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Original article

Serum carnitine levels of children with epilepsy: Related factors including valproate

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Abstract

Objective: This study measured the serum carnitine levels in patients with epilepsy and determined the factors contributing to low carnitine levels.

Methods: We measured the serum carnitine levels in 94 consecutive patients with epilepsy, including the free carnitine (FC) and acylcarnitine fractions, using an enzyme cycling method. We defined a low FC as a serum FC level < 36 μmol/L. Age, body mass index (BMI), standard deviation score of BMI (BMI-SDS), use of valproate, cognitive disorder, and feeding problems differed between patients with low and normal FC. In patients taking valproate, the associations of the serum FC level with the platelet count and serum ammonia and amylase levels were analyzed.

Results: Univariate analysis showed that a low BMI and BMI-SDS, the use of valproate, and cognitive disorder were more frequent in patients with a low FC. Logistic regression analysis revealed that a low BMI-SDS and cognitive disorders were independently associated with a low FC. Among the patients taking valproate, a low BMI-SDS and age were associated with a low FC. The serum FC and ammonia levels were inversely correlated, whereas no correlation was observed between the serum FC level and platelet count or serum amylase level.

Conclusion: A low BMI and cognitive disorders were related to a low FC in patients with epilepsy and the serum carnitine levels should be monitored in these patients.

Keywords: Free carnitine; Body mass index; Cognitive disorder; Valproate; Ammonia

1. Introduction

Carnitine is a small, water-soluble molecule that plays an important role in the mitochondrial oxidation of fatty acids by serving as a cofactor for shuttling long-chain fatty acids from the cytoplasm into the mitochondrial matrix. Carnitine deficiency can cause several disorders by impairing fatty acid oxidation. Mutations of the *SLC22A5* gene, which encodes the plasma membrane carnitine transporter, cause primary carnitine deficiency, which is characterized by progressive cardiomyopathy and skeletal muscle weakness. Secondary carnitine deficiency due to long-term use of pivalate-conjugated antibiotics has also attracted clinical attention. There have been several reports on acute encephalopathy or hypoglycemia associated with carnitine depletion due to chronic pivalate-conjugated antibiotic administration [1,2].

Valproic acid (VPA) is a branched chain carboxylic acid that has been used widely as a broad-spectrum

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antiepileptic drug (AED). VPA inhibits the biosynthesis of carnitine by decreasing the α-ketoglutarate concentration and is a potential cause of carnitine deficiency. Several studies have reported low carnitine levels in patients with epilepsy treated with VPA [3–8]. Some authors have recommended monitoring carnitine levels in patients taking VPA.

Measurements of the carnitine levels were not readily available in Japan until 2017. In 2018, measurements of two carnitine fractions, free carnitine (FC) and acylcarnitine, using an enzyme cycling method were listed in the public medical insurance system and have become easily accessible. We determined the serum carnitine levels in patients with epilepsy and examined the factors contributing to low carnitine levels, to clarify the need to monitor carnitine levels in patients with epilepsy.

2. Patients and Methods

We measured the serum carnitine levels in 94 consecutive patients with epilepsy who were followed in the Department of Pediatrics of Aichi Medical University Hospital between March and September 2018. The diagnosis of epilepsy and its treatment were determined by the consensus of three pediatric neurologists (AO, HK, and SN). This study was approved by the ethics committee of Aichi Medical University Hospital. Waiver of informed consent was also approved because we retrospectively analyzed the existing data with no identifiable private information and notification with opt-out was shown in the hospital.

The serum carnitine levels were measured together with the serum AED levels and hematological and blood chemistry parameters. In most patients treated with VPA, the serum ammonia and amylase levels were measured. Height and body weight were recorded at the time of blood sampling and body mass index (BMI) was calculated. We also calculated standard deviation score (SDS) of BMI, because BMI demonstrates a developmental change by age throughout the childhood. BMI-SDS was calculated using the Tools for Growth Evaluation of Children provided by the Japanese Society for Pediatric Endocrinology (available at http://jspe.umin.jp/medical/chart_dl.html.) No standard data was obtained for patients older than 17 years and 6 months. For such patients, BMI-SDS was calculated using the standard data of 17 years and 6 months of age, because standard values are presumed to change slightly until 20 years of age. Although the time of the blood sampling was not strictly regulated, it was usually performed several hours after the last dose of AED. No patient had evidence of any illness other than epilepsy at the time of blood sampling. The serum carnitine fractions (FC and the acylcarnitine level) were measured by an enzyme cycling method described elsewhere [9]. According to the 2018 Japanese guidelines for the diagnosis and treatment of carnitine deficiency, carnitine deficiency was defined as FC $<$ 36 μmol/L (http://www.jpeds.or.jp/uploads/files/20181207_shishin.pdf). Therefore, we defined a low FC as a serum FC level $<$ 36 μmol/L and a normal FC as a serum FC level $\geq$ 36 μmol/L.

Two patients had been given carnitine supplements and were excluded from the study. Ultimately, the study enrolled 92 patients [median age 11.6 years (range, 3 months – 20.5 years), 53 males]. The number of AEDs taken was one in 64 patients, two in 20, and three or more in eight. VPA was taken by 49 patients, levetiracetam by 28, topiramate by 12, and carbamazepine by 10. In this study, cognitive disorder was defined as an intelligence or developmental quotient $<$60. Feeding problem was defined as the need for a nasogastric tube or gastrostomy for feeding.

We first compared age, BMI, BMI-SDS, the use of VPA, cognitive disorder, and feeding problems between patients with low and normal FC using Fisher's exact probability test and the Mann–Whitney *U*test to compare categorical and numerical variables, respectively. Then, logistic regression analysis was performed to identify the contributors to a low FC. For patients taking VPA, we also compared age, BMI, BMI-SDS, the use of AEDs other than VPA, and cognitive disorder between patients with low and normal FC, followed by logistic regression analysis to identify the contributors to a low FC within this patient group. The associations of the serum FC level with the platelet count, and serum ammonia and amylase levels were analyzed using the Mann–Whitney *U*test and the Pearson's correlation test in patients treated with VPA. A p-value < 0.05 was considered to be statistically significant. The statistical analyses were performed using EZR ver. 1.33 (available at http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmed.html) [10].

3. Results

Of the 92 patients, 22 (24%) had a low FC. The median age was 114.5 months in the patients with a low FC and 156.4 months in those with a normal FC ($p > 0.05$) (Table 1). The median BMI was 15.8 (range 11.1–22.7) in patients with a low FC and 17.9 (13.2–25.3) in those with a normal FC. BMI and BMI-SDS were significantly lower in patients with a low FC ($p < 0.001$). The use of VPA and a cognitive disorder were more frequent in patients with a low FC ($p < 0.05$ and $p < 0.01$, respectively), whereas the rate of feeding problems did not differ between the two groups. Logistic regression analysis revealed that a lower BMI-SDS and cognitive disorders were independently associated with a low FC, whereas the use of VPA was not (Table 2).

Of the 49 patients treated with VPA, 16 (33%) had a low FC. In this patient subset, those with a low FC age were younger and had a lower BMI and BMI-SDS

Table 1
Clinical characteristics and serum free carnitine levels.

			FC < 36 μmol/L	FC ≥ 36 μmol/L	
All patients			N = 22	N = 70	
	Age (months)*		114.5 (3–247)	156.5 (9–240)	NS
	BMI *		15.8 (11.1–22.7)	17.9 (13.2–25.3)	P < 0.001
	BMI-SDS *		−0.92 (−7.66–0.72)	0.084 (−1.90–1.75)	P < 0.01
	Use of VPA				
		Yes	16 (73%)	33 (47%)	P < 0.05
		No	6 (27%)	37 (53%)	
	Cognitive disorder				
		Yes	8 (36%)	7 (10%)	P < 0.01
		No	14 (64%)	63 (90%)	
	Feeding problem				
		Yes	1 (5%)	1 (1%)	NS
		No	21 (95%)	69 (99%)	
Patients treated with VPA			N = 16	N = 33	
	Age (months) *		109.5 (3–206)	168 (27–240)	P < 0.01
	BMI *		15.6 (12.0–20.1)	18.2 (13.3–25.3)	P < 0.001
	BMI-SDS *		−0.92 (−7.66–0.72)	0.03 (−1.90–1.75)	P < 0.05
	Serum VPA level (μg/ml)*		70.5 (11–109)	75.0 (29–155)	NS
	Use of other AEDs				
		Yes	5 (31%)	11 (33%)	NS
		No	11 (69%)	22 (67%)	
	Cognitive disorder				
		Yes	6 (38%)	3 (9%)	P < 0.05
		No	10 (63%)	30 (91%)	
	Feeding problem				
		Yes	1 (6%)	1 (3%)	NS
		No	15 (94%)	32 (97%)	

FC: free carnitine, BMI: body mass index, BMI-SDS: body mass index standard deviation score, VPA, valproate, AED: antiepileptic drug, NS: not significant.
* Data are shown as median (range).

Table 2
Logistic regression analysis.

		Odds ratio	95% confidence interval	
All patients				
	Age (months)	0.992	0.981–1.01	NS
	BMI-SDS	0.435	0.235–0.807	P = 0.0083
	VPA	2.97	0.877–10.1	NS
	Cognitive disorder	4.65	1.10–19.6	P = 0.037
Patients treated with VPA				
	Age (months)	0.982	0.967–0.998	P = 0.027
	BMI-SDS	0.422	0.188–0.948	P = 0.036
	Cognitive disorder	2.48	0.426–14.4	NS

BMI-SDS: body mass index standard deviation score, VPA, valproate, NS: not significant.

($p < 0.01$, $p < 0.001$, and $p < 0.05$, respectively, Table 1). By contrast, the serum VPA levels did not differ between patients with low and normal FC. Cognitive disorders were more frequent in patients with a low FC ($p < 0.05$), while the rate of use of other AEDs or feeding problems did not differ between patients with a low and normal FC. Logistic regression analysis revealed that younger age and a decrease in BMI were associated with a low FC, whereas cognitive disorder was not (Table 2).

Fig. 1 shows the relationships between the serum FC level and the platelet count and serum ammonia and amylase levels in patients treated with VPA. The serum FC and ammonia levels were inversely correlated ($p < 0.01$). The patients with the two lowest serum FC levels had elevated serum ammonia levels, although the Mann–Whitney *U*test demonstrated no significant difference (Table 3). By contrast, no correlation was observed between the serum FC level and platelet count or serum amylase level and the Mann–Whitney *U*test showed no significant difference (Table 3).

4. Discussion

Our study found that BMI-SDS was associated with a low FC in patients with epilepsy taking AEDs. Cogni-

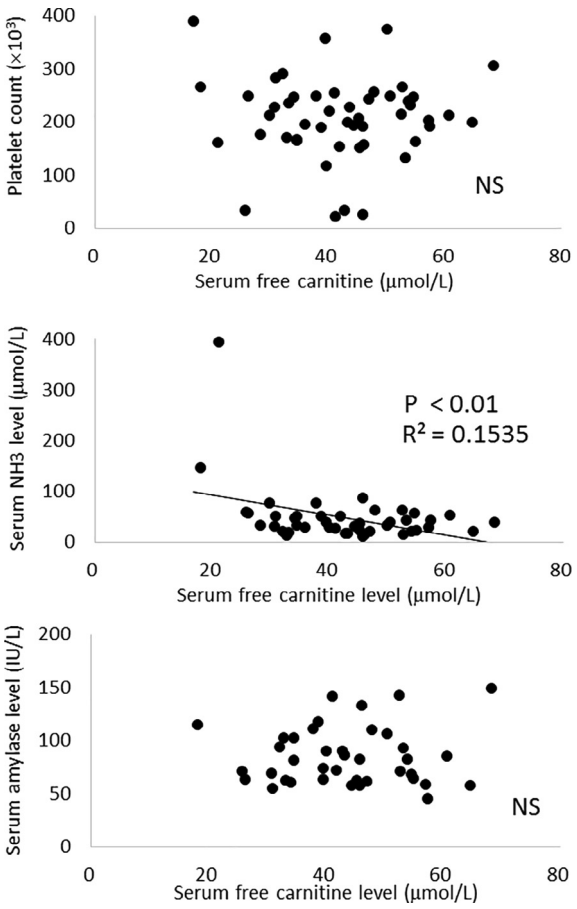

Fig. 1. The relation between serum free carnitine levels and platelet count, and serum ammonia and amylase levels in patients treated with valproate.

tive disorder was also independently associated with a low FC. Although univariate analysis showed that patients treated with VPA were more likely to have a low FC than those treated with other AEDs, no significant increase in a low FC was observed in the multivariate analysis. These data will be useful for selecting patients with epilepsy who require monitoring of the serum FC levels and carnitine supplementation.

Carnitine is an essential nutrient, which explains why a low BMI was correlated with low FC levels in our study. About 75% of the required amount is ingested from the diet and about 25% is supplied by biosynthesis. Most carnitine is distributed in tissues such as skeletal muscle and only 0.6% is distributed in blood. A low BMI is presumed to be correlated with two possible factors: decreased dietary intake of carnitine and storage in organs. The most common cause of a low BMI is malnutrition and the nutritional state is presumed to be relatively insufficient in patients with a low BMI, although this was not investigated in our study. The muscle volume is likely to be low in patients with a low BMI, which limits the carnitine storage capacity. A combination of these factors explains the correlation between a low FC and low BMI. Fukuda *et al.* reported that low body weight was correlated with carnitine deficiency in children with epilepsy [11], although they did not investigate the relationship between BMI and carnitine deficiency. By contrast, Fung *et al.* found no significant association between BMI and the serum carnitine levels [12]. Further studies are needed to clarify the relationship between BMI and carnitine deficiency.

Interestingly, cognitive disorder was more frequent in patients with a low FC, which might be explained by the fact that children with autism spectrum disorder (ASD) are at risk of developing nutritional deviations, and some patients with cognitive disorders also have ASD, even if a formal diagnosis has not been made. Shmaya *et al.* compared nutrient intake between children with ASD and their typically developing siblings and found that the children with ASD were more likely to suffer from nutritional deficiencies despite a higher BMI [13]. Castro *et al.* reported that children and adolescents with ASD consumed more calories than controls on average, had a limited food repertoire, and a high prevalence of inadequate calcium, sodium, iron, vitamin B_5, folate, and vitamin C intake [14]. These results suggest that insufficient carnitine intake may occur without a decrease in BMI. Studies of dietary habits are needed to clarify the reason for the low FC in children with cognitive disorders.

VPA has the potential to cause carnitine deficiency. In our study, a low FC was more frequent in patients treated with VPA than in those treated with other AEDs, although multivariate analysis failed to show a significant correlation. The reduced carnitine levels have been explained mainly by the hypothesis that VPA combines with carnitine to form valproylcarnitine, which is eliminated in the urine, decreasing the body store of carnitine and that VPA and its metabolites inhibit the biosynthesis of carnitine indirectly by decreasing the level of a-ketoglutarate, a cofactor for the enzyme, butyrobetaine hydroxylase [15]. Several authors have reported reduced serum carnitine levels in relation to

Table 3
The relation between serum free carnitine levels and platelet counts, and serum ammonia and amylase levels in patients treated with valproate.

	FC < 36 μmol/L	FC ≥ 36 μmol/L	
Platelet counts ($\times 10^3/\mu L$)	221 (35–391)	207 (23–376)	NS
Serum ammonia levels (μmol/L)	47 (13–395)	33 (12–88)	NS
Serum amylase levels (IU/L)	71 (55–115)	83 (46–149)	NS

VPA [3–8]. Verrotti *et al*. reported that the plasma total and free carnitine concentrations were significantly reduced in patients taking VPA compared with patients who did not take VPA [3]. They also reported that patients taking VPA had significantly higher hyperammonemia and that the plasma ammonia level was correlated with the VPA dosage, serum VPA level, and duration of VPA treatment. Coppola *et al*. found low plasma FC levels in 32 of 84 patients taking VPA and that VPA monotherapy was associated with a higher risk of hypocarnitinemia compared with carbamazepine [4]. However, some studies have reported opposite results. Hirose *et al*. found no significant difference in the total or free carnitine levels between VPA-treated patients without neurological abnormalities or nutritional problems and age-matched controls [16]. Nakajima *et al*. showed that VPA monotherapy did not result in decreases in FC or in the accumulation of long-chain acylcarnitines [17]. The relationship between the use of VPA and serum FC levels is complicated, and several factors influence the serum FC levels in patients treated with VPA, such as the use of other AEDs and nutritional state.

Researchers have focused on the relationship between carnitine levels and the adverse effects of VPA. Our study suggested that a low FC is related to elevated serum ammonia levels, whereas thrombocytopenia and serum amylase may not be related to a low FC. Several studies have found a correlation between a low FC and elevated serum ammonia levels [3,5,6,18]. Hamed *et al*. reported that the total and free carnitine levels were negatively associated with ammonia in patients treated with VPA and that the ammonia levels were correlated with patient age and the dose of VPA [5]. Similarly, Verrotti *et al*. found elevated ammonia concentrations and a low FC in patients treated with VPA and a correlation with the dose and duration of VPA treatment [3]. Moreover, several patients with hyperammonemic encephalopathy associated with VPA treatment have been reported [19–23]. Remarkably, the ammonia levels normalized in these patients after L-carnitine supplementation. We observed elevated ammonia levels in the two patients with the lowest FC levels, although neither patient had encephalopathy. On the other hand, no significant difference in serum ammonia levels was observed between children with normal and low FC in this study. This discrepancy may be explained by the hypothesis that threshold FC levels affecting urea cycle may be present. An elevation of serum ammonia levels will be observed in children with markedly low FC, whereas it will not be seen in those with slightly low FC. Carnitine is indirectly required for proper functioning of the urea cycle. The ω-oxidation metabolite, 4-en-VPA, which accumulates when carnitine is lacking, inhibits carbamoyl phosphate synthetase I, the first mitochondrial enzymatic step of the urea cycle. In addition, carnitine deficiency decreases the synthesis of N-acetyl glutamic acid, an important cofactor of carbamoyl phosphate synthetase I. Therefore, we should be aware of the possibility of hyperammonemia in patients with a low FC related to VPA, and monitor carnitine levels in this context. In contrast, the platelet count and serum amylase level were not related to the serum FC levels, suggesting that these adverse effects of VPA are caused by factors other than carnitine depletion.

This study had several limitations. First, the number of patients was insufficient to clarify the effects of VPA on carnitine levels. The results of the univariate and multivariate analyses were discrepant. We believe that the effects of VPA were underestimated because of the limited number of patients. Second, we did not obtain information on the patients' dietary intake, which is the main source of carnitine, and its effects on carnitine levels cannot be ignored. Moreover, we found that a low BMI was correlated with a low FC. Further studies are necessary to clarify the relationship between carnitine levels and dietary habits in children with epilepsy. In this study, only two fractions were measured: free carnitine and acylcarnitine. Studies using liquid chromatography-tandem mass spectrometry (LC-MS-MS) have revealed more complicated changes in carnitine metabolism. To elucidate the effects of various factors on carnitine, including AEDs, LC-MS-MS studies should be performed.

In conclusion, a low BMI and cognitive disorders were related to a low FC in patients with epilepsy. The correlation between VPA and a low FC cannot be denied, but it may be weak. Our results suggest that universal monitoring of serum carnitine levels may not be necessary in all patients with epilepsy, whereas monitoring of carnitine levels is necessary in patients with a low BMI or cognitive disorders regardless of treatment with VPA. Carnitine supplements are necessary in patients with a very low FC.

5. Funding source

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6. Potential conflicts of interest

The authors have no conflicts of interest relevant to this article to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2019.02.010.

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