we found that lower vitamin D level correlates with recurrence of childhood epileptic seizure albeit of normal macronutrient status and protocolary given antiepileptic drugs. This further emphasis vitamin D as a substance, which plays pivotal role in antiseizure physiology, as well as a high possibility that vitamin D, is a risk factor for recurrent seizure in epilepsy.

Low Hemoglobin Level and its Correlation with Recurrent Seizure

Lower hemoglobin level has been associated with increased prevalence of childhood seizure, especially in febrile seizure. Low hemoglobin are reported to often occurs in the first onset of seizure [17] and in also prevalent epileptic children [18]. A meta-analysis showed that low serum hemoglobin due to iron-deficiency anemia increased the risk of febrile seizure [32].

In this study, hemoglobin was used as micronutrient parameter, since it may represent chronic iron deficiency and vitamin B12 deficiency due to the setting of inclusion and exclusion criteria. In childhood, low hemoglobin is associated to prior chronic condition; e.g. iron deficiency, vitamin B12 deficiency, infection, tumor, and hematological disorder. Studies showed that the risk of seizure in low serum hemoglobin is more associated with iron deficiency [33–35]. Other study also showed that iron deficiency anemia may have possible role in both occurrence and recurrence of febrile seizure[36]. Seizure featuring megaloblastic anemia caused by vitamin B12 deficiency is exists at very rare prevalence, and often found in infants with hemoglobin level lower than 6.0 mg/dL [37]. I this study, we were able to exclude the possibility of anemia caused by severe disease by including only participants whose macronutrient-sufficient and no comorbidities.

Pathophysiology of seizure in in low-hemoglobin level is still not clearly understood and process is often associated to other hemoglobin-related molecule than the hemoglobin itself, especially iron. Iron deficiency-caused anemia is known to increase seizure susceptibility

in childhood [32]. Animal study showed that this process is thought to be a product of sex-specific and time-specific manners [38], suggesting a chronic origin. Seizures due to low hemoglobin level is associated with significant instability of brain neurotransmitters, such as norepinephrine, dopamine, glutamate, gamma-aminobutyric acid (GABA), and serotonin [39–42]. Again, studies showed that lower iron level, but not hemoglobin, affects this process most, as shown with extracellular dopamine and norepinephrine [43].

Contradictory findings showed that blood parameter such as hemoglobin might not be associated with the recurrence of seizure itself although related to the first onset of seizure [35,44]. This suggest that epileptic children with recurrent seizure might have already exposed to prolonged low hemoglobin-inducing problem which remain undetected until the latest diagnosis of anemia is established. Hence, further and careful investigation of hemoglobin content and its associated micronutrients status in epileptic children is needed to prevent recurrence seizure in this situation.

Low Calcium Level and its Correlation with Recurrent Seizure

Calcium in the form of Ca2+ is an important voltage stabilizer of neuron. In brain, calcium presents at extracellular space as well as in the neuron intracellular compartment. In the neuron intracellular compartment, it may present as organelle-stored calcium or non-stored calcium [45]. In presynaptic neuron, intracellular Ca2+ must be kept at critically balanced level because higher or lower concentration of Ca2+ may induce excitability of certain neuron. A study showed suppression of neuronal excitability through activation of several calcium-gated potassium currents, which made possible through the rise of non-stored intracellular calcium [46]. Model on how overall calcium affect neuronal excitability is complex and still limited, however, in the extracellular compartment it is suggested that calcium sensing receptors (CaSR) affects neuronal action potentials, resting membrane potential, firing threshold, firing frequency, and finally, neurotransmitter release [45]. Hence, decrease on extracellular calcium may cause increase in neuronal excitability which further result as proconvulsive situation.

Hypocalcemia has been thought to provoke recurrent seizure in epileptic children due to increased neuron excitability [47]. There are broad evidence that epileptic children had a lower calcium level than normal population [48]. However, due to the use of AED, these findings is counterintuitive, since opponents of these findings may find that lower calcium level in epileptic population may be obtained due to pharmacodynamics of antiepileptic drugs towards cytochrome P-450 [49,50].

Some studies reported that hypocalcemia is an important side effect of long-term antiepileptic drugs administration in epileptic children [51–53]. Antiepileptic drug such as carbamazepine, lamotrigine, levetiracetam, oxcarbazepine, phenobarbital, phenytoin, topiramate, and valproic acid can affect bone biochemistry and reduce its calcium and phosphate contents [54,55].

Study showed that in childhood, drug-induced hypocalcemia is mostly due to longer antiepileptic drug administration duration than its dose [56]. However, another study showed that under the presence of hypocalcemia, even without antiepileptic drug the patient still can experience seizure [57]. These contradicting findings showed the importance of serum calcium monitoring in epileptic children. Further studies are still needed to explore whether seizure recurrence in low serum calcium condition is tied to antiepileptic drug side effects or caused by existing calcium deficiency.

Study Limitation

The limitation of this study is mainly due to the very low number of samples. The case-control design was selected under the knowledge of already low prevalence of controlled seizure in the hospital where this study took place. However, we found that although eligibility criteria for participants have been made as loose as possible, the number of participants remaining in inclusion group is still low. The exclusion of population with comorbidities, which may trigger secondary micronutrient-deficiency, may suggest a bigger role of those comorbidities instead of pure nutritional intake problem. This study also only used data from participants using the conventional antiepileptic drugs (phenytoin, phenobarbital, and valproic acid). Hence, this study cannot confirm situation in the population in which newer antiepileptic drugs are used. Because of this, our conclusion may be only applicable in certain situation. While our study results

are satisfying, it requires more study to accept the idea whether lower hemoglobin level, serum calcium, and serum 25-dehydroxycholecalciferol is the direct cause of recurrent seizure in antiepileptic drug controlled, macronutrient sufficient, epileptic children. Hence, due to limitations mentioned, we cannot statistically confirm the causative effect between our analyzed variables. Hence, further studies are required to solve these problems.

CONCLUSION

Lower hemoglobin level, serum calcium level, and vitamin D in the form of 25-(OH)D are significantly lower micronutrient found in epileptic children with recurrent seizure. Findings of this study still needs further investigation in to confirm possible direct causative association between micronutrient deficiency and seizure recurrence.

CONFLICTS OF INTEREST

All authors declared no conflict of interest related to financial support or relationship during the proposal writings, data collection, analysis, and manuscript writings of this study.

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