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CLINICAL STUDY

Clinical significance of different carnitine levels for improving the prognosis of patients undergoing hemodialysis

Yu-Mei Zhang^a, Li Zhuo^a, Jing Hu^a, Gang Cui^b, Ling Zhang^a, Xiang-Lin Zhang^b and Wen-Ge Li^a

^aDepartment of Nephrology, China-Japan Friendship Hospital, Beijing, China; ^bDepartment of Pharmacy, China-Japan Friendship Hospital, Beijing, China

ABSTRACT

Objective: To investigate plasma-free carnitine (Fc), acylcarnitine (Ac), and total carnitine (Tc) levels in patients undergoing hemodialysis (HD), and to explore their clinical significance.

Methods: A total of 20 subjects were in the normal control group and 133 patients undergoing HD were divided into medicated (received carnitine treatment) and non-medicated groups. The medicated group was further divided into three subgroups according to Fc level: Fc = 80–199, 200–299, and ≥ 300 $\mu\text{mol/L}$. We used non-derivative tandem mass spectrometry to determine carnitine levels, and clinical symptoms such as weakness, hypotension, and muscle cramps were recorded during dialysis.

Results: Fc and Tc levels were significantly lower in the non-medicated group than in the control group, whereas Fc, Ac, and Tc levels were higher in the medicated than non-medicated group ($p < .05$). The medicated group had fewer symptoms during dialysis than the non-medicated group such as weakness, hypotension, and muscle cramps ($p < .05$). An additional comparison showed that the incidence rates of hypotension and muscle cramps in the Fc < 80 –199 $\mu\text{mol/L}$ group were significantly lower than those in the Fc ≥ 300 $\mu\text{mol/L}$ medicated and non-medicated groups.

Conclusions: Patients undergoing HD have low carnitine levels. L-Carnitine can effectively increase Fc concentration and improve clinical symptoms; however, only the proper Fc range can reduce complications caused by dialysis. Thus, this range needs to be determined.

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Carnitine; maintenance hemodialysis; weakness; hypotension; muscle cramps

Introduction

Carnitine is an amino acid derivative that helps produce energy for muscle and cell metabolism by mediating the mitochondrial β -oxidation of fatty acids. It is mainly derived from the diet and by endogenous biosynthesis, primarily by the liver and kidney. Healthy people rarely lack carnitine, but carnitine homeostasis can be disturbed in patients receiving long-term hemodialysis (HD).¹ These patients typically have concomitant carnitine insufficiency in plasma and tissues due to impaired carnitine biosynthesis, reduced protein uptake, and increased removal of carnitine by dialysis, resulting in reduced carnitine activity.^{2,3} Carnitine deficiency worsens in patients undergoing long-term dialysis and can cause energy metabolic disorders and symptoms such as weakness, fatigue, dialysis hypotension, and muscle cramps in the short term; and angina, arrhythmia, heart failure, and limited erythropoietin effects during long-term deficiency. These symptoms are easily confused

with uremia caused by retained toxins, but determining plasma carnitine levels is diagnostic.

In this study, we compared plasma carnitine levels in normal subjects and patients undergoing HD to determine its clinical significance and to provide a basis for carnitine supplementation in patients undergoing HD.

Subjects and methods

Patients

A total of 20 patients (our hospital healthcare workers) were in the normal control group; thus, 20 plasma samples were collected. A total of 133 patients were in the maintenance HD (MHD) group. These patients received MHD in the blood purification center of the China-Japan Friendship Hospital during October 2014, and were divided into medicated (received carnitine treatment) and non-medicated groups. HD conditions: performed three times/week, 4 h/once; HD unit

area $\geq 1.5 \text{ m}^2$; dialysate flow, 500 mL/min; blood flow, 200–300 mL/min.

General information records

Basic patient information including age, sex, weight, primary disease, and date the patients accepted MHD treatment were recorded. The patients' clinical symptoms such as weakness after dialysis, low blood pressure, and muscle cramps during dialysis were also recorded.

Reagents

The NeoBase™ Non-derivatized MSMS Kit (PerkinElmer, Waltham, MA) contains mobile phase solvents, extract liquid, 96-well microtiter plates, microtiter plate covers, high-low quality product, and one bottle of acylcarnitine (Ac) stable isotopes containing 13 kinds of Ac stable isotopic internal standards: 2H_9 -free carnitine, 2H_3 -acetylcarnitine, 2H_3 -propionyl carnitine, 2H_3 -butyryl carnitine, 2H_9 -isovaleryl carnitine, 2H_6 -glutaryl carnitine, 2H_3 -caproyl carnitine, 2H_3 -capryloyl carnitine, 2H_3 -decanoyl carnitine, 2H_3 -dodecanoyl carnitine, 2H_3 -myristoyl carnitine, 2H_3 -palmitoyl carnitine, and 2H_3 -stearyl carnitine.

Instruments

Acquity UPLC ultra performance liquid chromatography (Waters Co, Billerica, MA); Quattro Premier XE three-level series of four-pole mass spectrometer with ESI (Waters) and the Masslynx 4.1 data processing system (Waters); and the MB 100-4A Micro hole plate constant temperature oscillator (Hangzhou Allsheng Instruments Co., Ltd, Zhejiang, China) were used for the analysis.

Sample processing

In accordance with the method in the NeoBase™ Non-derivatized MSMS Kit (PerkinElmer, Waltham, MA), we collected morning fasting venous blood samples from the control group and fasting venous blood before HD from the HD group. The medicated group was given 1 g L-carnitine after each HD session. Venous blood was dropped on 10 mm diameter filter paper, allowed to dry at room temperature, and stored at -20°C .

Testing method

The filter paper containing dried blood (equivalent to $3.2 \mu\text{L}$ whole blood) was placed in a 96-well microtiter plate. Then $100 \mu\text{L}$ Ac isotopic internal standard working

solution was added and the sample was subjected to constant temperature oscillator 700 rpm at 45°C for 45 min. A $75 \mu\text{L}$ aliquot of the extracted liquid was removed, placed on a heat-resistant plate, and sealed with aluminum foil for testing.

Instrument settings

The following kit conditions were used: velocity gradient, 0–0.22 min at 0.15 mL/min; 0.22–1.0 min at 0.023 mL/min; and 1.0–1.5 min at 0.60 mL/min. The electrospray cation scan mode and multiple reaction monitoring mode were used for data collection. Each sample was measured for 1.5 min and the injection volume was $10 \mu\text{L}$.

Statistical analysis

The mass spectrum response values were processed using Masslynx V4.1. software. Ac levels were quantified by calculating ionic strength divided by the ionic strength of the isotopic internal standard. SPSS 19.0 software (SPSS Inc., Chicago, IL) was used to analyze the differences in spectrometry parameters. Measurement data are expressed as mean \pm standard deviation. The two groups were compared using the *t*-test and the count data were analyzed with the χ^2 test. A *p* value $<.05$ was considered statistically significant.

Results

General data analysis

1. Normal control group: The mean age of the 20 healthcare workers (nine males and 11 females) was 29.65 ± 4.80 years (range, 22–43 years) and the mean weight was 58.48 ± 9.95 kg.
2. HD group: One-hundred and thirty-three patients (68 females and 65 males) who received MHD in the department of blood purification center of China-Japan Friendship Hospital in March 2015. The mean age was 61.24 ± 13.45 years (range, 25–86 years) and the mean weight was 65.85 ± 16.24 kg. A total of 38 (28.57%) cases had chronic glomerulonephritis, 35 (26.32%) had diabetic nephropathy, 21 (15.79%) had hypertensive renal damage, 12 (9.02%) had tubulointerstitial nephropathy, seven (5.26%) had polycystic kidney, two (1.50%) had renal tumors, and 18 (13.53%) had other kidney diseases. The patients were divided into medicated and non-medicated groups. A total of 66 non-medicated cases had been undergoing MHD for 62.16 ± 69.70 months (range, 1–264

months); 25 (34.85%) of the 66 patients had <1 year dialysis history, 11 (16.67%) had a dialysis history of 1–3 years, and 32 (48.48%) had >3 years of dialysis history. A total of 67 medicated cases received 1 g L-carnitine intravenously after each dialysis session. The mean MHD history of the medicated group was 80.06 ± 69.22 months (range, 4–324 months) and their medication history was >2 months.

Carnitine levels in the two groups

The results are shown in Figure 1. The plasma-free carnitine (Fc) and total carnitine (Tc) levels in the non-medicated group were lower than those in the normal control group ($p < .05$). The Ac level in the non-medicated group tended to be higher than that in the control group. Fc, Ac, and Tc levels in the medicated group were higher than those in the non-medicated group ($p < .05$).

Clinical symptoms in the HD group

Dialysis hypotension refers to a decrease in systolic blood pressure >20 mmHg during dialysis or a decrease in mean arterial pressure >10 mmHg with symptoms of low blood pressure.⁴ We divided the medicated group into three subgroups based on Fc level: $Fc = 80$ –199; 200–299 and $Fc \geq 300$ $\mu\text{mol/L}$. The detailed data are shown in Table 1. The incidence of weakness increased with Fc level after dialysis. The incidence of weakness in

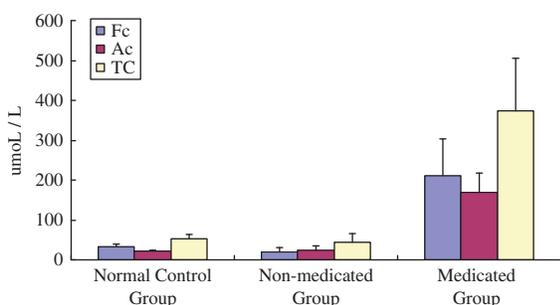


Figure 1. Carnitine levels in the medicated and non-medicated groups ($\mu\text{mol/L}$). Abbreviations: Fc: free carnitine; Ac: acylcarnitine; Tc: total carnitine.

Table 1. Clinical symptoms in the groups.

Groups ($\mu\text{mol/L}$)	Cases (incidence%)			Total
	Weakness	Hypotension	Muscle cramps	
Non-medicated group	26 (39.39)	33 (50.00)	39 (59.09)	66
Medicated group				
Fc = 80–199	7 (20.00)	6 (17.14)	7 (20.00)	35
Fc = 200–299	6 (33.33)	8 (44.44)	6 (33.33)	18
Fc ≥ 300	7 (50.00)	8 (57.14)	9 (64.28)	14

the $Fc \geq 300$ $\mu\text{mol/L}$ group tended to be higher than that in the non-medicated group. Similarly, the incidence rates of hypotension and muscle cramps also increased during dialysis as Fc level increased. The $Fc \geq 300$ $\mu\text{mol/L}$ group had more cases of hypotension and muscle cramps than those in the non-medicated group (χ^2 test; $p < .05$). The incidence rates of hypotension and muscle cramps in the subgroups were significantly different (by χ^2 test). We used the Bonferroni method to compare the pairs of subgroups; the significance level was adjusted to $\alpha = 2\alpha/k$ ($k - 1$) = 0.008, where k is the number of groups (four in this study). The significant data were marked in the 2×2 chi-square test. The Fc 80–199 $\mu\text{mol/L}$ group (17.14%) had a lower incidence of hypotension than the $Fc \geq 300$ $\mu\text{mol/L}$ (57.14%) and non-medicated groups (50.00%) (χ^2 test; $p < .05$). No other comparisons were significant. The incidence of muscle cramps during dialysis in the Fc 80–199 $\mu\text{mol/L}$ group of patients undergoing HD was lower than that in the $Fc \geq 300$ $\mu\text{mol/L}$ (64.28%) and non-medicated groups (59.09%) (χ^2 test; $p < .05$). No other comparisons were significant.

Discussion

Most carnitine is obtained from dietary sources in humans and approximately 25% comes from *de novo* biosynthesis. Carnitine is obtained by consuming animal-based products including red meats, poultry, fish, and dairy products, whereas negligible quantities are available from plant-derived foods. On the other hand, carnitine is produced from lysine and methionine by a variety of enzymes in the liver, kidneys, and brain. The kidney is the main carnitine excretory organ, and a healthy person excretes 100–400 $\mu\text{mol/Lol}$ daily in the urine. However, to reduce L-carnitine loss and maintain adequate endogenous concentrations, >95% of filtered L-carnitine is reabsorbed by the proximal tubules and returned to the bloodstream.

Tandem mass spectrometry is an efficient, rapid, and accurate technique to detect substances; thus, it was used to analyze the amino acid, organic acid, carnitine, and fatty acid levels in dried blood on filter paper. Plasma carnitine levels slightly differed among studies, as carnitine level can change due to diet, race, age, and method of determination.

Carnitine deficiency is common in patients with end-stage renal disease receiving HD treatment.⁵ We found that Fc and Tc levels in non-medicated patients undergoing HD were lower than those in the control group. Patients may have low Tc and Fc levels due to lower protein and meat intake, which results in lower carnitine intake; less absorption by the gastrointestinal

tract; reduced carnitine synthesis in the kidneys; low renal tubular reabsorption; carnitine transport disorder; and the fact that it is highly dialyzable because of its low molecular weight and lack of protein binding in the plasma. Plasma carnitine levels generally increase in patients with chronic renal failure who do not undergo HD, but decrease in patients who accept HD treatment. In fact, a single HD session can decrease the plasma carnitine concentration by as much as 75%.⁶ We examined 75 non-medicated patients undergoing HD, 25 (20%) of whom had been undergoing HD for <1 year, with a minimum of one month.

Carnitine is a water-soluble zwitterionic quaternary amine (beta-hydroxy-gamma trimethyl aminobutyric acid; molecular weight, 161.2 Da) that exists as Fc and Ac in serum. Fc accounts for 85.2% of total carnitine, and Fc and Ac together constitute Tc. Carnitine exists as D- and L-carnitine enantiomers; however, only the L-isomer is physiologically active. L-carnitine is a naturally occurring compound found in all mammals. The most important biological function of L-carnitine is transport of fatty acids into the mitochondria for β -oxidation.⁷ As L-carnitine plays an important role in biological metabolism, it is frequently used in clinical treatment. Administering carnitine is beneficial to patients with angina, ischemia-induced cardiac insufficiency, cardiogenic shock, cardiomyopathy, and myocardial infarction.⁸ A hyperbolic increase in plasma carnitine concentration is observed after completing a dialysis session, as it is released from tissue stores. Repeated dialysis treatment over an extended period results in a deficiency of tissue carnitine.⁹ Carnitine insufficiency in patients on long-term HD affects dialysis-related symptoms such as skeletal myopathy, cardiomyopathy, poor exercise performance, anemia, and dialytic complications, such as hypotension, cramps, weakness, and fatigue,^{10,11} which decrease dialysis tolerance and reduce the long-term survival rate. Consequently, carnitine therapy has been used and extensively studied in patients with ESRD. Although a large number of clinical studies have shown that administering carnitine can alleviate many dialysis-related symptoms in patients undergoing HD, controversy regarding its use persists because of conflicting results. These different results may be related to a lack of randomization and control groups, dose heterogeneity, duration and method of carnitine administration, nonstandard measures of symptom improvement, different laboratory or clinical endpoints, variation in the patient population, or patient response. However, it has been suggested that L-carnitine may have to be administered at doses that achieve supra-physiological concentrations in plasma

and target tissues to fully exploit its pharmacologic potential.^{12,13}

This study found that Fc and Tc levels in non-medicated patients undergoing HD were lower than those in the control group. The Fc, Ac, and Tc levels of patients increased significantly after 1 g L-carnitine was administered intravenously. Plasma carnitine levels decreased after HD therapy but significantly increased after administering L-carnitine, demonstrating that L-carnitine can effectively make up for the loss of carnitine in patients undergoing HD.

Fewer clinical symptoms, such as weakness, hypotension, and muscle cramps, were observed after dialysis in patients undergoing HD in the medicated group compared to the non-medicated group. The incidence of weakness increased after dialysis as Fc level increased. The incidence of weakness in the $Fc \geq 300 \mu\text{mol/L}$ group tended to increase more than that in the non-medicated group (by χ^2 test; $p > .05$). Similarly, the incidence rates of hypotension and muscle cramps also increased during dialysis as Fc levels increased. In this case, the incidence rates of hypotension and muscle cramps in the $Fc \geq 300 \mu\text{mol/L}$ group were also higher than those in the non-medicated group (by χ^2 test; $p < .05$). The incidence of hypotension during dialysis in the Fc 80–199 $\mu\text{mol/L}$ group (17.14%) was lower than that in the $Fc \geq 300 \mu\text{mol/L}$ (57.14%) and non-medicated groups (50.00%) ($p < .05$). No other comparisons were significant. The incidence of muscle cramps in the Fc 80–199 $\mu\text{mol/L}$ group (20.00%) was lower than that in the $Fc \geq 300 \mu\text{mol/L}$ (64.28%) and non-medicated groups (59.09%) ($p < .05$). No other comparisons were significant. Our results showed that L-carnitine effectively improved hypotension and muscle cramp symptoms during dialysis in the Fc 80–199 $\mu\text{mol/L}$ group. The $Fc \geq 300 \mu\text{mol/L}$ group had the highest incidence rates of hypotension and muscle cramp compared to those in the non-medicated group.

In conclusion, patients undergoing HD generally have low levels of carnitine. We determined Fc levels in patients before dialysis and found that their levels were lower than those in the control group. We speculate that reductions in carnitine would be more noticeable after dialysis, so we recommend that doctors monitor carnitine levels in patients undergoing HD. L-Carnitine is important in clinical medicine, and our results demonstrate that it effectively increases blood Fc concentrations and improves clinical symptoms. This comprehensive evaluation results suggest that the appropriate range of free carnitine can improve complications in patients undergoing dialysis; however, beyond a certain range its effects may be

counterproductive. Thus, the appropriate Fc range requires further study.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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