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Original article

Carnitine deficiency: Risk factors and incidence in children with epilepsy

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Abstract

Background: Carnitine deficiency is relatively common in epilepsy; risk factors reportedly include combination antiepileptic drug (AED) therapy with valproic acid (VPA), young age, intellectual disability, diet and enteral or parenteral feeding. Few studies have examined the correlation between each risk factor and carnitine deficiency in children with epilepsy. We examined the influence of these risk factors on carnitine deficiency, and identified a formula to estimate plasma free carnitine concentration in children with epilepsy.

Methods: Sixty-five children with epilepsy and 26 age-matched controls were enrolled. Plasma carnitine concentrations were measured using an enzyme cycling assay, and correlations were sought with patients' other clinical characteristics.

Results: Carnitine deficiency was found in approximately 17% of patients with epilepsy and was significantly associated with carnitine-free enteral formula only by tube feeding, number of AEDs taken (independent of VPA use), body weight (BW), body height and Gross Motor Function Classification System (GMFCS) score. Stepwise multiple linear regression analysis indicated that carnitine concentration (in μmol/L) could be accurately estimated from a formula that does not require blood testing: $42.44 + 0.14 \times$ (BW in kg) $- 18.16 \times$ (feeding) $- 3.19 \times$ (number of AEDs), where feeding was allocated a score of 1 for carnitine-free enteral formula only by tube feeding and 0 for taking food orally ($R^2 = 0.504$, $P < 0.001$).

Conclusions: Carnitine-free enteral formula only by tube feeding, multiple AED treatment and low BW are risk factors for carnitine deficiency in children with epilepsy. L-carnitine should be administered to children at risk of deficiency to avoid complications. Treatment decisions can be informed using an estimation formula that does not require blood tests.

Keywords: Carnitine deficiency; Childhood epilepsy; Tube feeding; Antiepileptic drugs; Intellectual disability; Estimated formula

1. Introduction

Carnitine deficiency is relatively common in patients with epilepsy. Carnitine, a water-soluble quaternary amine, has important intracellular functions, but is only biologically active in the L-isoform. Approximately 75% of carnitine is obtained from the diet and the remainder from endogenous biosynthesis; the major tissue reservoir of carnitine is skeletal muscle [1,2]. Carnitine deficiency is defined as a plasma free carnitine concentration $\leqslant$20 μmol/L or a plasma esterified-to-free carnitine ratio of $\geqslant$0.4 in infants 1 week after birth. Carnitine deficiency

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may be classified as primary or secondary, and it may be associated with genetically determined metabolic errors, acquired disease or iatrogenic factors such as drug administration [3].

Numerous studies have shown that total or free plasma carnitine concentrations, or both, are significantly lower in patients taking antiepileptic drug (AED) combinations that include valproic acid (VPA), or VPA monotherapy [4–6]. The risk factors for carnitine deficiency are reported to include multiple AED therapy (including VPA), age <10 years, neurological disability (intellectual disability, cerebral palsy and microcephaly), a diet deficient in meat and dairy products, tube feeding and intravenous hyperalimentation [7]. Few studies have examined the correlation between each risk factor and carnitine deficiency in children with epilepsy. We sought to establish the strength of the relationship between each risk factor and carnitine deficiency, and generate a formula to estimate the plasma free carnitine concentration for children with epilepsy.

2. Materials and methods

2.1. Study population

Patients were recruited from those under the care of the Ehime University Hospital and Ehime Rehabilitation Center for Children between April 2012 and March 2013. Inclusion criteria were: (1) children with epilepsy aged from 1 to 15 years; (2) taking mono- or poly-AED therapy; and (3) AED treatment for more than 6 months. Exclusion criteria were: (1) children having special dietary therapies including ketogenic diet; (2) the use of dietary carnitine supplements or carnitine-containing formula; (3) steroid therapy; and (4) a diagnosis of one or more progressive degenerative, musculoskeletal or metabolic diseases, including primary carnitine deficiency. Sixty-five children with epilepsy were enrolled. For controls, serum samples were collected from 26 age-matched patients who had undergone blood tests for the assessment of their underlying diseases such as psychosomatic disorder, headaches, orthostatic hypotension or as part of a general health check-up; all investigations performed in samples obtained from control patients were within their normal ranges. Participant characteristics are described in Table 1. All children or their parents provided informed consent to participate.

2.2. Sample and data collection

Blood samples were immediately sent to a laboratory (Bio Medical Laboratories, Tokyo, Japan) for the measurement of plasma total carnitine, free carnitine and acylcarnitine concentrations. These were measured using an assay that uses nicotinamide adenine dinucleotide $(NAD)^+$ and thio-NAD^+ as coenzymes with carnitine dehydrogenase for the reduction of L-carnitine; the resultant enzymatic cycling leads to the accumulation of thio-NADH at a constant rate that is proportional to the concentration of L-carnitine, and can be measured as the increase in absorbance at 415 nm, as previously described [8]. The samples were also used to measure the serum concentrations of ammonia, glucose, creatinine and lactate, and liver function tests. On the same day, the following physical parameters were recorded: body height (BH; cm), body weight (BW; kg), age (months), Gross Motor Function Classification System score (GMFCS score) [9,10], meal patterns and feeding methods (carnitine-free enteral formula only by tube feeding or taking food orally), number of AEDs and VPA therapy. The GMFCS is a grading system that describes the ability of children from birth to 18 years of age to function and move in their daily life. GMFCS level I equates to being able to run and jump, level II to being able to stand and walk, level III to being able to crawl and kneel; level IV being able to sit; and level V, lies but is able to roll.

Table 1
Summary of the demographic and clinical characteristics of patients and controls.

	Patients with epilepsy	Controls
Number of patients	65	26
Male:Female	29:36	15:11
Age (months)	113.7 ± 6.0	107.4 ± 9.1
Body height (cm)	122.7 ± 3.1	131.4 ± 4.9
Body weight (kg)	25.1 ± 3.1	33.2 ± 2.9
GMFCS score	2.8 ± 0.2	1.3 ± 0.2
Tube feeding with carnitine-free enteral formula only (*n*)	17 (26.2%)	0 (0%)
Number of AEDs prescribed	1.6 ± 0.1	–
VPA use (*n*)	38 (58.5%)	–

Data are presented as the mean ± standard error, or number (proportion).
Abbreviations: GMFCS, Gross Motor Function Classification System; AED, anti-epileptic drug; VPA, valproic acid.

2.3. Statistical analysis

Clinical and laboratory data were processed using IBM SPSS Statistics version 20 (IBM, Chicago, IL, USA). Data were analyzed using one-way analysis of variance (ANOVA) followed by Scheffe's multiple comparisons test, simple linear regression analysis and multiple linear regression analysis (stepwise method). Power analysis was undertaken using G*Power 3.1.9.2 (Heinrich Heine, Universität Düsseldorf, Germany). Results are expressed as means ± standard error, and P values of <0.05 were considered significant.