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Factors associated with blood carnitine levels in adult epilepsy patients with chronic valproic acid therapy

Masanori Saito *, Takeya Takizawa, Hitoshi Miyaoka

Department of Psychiatry, Kitasato University School of Medicine, Japan

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ABSTRACT

Aims: Valproic acid (VPA) is a widely used antiepileptic drug for the treatment of epilepsy, seizures, and bipolar and psychiatric disorders. A deficiency of carnitine, a compound involved in energy production, is associated with chronic VPA use. However, the clinical factors affecting blood carnitine levels and their pathophysiology remain unclear. Hence, we aimed to identify the factors that correlated with serum carnitine levels in epilepsy patients receiving chronic VPA therapy.

Methods: This observational study included 138 epilepsy patients receiving chronic VPA therapy. Serum total and free carnitine levels, routine blood tests and drug concentrations were assessed. The correlation between carnitine levels and other factors were calculated using Spearman's rank correlation coefficients, and a principal component analysis (PCA) and a multiple linear regression analysis were performed.

Results: Overall, serum free carnitine levels showed significant negative correlations with epilepsy duration, VPA treatment duration, daily VPA dose, and blood VPA concentration. A significant positive correlation was observed with erythrocyte count, hemoglobin levels, and creatinine levels. Of the 138 patients, 21 (15.2 %) with serum free carnitine levels of <20 μmol/L had significantly longer disease duration, a higher daily VPA dose, and lower blood clobazam concentrations. In the 48 VPA monotherapy patients, serum free carnitine levels showed a significant negative correlation with disease duration and duration of VPA therapy. Furthermore, in the 2.1 % patients receiving VPA monotherapy, serum free carnitine levels were <20 μmol/L. PCA resulted in seven factor solution (eigenvalue >1; 71.67 % explained variance). Component 1 clearly revealed the maximal loading for serum free carnitine level (.792) and the most negative loading for disease duration of epilepsy (−.595). A linear regression analysis revealed that the duration of epilepsy, serum creatinine level, and daily dose of VPA were significant ($p < .01$) factors that affected serum free carnitine levels.

Conclusions: The effects of combination therapy with VPA and other anti-epileptic drug(s) on carnitine levels are higher than that of VPA monotherapy. Additionally, epilepsy duration may affect serum free carnitine level.

1. Introduction

Valproic acid (VPA) has been established as an effective anti-epileptic drug (AED) since the 1970s (Gram et al., 1977). VPA is well-tolerated and widely prescribed for epilepsy, bipolar affective disorder, and migraine prevention. VPA therapy has been reported to be associated with carnitine deficiency (Matsuda et al., 1986). Carnitine is an essential cofactor in the metabolism of fatty acids into acyl-carnitines, and aids in their transport into the mitochondrial matrix, where they are used for energy production in the Krebs cycle (Mock and Schwetschenau, 2012).

The clinical presentation of carnitine deficiency in pediatric patients was first described by Winter et al. (Winter et al., 1987), and was reported to include hypotonia, failure to thrive, and recurrent infections. Rodriguez-Segade et al. (1989) were the first to report the prevalence of carnitine deficiency in adult epilepsy patients treated with various AEDs, and found that 26 of 34 (76.5 %) epilepsy patients undergoing VPA therapy had a deficiency of serum free carnitine. In Hug et al.'s study (1991) involving a large cohort of 471 pediatric patients receiving AED therapy, 46 (9.8 %) were reported to be carnitine deficient. In

Abbreviations: VPA, Valproic acid; PCA, principal component analysis; AED, anti-epileptic drug; CLB, clobazam; RBC, red blood cell; CRE, creatinine; Baso, basophil; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; BMI, body mass index.

* Corresponding author at: 1-15-1 Kitazato, Minami-ku, Sagamihara, Kanagawa Pref., 252-0374, Japan.
E-mail address: 7n2ecx-msaito@umin.ac.jp (M. Saito).

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another study conducted among Japanese pediatric patients receiving AEDs, Okumura et al. (2019) reported that 16 of 49 (32.7 %) patients treated with VPA therapy had serum free carnitine levels of <36 μmol/L. Thus, VPA is now widely known to affect serum total or free carnitine levels.

Besides VPA, other acquired factors reported to affect serum carnitine levels include nutrition (Evans and Fornasini, 2003), renal function (Matsuda et al., 1986), hemodialysis (Hurot et al., 2002), intestinal resection, severe infection, liver disease (Kelly, 1998), and anti-epileptic drugs such as phenobarbital, carbamazepine, and phenytoin (Hug et al., 1991).

However, it is not yet known whether the level of carnitine in blood is affected by factors such as demographics, blood levels of transaminases and ammonia, combination therapy or polytherapy with VPA and other anti-epileptic drug(s), duration of epilepsy, type of epilepsy, type of seizures, and the frequency of seizures. To this end, we conducted an observational study among epilepsy patients receiving chronic VPA therapy and sought to identify the factors that correlated with serum carnitine levels in these patients.

2. Materials and methods

2.1. Patients

In total, 552 epilepsy patients regularly consulted the neuropsychiatric center at Kitasato University East Hospital between 2018 and 2019. Among them, 146 patients who were prescribed an AED, including VPA, for more than one year before this study were short-listed. Two patients who were hepatitis C carriers, one patient with acute cholecystitis, and one patient with alcoholism were excluded. Additionally, four patients who refused to provide blood samples were also excluded. The final study population consisted of 138 patients. None of the patients included in the study population had any known congenital metabolic defect or organ failure. None of the patients were vegetarian or receiving total parenteral nutrition, which is known to affect the blood carnitine level (Evans and Fornasini, 2003).

The collected clinical data included each patient's age, sex, height, body weight, age at epilepsy onset, duration of epilepsy, self-reported seizure frequency, duration of treatment with VPA, epilepsy type, seizure type, etiology of seizure disorder, comorbidities, and all prescriptions at the time of blood sampling. The demographic and clinical characteristics of the study patients are described in Table 1.

2.2. Blood collection and analysis

Blood samples were collected as a part of diurnal routine check-ups at 2–4 h after a meal and 1–9 h after drug intake.

The parameters that were analyzed included complete blood count, routine blood chemistry (including total protein, albumin, total bilirubin, cholesterols, triglyceride, blood urea nitrogen, creatinine, uric acid, sodium, potassium, chlorine, calcium, ammonia, activities of transaminases, alkaline phosphatase, gamma-glutamyl transpeptidase, lactate dehydrogenase, and creatine phosphokinase), daily dose and concentrations of all AEDs, and serum total and free carnitine levels.

Table 1
Demographic and clinical characteristics of the study patients.

	All participant (N = 138)		Male (N = 66)		Female (N = 72)	
Age, Median (renge)	42.4	(17.5–79.5)	39.8	(17.5–69.7)	44.8	(19.1–79.5)
Height, Median (renge)	162.7	(141.2–182.8)	168.2	(150.0–182.8)	156.8	(141.2–169.0)
Body weight, Median (renge)	64.2	(35.0–145.4)	69.3	(35.0–145.4)	59.5	(36.0–94.1)
BMI, Median (renge)	24.3	(14.8–47.5)	24.3	(15.0–47.5)	24.4	(14.8–36.9)
Age at epilepsy onset, Median (renge)	11.8	(0.0–74.9)	12.5	(0.0–61.8)	11.2	(0.1–74.9)
Disease duration of epilepsy, Median (renge)	30.6	(2.9–77.5)	27.4	(2.9–52.3)	33.4	(4.3–77.5)
Self-reported seizure frequency (/month), Median (renge)	10.7	(0–320)	9.7	(0–320)	11.7	(0–300)
Duration of VPA treatment, Median (renge)	22.6	(1.6–53.0)	21.3	(2.3–52.2)	23.9	(1.6–53.0)
Epilepsy type, n, (%)						
Focal	84	(60.9 %)	40	(60.6 %)	44	(61.1 %)
Generalized	46	(33.3 %)	23	(34.8 %)	23	(31.9 %)
Combined	5	(3.6 %)	1	(1.5 %)	4	(5.6 %)
Unclassified	3	(2.2 %)	2	(3.0 %)	1	(1.4 %)
Seizure type[†], n, (%)						
Focal	86	(62.3 %)	37	(56.1 %)	49	(68.1 %)
SGS	78	(56.5 %)	36	(54.5 %)	42	(58.3 %)
GTCs	58	(42.0 %)	30	(45.5 %)	28	(38.9 %)
Other GS	24	(17.4 %)	11	(16.7 %)	13	(18.1 %)
Etiology of seizure disorder, n, (%)						
Genetic	7	(5.1 %)	3	(4.5 %)	4	(5.6 %)
Unknown	96	(69.6 %)	45	(68.2 %)	51	(70.8 %)
Structural	26	(18.8 %)	15	(22.7 %)	11	(15.3 %)
Infectious	6	(4.3 %)	3	(4.5 %)	3	(4.2 %)
Immune	3	(2.2 %)	0	(0.0 %)	3	(4.2 %)
Metablic	0	(0.0 %)	0	(0.0 %)	0	(0.0 %)
Comorbidity[†], n, (%)						
Intellectual Disability	68	(49.3 %)	32	(48.5 %)	36	(50.0 %)
Autism Spectrum Disorder	12	(8.7 %)	7	(10.6 %)	5	(6.9 %)
Attention-Deficit/Hyperactivity Disorder	3	(2.2 %)	2	(3.0 %)	1	(1.4 %)
Cerebral Palsy	12	(8.7 %)	7	(10.6 %)	5	(6.9 %)

Abbreviations: BMI, body-mass index; SGS, secondarily generalized seizure; GTCs, secondarily generalized seizure; GS, generalized seizure.
[†] Each category contains overlap.

The serum total and free carnitine levels were tested at SRL Co. LTD (Tsushima and Kase, 2000; Umemori et al., 2017).

2.3. Statistical analysis

All statistical analyses were performed using Excel (Microsoft, Redmond, WA, USA) or SPSS 26.0 (SPSS, Chicago, IL, USA).

Correlation between carnitine levels and other factors were calculated using Spearman's rank correlation coefficients. We performed non-parametric (Mann-Whitney) tests to compare low carnitine (free carnitine <20 μmol/L) vs. normal carnitine groups (free carnitine >19 μmol/L), defined as per Winter et al.'s classification (Winter et al., 1987; Buist, 2016), and monotherapy (VPA alone) vs. combination therapy (VPA + other AEDs). For categorical variables, the $\chi 2$ test was used for comparisons.

Further, a principal component analysis (PCA) was performed on the factors that had a significant correlation with serum total and/or free carnitine level. The aim of this PCA was to search for a possible component or eigenvector in which the factor loadings of serum total- and/or free carnitine levels are maximal. This component was supposed to include the variables with high loadings that have "true" correlations with serum carnitine levels (Glantz and Slinker, 2001).

Owing to the small sample size, the blood concentrations of clobazam (CLB) were not used in the PCA. Only data from 116 of the 138 patients, who had no missing values for any independent variable, were used for the PCA.

Then, a multiple linear regression analysis with stepwise method was performed. In this analysis, variables were chosen from the PCA component that had largest factor loadings for serum carnitine levels. More briefly, a PCA-assisted selection of variables that were supposed to have true correlations with serum carnitine levels, was performed.

Considering the multiplicity of the test and type I error, the significance level was set to $p < 0.01$ in the correlation analysis and comparison test.

2.4. Ethics statement

This study was approved by the institutional review board for observational and epidemiological studies (KMEO B18−275). In addition, this study was conducted in accordance with the World Medical Association Code of Ethics (Declaration of Helsinki), and participants gave informed consent to participate in the study.

3. Results

3.1. Correlation studies and multiple comparison

3.1.1. Factors affecting serum carnitine levels

Factors significantly correlated with serum total or free carnitine levels are shown in Table 2. Serum free carnitine levels showed a significant negative correlation with the duration of epilepsy (r_s= -0.48, $p = 2.8\times10^{-9}$), duration of VPA treatment (r_s=-0.35, $p = 2.8\times10^{-5}$), and daily dose of VPA (r_s= -0.42, $p = 4.1\times10^{-7}$). They showed a significant positive correlation with the concentration of CLB ($n = 15$) ($r_s = 0.67$, $p = 0.006$), red blood cell (RBC) count ($r_s = 0.45$, $p = 2.4\times10^{-8}$), creatinine (CRE) levels ($r_s = 0.40$, $p = 9.1\times10^{-7}$), and total bilirubin levels ($r_s = 0.38$, $p = 3.2\times10^{-6}$).

3.1.2. Normal carnitine group versus low carnitine group

Compared to the normal carnitine group, the low carnitine group showed a significantly longer duration of epilepsy ($u = 556.0$, $p = 2.1\times10^{-4}$), higher self-reported seizure frequency ($u = 667.0$, $p = 0.004$), higher daily dose of VPA (u=565.5, $p = 7.0\times10^{-5}$), lower concentrations of CLB ($u = 1.0$, $p = 0.006$), higher mean corpuscular volume of RBC (u=792.0, $p = 0.009$), higher erythrocyte mean corpuscular volume (MCV) (u=688.0, $p = 0.001$), higher γ-GTP

Table 2
Correlations with serum total or free carnitine levels.

	Total carnitine		Free carnitine	
	r_s	p	r_s	p
Age	**−0.25**	**0.004**	**−0.29**	**5.1 × 10−4**
Height	0.20	0.034	0.18	0.050
Body weight	0.19	0.027	0.19	0.028
BMI	0.07	0.473	0.08	0.377
Age at epilepsy onset	0.22	0.011	0.22	0.011
Disease duration of epilepsy	**−0.44**	**1.0 × 10−7**	**−0.48**	**2.8 × 10−9**
Self-reported seizure frequency (/month)	**−0.26**	**0.004**	**−0.26**	**0.003**
Duration of VPA treatment	**−0.32**	**1.4 × 10−4**	**−0.35**	**2.8 × 10−5**
Daily dose (mg/day)				
VPA	**−0.36**	**1.2 × 10−5**	**−0.42**	**4.1 × 10−7**
LEV	−0.23	0.220	−0.25	0.170
CBZ	−0.30	0.032	−0.28	0.045
CLB	0.16	0.545	0.19	0.456
ZNS	−0.42	0.086	−0.41	0.095
PER	–	–	–	–
TPM	−0.42	0.264	−0.31	0.423
Concentration (μg/mL)				
VPA	−0.19	0.030	**−0.26**	**0.003**
LEV	−0.11	0.590	−0.14	0.487
CBZ	0.01	0.938	0.04	0.797
CLB	0.59	0.022	**0.67**	**0.006**
ZNS	−0.30	0.247	−0.33	0.202
PER	–	–	–	–
TPM	0.03	0.932	−0.07	0.865
NZP	−0.80	0.200	−0.8	0.200
Complete blood count (CBC)				
WBC, (10^2/μL)	0.13	0.141	0.12	0.163
Neutro, (%)	0.12	0.155	0.13	0.133
Eo, (%)	0.09	0.288	0.11	0.196
Ly, (%)	−0.10	0.231	−0.13	0.138
Mo, (%)	−0.03	0.684	0.00	0.963
Baso, (%)	0.09	0.268	0.12	0.153
Neutro counts	0.15	0.085	0.14	0.093
Ly counts	0.05	0.586	0.02	0.803
RBC, (10^4/μL)	**0.46**	**1.2 × 10−8**	**0.45**	**2.4 × 10−8**
Hb, (fL)	**0.38**	**5.4 × 10−6**	**0.35**	**2.6 × 10−5**
MCV, (fL)	−0.22	0.010	**−0.25**	**0.003**
MCHC, (%)	0.18	0.039	0.16	0.059
PLT, (10^4/μL)	0.03	0.699	0.07	0.407
Blood chemistry				
TP, (g/dL)	0.09	0.317	0.05	0.527
ALB, (g/dL)	0.41	0.011	0.40	0.014
T-Bil, (mg/dL)	**0.38**	**4.2 × 10−6**	**0.38**	**3.2 × 10−6**
LDH, (U/L)	0.15	0.087	0.10	0.246
AST, (U/L)	**0.22**	**0.009**	0.18	0.037
ALT, (U/L)	**0.26**	**0.002**	**0.23**	**0.006**
ALP, (U/L)	−0.17	0.050	−0.15	0.070
γ-GTP, (U/L)	−0.21	0.015	**−0.26**	**0.002**
CPK, (U/L)	**0.22**	**0.009**	0.21	0.015
BUN, (mg/dL)	**0.24**	**0.005**	0.19	0.028
CRE, (mg/dL)	**0.44**	**7.3 × 10−8**	**0.40**	**9.1 × 10−7**
UA, (mg/dL)	**0.39**	**2.6 × 10−6**	**0.36**	**1.6 × 10−5**
Na, (mEq/L)	0.18	0.037	0.15	0.086
K, (mEq/L)	−0.01	0.898	−0.03	0.712
Cl, (mEq/L)	0.13	0.117	0.12	0.152
Ca, (mg/dL)	**0.30**	**3.6 × 10−4**	**0.29**	**5.5 × 10−4**
TC, (mg/dL)	−0.04	0.614	−0.10	0.266
LDL-C, (mg/dL)	0.10	0.386	0.05	0.640
TG, (mg/dL)	0.08	0.364	0.06	0.475
BS, (mg/dL)	−0.16	0.070	−0.15	0.074
NH_3, (μg / dL)	−0.06	0.460	−0.04	0.631

Abbreviations: BMI, body-mass index; AED, antiepileptic drug; VPA, valproate; LEV, levetiracetam; CBZ, car- bamazepine; CLB, clobazam; ZNS, zonisamide; PER, perampanel; TPM, topiramate; NZP, nitrazepam; WBC, White Blood Cell

count; Neutro, neutrophil; Eo, eosinophil; Ly, lymphocyte; Mo, monocyte; Baso, basophil; RBC, red blood cell count; Hb, hemoglobin; MCV, erythrocyte mean corpuscular volume; MCHC, erythrocyte mean corpuscular hemoglobin concentration; PLT, platelets; TP, total protein; ALB, albumin; T-Bil, total billirubins; LDH, lactate dehydorogenase; AST, asparate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, gamma-glutamyl transpeptidase; CPK, creatine phosphokinase; BUN, blood urea nitrogen; CRE, creatinine; UA, uric acid; Na, sodium; K, potassium; Cl, chlorine; Ca, calcium; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglyceride; BS, blood suger; NH3, ammonia; rs, Spearman's rank correlation coefficient; p, p-values. Significant P-values ($p < 0.01$) is boldface and underlined.

(u=720.0, $p = 0.003$), and lower uric acid levels (u=739.0, $p = 0.005$).

3.1.3. Serum carnitine levels among patients receiving VPA monotherapy versus combination therapy

Of the 138 patients included in the study, 48 patients (20 males, 28 females) were administered VPA monotherapy for more than one year. Their mean age was 41.9 years (SD=±14.2; range, 19–79.5 years) and daily dose of VPA was 756 mg/day (±361, 100–1600 mg/day). The duration of VPA monotherapy was 20.6 years (±13.5, 1.58–52.25 years) and free carnitine level was 39.4 μmol/L (±9.8, 16.4–56.9). Among those 48 patients, only one patient had serum carnitine level of <20 μmol/L (2.1 %).

Table 3
Comparison between VPA monotherapy and combination therapy of other AED(s).

	VPA alone (n = 48)		VPA + Other AED(s) (n = 90)			
	Mean	SD	Mean	SD	u	p
Age	41.9	(±14.2)	42.6	(±13.7)	2111.5	0.828
Height	161.7	(±8.3)	163.2	(±9.3)	1376.5	0.352
Body weight	64.7	(±15.7)	63.9	(±16.7)	2050.0	0.623
BMI	24.5	(±4.6)	24.2	(±5.5)	1421.5	0.503
Age at epilepsy onset	14.8	(±15.5)	10.3	(±11.8)	1616.0	0.029
Disease duration of epilepsy	27.0	(±16.0)	32.4	(±13.1)	1651.0	0.044
Self-reported seizure frequency (/month)	0.2	(±0.8)	16.4	(±59.0)	883.0	**$3.2 \times 10{-}6$**
Duration of VPA treatment	20.6	(±13.5)	23.8	(±13.2)	1822.5	0.157
Serum total carnitine (μmol/L)	48.4	(±11.0)	37.5	(±13.8)	1117.0	**$3.1 \times 10{-}6$**
Serum free carnitine (μmol/L)	39.4	(±9.8)	29.7	(±11.5)	1117.5	**$3.2 \times 10{-}6$**
Daily dose of VPA (mg/day)	756.3	(±361.4)	965.6	(±506.2)	1652.0	0.022
Blood concentration of VPA (μg/mL)	49.1	(±30.1)	62.0	(±27.9)	1613.0	0.023
Complete blood count (CBC)						
WBC, (10^2/μL)	6200.0	(±1904.3)	5467.8	(±1748.1)	1624.5	0.017
Neutro, (%)	56.4	(±9.5)	54.2	(±9.1)	1882.5	0.215
Eo, (%)	2.5	(±1.7)	3.0	(±3.0)	2108.5	0.818
Ly, (%)	34.0	(±8.8)	35.1	(±8.0)	2042.0	0.598
Mo, (%)	7.1	(±6.4)	6.9	(±1.7)	1663.0	0.026
Baso, (%)	0.8	(±0.3)	0.8	(±0.4)	2037.5	0.582
Neutro counts	3557.9	(±1552.6)	3038.7	(±1351.1)	1693.0	0.037
Ly counts	2055.3	(±682.7)	1853.9	(±536.7)	1789.0	0.097
RBC, (10^4/μL)	464.1	(±58.9)	438.4	(±49.1)	1526.5	**0.005**
Hb, (fL)	13.8	(±2.0)	13.7	(±1.6)	2008.0	0.497
MCV, (fL)	86.7	(±11.0)	91.9	(±5.1)	1164.5	**$8.6 \times 10{-}6$**
MCHC, (%)	33.3	(±4.5)	34.1	(±1.0)	2045.0	0.607
PLT, (10^4/μL)	21.8	(±5.2)	20.8	(±4.8)	1880.0	0.211
Blood chemistry						
TP, (g/dL)	7.1	(±0.5)	7.1	(±0.4)	2011.0	0.504
ALB, (g/dL)	4.1	(±0.6)	4.2	(±0.4)	160.5	0.889
T-Bil, (mg/dL)	0.5	(±0.2)	0.4	(±0.2)	1344.0	**$1.6 \times 10{-}4$**
LDH, (U/L)	174.8	(±44.1)	172.3	(±31.9)	2131.0	0.897
AST, (U/L)	19.6	(±5.7)	19.6	(±7.0)	2086.0	0.740
ALT, (U/L)	20.7	(±14.5)	20.4	(±13.6)	2091.5	0.759
ALP, (U/L)	208.7	(±68.3)	239.1	(±105.8)	1853.0	0.170
γ-GTP, (U/L)	46.5	(±54.7)	86.7	(±96.5)	1213.0	**$2.3 \times 10{-}5$**
CPK, (U/L)	124.0	(±135.2)	99.1	(±62.6)	1981.5	0.425
BUN, (mg/dL)	13.5	(±5.5)	11.4	(±4.4)	1666.5	0.027
CRE, (mg/dL)	0.7	(±0.2)	0.6	(±0.2)	1654.0	0.024
UA, (mg/dL)	5.7	(±1.5)	4.4	(±1.4)	1083.0	**$3.9 \times 10{-}6$**
Na, (mEq/L)	139.8	(±2.2)	138.6	(±4.4)	1982.5	0.425
K, (mEq/L)	4.0	(±0.2)	4.1	(±0.3)	1705.5	0.041
Cl, (mEq/L)	103.4	(±2.8)	102.8	(±4.7)	1984.5	0.431
Ca, (mg/dL)	9.3	(±0.4)	9.2	(±0.4)	1903.5	0.250
TC, (mg/dL)	192.4	(±39.7)	199.8	(±40.0)	1870.5	0.231
LDL-C, (mg/dL)	113.7	(±34.7)	121.2	(±37.9)	693.5	0.461
TG, (mg/dL)	160.0	(±115.7)	142.0	(±101.6)	1956.0	0.417
BS, (mg/dL)	109.3	(±65.3)	102.6	(±21.0)	1987.5	0.503
NH_3, (μg / dL)	44.9	(±16.9)	63.3	(±30.5)	1387.5	**$5.5 \times 10{-}4$**

Abbreviations: BMI, body-mass index; AED, antiepileptic drug; VPA, valproate; LEV, levetiracetam; CBZ, car- bamazepine; CLB, clobazam; ZNS, zonisamide; PER, perampanel; TPM, topiramate; NZP, nitrazepam; WBC, White Blood Cell count; Neutro, neutrophil; Eo, eosinophil; Ly, lymphocyte; Mo, monocyte; Baso, basophil; RBC, red blood cell count; Hb, hemoglobin; MCV, erythrocyte mean corpuscular volume; MCHC, erythrocyte mean corpuscular hemoglobin concentration; PLT, platelets; TP, total protein; ALB, albumin; T-Bil, total billirubins; LDH, lactate dehydorogenase; AST, asparate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, gamma-glutamyl transpeptidase; CPK, creatine phosphokinase; BUN, blood urea nitrogen; CRE, creatinine; UA, uric acid; Na, sodium; K, potassium; Cl, chlorine; Ca, calcium; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglyceride; BS, blood suger; NH3, ammonia; u, u-values (Mann-Whitney tests); p, p-values. Significant P-values ($p < 0.01$) is boldface and underlined.

Next, the correlation between serum free carnitine level and other factors was examined in the VPA monotherapy group. In this group, serum free carnitine levels showed significant negative correlations with the duration of epilepsy (r_s=-0.42, $p = 0.003$) and the duration of VPA therapy (r_s=-0.44, $p = 0.002$), and positive correlations with basophil (Baso) ($r_s = 0.42$, $p = 0.003$), erythrocyte mean corpuscular hemoglobin concentration (MCHC) ($r_s = 0.37$, $p = 0.010$) and CRE levels ($r_s = 0.45$, $p = 0.001$).

Carnitine levels of <20 μmol/L were observed in 20 of the 90 patients treated with VPA in combination with other AED(s) (22.2 %). The proportion of patients with low carnitine levels was higher among epilepsy patients receiving combination treatment of VPA with other AEDs than among those receiving VPA monotherapy [$\chi^2(1) = 9.841$, $p = 0.002$]. Table 3 depicts the comparison between patients treated with VPA monotherapy and combination therapy. When compared to the VPA monotherapy group, the combination therapy group showed significantly higher seizure frequency (u = 883.0, $p = 3.2\times10^{-6}$) and lower serum free carnitine levels (u = 117.5, $p = 3.2\times10^{-6}$); however, the daily dose of VPA (u = 1652.0, $p = 0.022$) and blood concentration of VPA (u=1613.0, $p = 0.023$) did not show any significant difference.

3.2. Multivariate analysis

3.2.1. Principal component analysis

As shown in Table 4, PCA showed seven components whose eigenvalues exceeded 1.0 (Coste et al., 2005). The component that had the largest factor loading for serum free carnitine was component 1.

Component 1 (eigenvalue = 5.00, 25.02 % of variance) showed the largest factor loading for serum free carnitine (loading = 0.792). Factors such as serum total carnitine (0.765), RBC (0.627) serum uric acid (0.618), and serum CRE (0.599) were also strongly associated with component 1. Component 1 was negatively associated with the duration of epilepsy (-0.595), duration of VPA treatment (-0.477), daily dose of VPA (-0.443), and blood concentration of VPA (-0.408). The findings suggest that this is the key component that is associated with serum carnitine levels and variables that could truly affect serum carnitine levels.

Component 2 (eigenvalue = 2.31, 11.55 % of variance) was strongly associated with patients' age (loading = 0.728), blood urea nitrogen (0.666), and MCV of RBC (0.505). Component 2 seemed to represent age-related factors.

Component 3 (eigenvalue = 2.14, 10.72 % of variance) was associated with serum gamma-glutamyl transpeptidase (loading = 0.681), serum alanine aminotransferase (0.589), and serum aspartate aminotransferase (0.490). Component 3 seemed to represent hepatic factors.

Component 4 (eigenvalue = 1.41, 7.05 % of variance) was associated with blood concentration of VPA (loading = 0.632), frequency of seizures (0.598), and daily dose of VPA (0.441). Thus, the blood concentration of VPA and the daily dose of VPA seemed to have a close relationship with the frequency of seizures.

Component 5 (eigenvalue = 1.34, 6.68 % of variance) was positively associated with blood hemoglobin (loading = 0.425) and negatively associated with serum aspartate aminotransferase level (-0.447). The interpretation of component 5 seemed difficult.

Component 6 (eigenvalue = 1.08, 5.41 % of variance) was associated with the daily dose of VPA (loading = 0.532) and serum uric acid (0.433). The interpretation of component 6 also seemed difficult.

Component 7 (eigenvalue = 1.05, 5.25 % of variance) was associated with serum creatine phosphokinase (loading = 0.738).

3.2.2. Multiple linear regression analysis

A regression analysis with PCA-assisted selection of variables was performed. Based on the variables with high factor loadings (>0.4 or <-0.4) in the component 1 mentioned above, a stepwise multiple linear regression model was built. Table 5 summarizes these results. Serum free carnitine levels were significantly affected by the duration of epilepsy (β=-0.30, $p = 5.3\times10^{-5}$, VIF = 1.20), daily dose of VPA (β=-0.22, $p = 0.002$, VIF=1.09), and serum CRE levels ($\beta = 0.22$, $p = 0.003$,

Table 4
Principal component analysis with variables for which correlations have been obtained.

	Component 1	Component 2	Component 3	Component 4	Component 5	Component 6	Component 7
Eigenvalue	5.00	2.31	2.14	1.41	1.34	1.08	1.05
Cumulative (% of variance)	25.02	36.58	47.30	54.34	61.02	66.42	71.67
Component loadings							
Serum free carnitine (μmol/L)	***0.792***	−0.010	−0.342	0.141	−0.017	−0.185	−0.220
Serum total carnitine (μmol/L)	***0.765***	0.070	−0.321	0.211	0.008	−0.147	−0.243
RBC, (10^4/μL)	***0.627***	−0.461	0.337	−0.002	0.395	−0.007	0.072
UA, (mg/dL)	***0.618***	0.238	−0.002	−0.229	0.199	0.433	0.012
CRE, (mg/dL)	***0.599***	0.451	−0.049	0.124	0.147	0.302	0.059
Disease duration of epilepsy	***−0.595***	0.360	0.286	−0.084	0.375	−0.159	0.044
Hb, (fL)	***0.579***	−0.213	0.545	0.101	0.425	−0.130	0.085
Ca, (mg/dL)	***0.568***	−0.192	0.237	−0.098	0.303	0.023	0.287
T-Bil, (mg/dL)	***0.528***	0.216	−0.168	0.047	0.104	−0.152	0.002
Duration of VPA treatment	***−0.477***	0.253	0.309	−0.018	0.258	−0.272	−0.261
Age	−0.275	0.728	0.101	−0.103	0.185	−0.017	0.231
BUN, (mg/dL)	0.197	0.666	−0.289	0.334	0.127	0.222	−0.051
MCV, (fL)	−0.189	0.505	0.252	0.209	0.087	−0.280	−0.027
γ-GTP, (U/L)	−0.047	0.002	0.681	−0.191	−0.314	0.225	−0.232
ALT, (U/L)	***0.588***	0.123	0.589	0.072	−0.313	−0.027	−0.214
AST, (U/L)	***0.458***	0.390	0.490	0.191	−0.447	−0.009	0.024
Blood concentration of VPA (μg/mL)	***−0.408***	−0.210	0.095	0.632	0.175	0.157	0.071
Self-reported seizure frequency	−0.139	−0.210	0.069	0.598	0.051	−0.195	−0.240
Daily dose of VPA, (mg/day)	***−0.443***	−0.164	0.159	0.441	−0.005	0.532	0.090
CPK, (U/L)	0.228	0.024	0.021	0.228	−0.374	−0.284	0.738
Goodness of fit							
KMO	0.619						
Bartlett	$\chi2(190) = 1397.119$, 1.002×10^{-182}						

Abbreviations: RBC, red blood cell count; UA, uric acid; T-Bil, total billirubins; CRE, creatinine; Hb, hemoglobin; Ca, calcium; T-Bil, total billirubins; BUN, blood urea nitrogen; MCV, erythrocyte mean corpuscular volume; γ-GTP, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, asparate aminotransferase; VPA, valproate; CPK, creatine phosphokinase; KMO, Kaiser-Meyer-Olkin Measure of Sampling; Bartlett, Bartlett test of sphericity.

Table 5
Multiple regression analysis.

	Unadjusted		Adjusted		
Independent	B	SE	β	p	VIF
Constant				*0.097*	
Disease duration of epilepsy	−0.25	0.06	−0.30	5.3×10^{-5}	1.20
CRE, (mg/dL)	16.12	5.24	0.22	0.003	1.17
Daily dose of VPA (mg/day)	−0.01	0.00	−0.22	0.002	1.09
RBC, (10^4/μL)	0.04	0.02	0.17	0.020	1.16
T-Bil, (mg/dL)	10.71	4.80	0.16	0.028	1.26
R	0.67				
R^2	0.45				
Adjusted R^2	0.43				
AIC	584.52				
BIC	601.81				

Abbreviations: VIF, Variance Inflation Factor; VPA, valproate; RBC, red blood cell count; T-Bil, total billirubins; CRE, creatinine; AIC, Akaike's Information Criterion; BIC, Bayesian Information Criterion. The dependent variable was serum free carnitine value. $F(5) = 20.904$, $p < 0.0001$.

VIF=1.17). The overall *p* value of this regression model was under 0.0001 ($F(5) = 20.904$, $p < 0.0001$, R=0.67, $R^2 = 0.45$).

4. Discussion

4.1. The effects of VPA combination therapy versus VPA monotherapy on serum carnitine levels

VPA can bind carnitine to form compounds that are excreted in the urine, resulting in carnitine depletion (El-Hattab and Scaglia, 2015). In patients with normal renal function, a higher dose of VPA is expected to result in higher excretion of VPA-carnitine compounds. Our data, shown in Table 2, seems consistent with this finding. Moreover, daily dose of VPA was observed to be negatively correlated with serum free carnitine levels in our study.

Serum VPA concentrations were negatively correlated with serum free carnitine levels, whereas no significant difference in serum VPA concentration was observed between the normal and low carnitine groups. This is not surprising because VPA concentrations depend not only on the excretion of VPA-carnitine compounds but also on various other factors.

Surprisingly, in the 48 patients treated with VPA monotherapy, there was no correlation of either the daily dose of VPA or VPA concentration with serum carnitine levels, and only one patient in this group had serum carnitine levels of <20 μmol/L. The total and free carnitine levels in patients receiving combination therapy were significantly lower than in patients receiving monotherapy (Table 3). Taken together, our results indicate that a combination of VPA and other AED(s) could reduce serum carnitine levels in a VPA dose-dependent manner.

The precise mechanism underlying serum carnitine reduction by VPA and co-prescribed drug(s) is currently unclear. Rodriguez-Segade et al. (1989) reported that up to 26.8 % of patients treated with AED(s) other than VPA were deficient in serum free carnitine; however, the pathogenesis was undetermined. Based on our data, it appears that CLB could attenuate VPA-induced carnitine reduction, though the mechanism is yet to be clarified. Gabapentin and pregabalin can interfere with the membrane transporter OCTN1, which is involved in carnitine transport (Pochini et al., 2019). Therefore, it is possible that this action is exerted though a mechanism involving OCTN2 or carnitine/acylcarnitine carrier (CAC).

4.2. What did PCA reveal?

Among seven components that were obtained by PCA, the maximal factor loading for serum free carnitine (0.792) was shown in component 1, and the item with the highest absolute value of factor loading was serum free carnitine. This suggests that component 1 could be the only eigenvector that is associated with serum free- (as well as total-) carnitine level. Therefore, in multiple regression analysis, possible variables that could significantly affect serum free carnitine level should be selected from the items with high factor loadings in component 1.

The aim of the PCA was to assist regression analysis. We did not intend to reveal potentially important components that underlie chronic VPA treatment. However, some interesting points have been elucidated. For example, age was not strongly associated with component 1 (factor loading was -0.275) but it was associated with component 2 (0.728). By its nature, each PCA component is independent and uncorrelated. Therefore, age has been suggested to have only a minor effect on serum free carnitine level, though rank-correlation coefficient between serum free carnitine and age was significant (Table 2). Likewise, alanine aminotransferase (strongly associating component 3), gamma-glutamyl transpeptidase (strongly associating component 3), seizure frequency (strongly associating component 4), and the blood concentration of VPA (strongly associating component 4) have been suggested to have only minor effects on serum free carnitine level, though the rank-correlation coefficients were significant.

4.3. Disease duration and serum carnitine levels

The most surprising finding of the study was the significant negative correlation between the duration of epilepsy with serum total and free carnitine levels (Table 2). This negative correlation was seen in VPA monotherapy patients. Moreover, the duration of disease in patients belonging to the low carnitine group was significantly longer than that in patients with normal carnitine levels. The absolute value of the factor loading of disease duration (-0.595) was larger than that of the daily dose of VPA (-0.443) and blood concentration of VPA (-0.408). Moreover, the duration of epilepsy was shown to be a significant factor in our multiple linear regression analysis. Therefore, we can conclude that the effect of the duration of epilepsy on serum total and free carnitine levels is not negligible. Further studies are needed to address why and how the duration of epilepsy is negatively correlated with serum total and free carnitine levels.

4.4. Renal function and serum carnitine levels

Both total and free serum carnitine levels showed a significant positive correlation with serum creatinine levels (Table 2). The PCA showed that serum CRE level was associated with component 1 (factor loading was 0.599), and serum CRE level significantly affected free carnitine level in our multiple linear regression analysis. These results are consistent with the observation that CRE excretion might be proportional to the excretion of carnitine-VPA compounds when renal function is normal (El-Hattab and Scaglia, 2015).

However, this view could be challenged by the fact that free carnitine levels are often low in patients with renal failure. Therefore, the positive correlation between serum carnitine level and serum creatinine level may only be observed in patients with normal renal function; further studies are required to confirm this hypothesis.

In pediatric patients with VPA-related hyperammonemia, decreased reabsorption of free carnitine in the kidney could be a cause of low serum carnitine levels (Matsuda et al., 1986). However, in adult patients treated with chronic VPA therapy, the reabsorption of free carnitine is yet to be elucidated.

4.5. Serum carnitine levels and serum ammonia levels

Haidukewych et al. (1985) reported that among 157 patients receiving chronic VPA therapy, the ammonia concentrations were found to be linearly and directly correlated with VPA plasma concentration, and the group with elevated ammonia levels had a significantly higher

mean plasma concentration of VPA. Itoh et al. (2012) reported a significant positive correlation between plasma ammonia levels and VPA dose, serum trough total VPA concentrations, and serum trough free VPA concentrations. Supplementation of L-carnitine has been reported to be effective for the treatment of hyperammonemia and hyperammonemia encephalopathy (Rigamonti et al., 2014; Cattaneo et al., 2017; Nozu et al., 2018). VPA-induced hyperammonemia encephalopathy can arise due to VPA intake even within the recommended range and normal blood concentration and can be treated with the dietary L-carnitine supplementation (Mock and Schwetschenau, 2012), indicating that serum carnitine levels are negatively correlated with plasma ammonia levels.

However, in our patients, no such correlation was observed (Table 2). It is possible that this negative correlation between serum carnitine levels and plasma ammonia concentrations would only be observed in extraordinary conditions such as VPA-induced hyperammonemia.

4.6. Other considerations

A low body mass index (BMI) and cognitive disorders were reported to be related to low free carnitine levels in pediatric patients with epilepsy (Okumura et al., 2019). In our patients, however, BMI, as well as comorbidities such as mental retardation, autism, and attention deficit hyperactivity disorder, did not significantly affect serum total or free carnitine levels. In our study, most patients with severe comorbidities lived in care homes and received well-balanced nutrition. This may be why we were unable to evaluate the relationship between poor nutrition and a low BMI with carnitine in our study.

4.7. Limitations

First, this was an uncontrolled naturalistic observational study with cross-sectional data sampling. The effect of controlled changes in VPA dose or other factors remains unknown. Additionally, the longitudinal correlation between serum carnitine levels and other factors is also unknown. Second, due to the restrictions in Japanese health insurance systems, serum carnitine levels were not determined in epileptic patients for whom VPA was not prescribed. Third, the timing of blood sampling was not strictly determined. Fourth, the overall frequency of seizures was counted by the patients, their parents, and caregivers. Although a seizure diary is a powerful tool for everyday clinical practice, it may not be fully accurate, which could have affected our results. Fifth, even though no patient was confirmed to suffer from malnutrition, the precise nutritional levels were not systematically assessed in any patient. Because the majority of carnitine intake is from food, we should further perform detailed assessments on food intake in our patients, using assessment tools such as ASA24. Finally, this study is lacking the assessment of patients' muscle volume that could affect both serum carnitine and creatinine levels.

4.8. Conclusion

Our results demonstrate that the effects of a combination therapy of VPA and other AED(s) on serum total and free carnitine levels appears to be higher than that of VPA monotherapy. In addition, the duration of epilepsy may affect serum total and free carnitine levels.

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Author Contributions

M.S. was critically involved in data collection and analysis and wrote the first draft of the manuscript. T.T. was involved in data analysis and contributed to the interpretation of the data and the writing of the manuscript. H.M. supervised the entire project, collected the data, and was critically involved in the design, analysis, and interpretation of the data. All authors contributed and approved the final manuscript.

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Appendix A. Supplementary data

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