Renal Replacement Therapy

RESEARCH

Decreased level of serum carnitine might lead to arteriosclerosis progression via the accumulation of advanced glycation end products in maintenance hemodialysis patients

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Abstract

Background: Carnitine is reported to improve insulin resistance and reduce oxidative stress. Hyperglycemia and increased oxidative stress are well known to promote the production of advanced glycation end products (AGEs) that can lead to arteriosclerosis in patients with maintenance hemodialysis (HD). In the present study, we aimed to determine whether decreased level of serum carnitine accelerated arteriosclerosis and to clarify the relationships between carnitine, AGEs, and arteriosclerosis in HD patients.

Methods: We recruited 116 patients (65 men and 51 women, 62 ± 13 years) undergoing HD three times a week. We measured pre-HD serum free carnitine prior to the first weekly session. AGE level was quantitatively evaluated by measuring skin autofluorescence (SAF) with AGE reader. Arteriosclerosis was evaluated by measuring carotid intima-media thickness (cIMT). Relationships between free carnitine, SAF, and cIMT were analyzed, and significant limiting factors for arteriosclerosis were identified using univariate and multivariate regression analyses.

Results: Free carnitine ranged from 14.9 to 53.3 μmol/L (mean, 28.6 ± 8.1 μmol/L). Free carnitine was negatively correlated with SAF ($r = -0.223$, $P = 0.017$) and cIMT ($r = -0.252$, $P = 0.006$). SAF was positively correlated with cIMT ($r = 0.263$, $P = 0.005$). Free carnitine was identified as a significant independent limiting factor for cIMT ($\beta = -0.194$, $P = 0.037$).

Conclusions: Decreased level of serum carnitine might lead to the progression of arteriosclerosis via the AGE accumulation in HD patients.

Keywords: Advanced glycation end products, Arteriosclerosis, Carnitine, Hemodialysis

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Background

In maintenance hemodialysis (HD) patients, oxidative stress and hyperglycemia are known to contribute to the formation of advanced glycation end products (AGEs) that are formed by non-enzymatic glycation reactions between reduced sugars and biological macromolecules such as proteins [1, 2]. In addition, AGEs are slowly metabolized in HD patients, given the role of the kidney in their degradation and excretion [3]. Consequently, AGE accumulation accelerates in the tissues and organs of HD patients [4]. AGEs, binding to AGE receptors on vascular endothelial cells, generate reactive oxygen species and induce vascular inflammation, which can accelerate the progression of arteriosclerosis [5]. Indeed, a number of studies have found that the progression of arteriosclerosis is more accelerated in HD patients due to the high accumulation of AGEs relative to age- and sex-matched healthy individuals [4].

Carnitine is an essential cofactor for the transport of long-chain fatty acids and β-oxidation in mitochondria [6]. It enhances the oxidative utilization of glucose through the acceleration of pyruvate uptake in mitochondria [7]. Moreover, it improves insulin sensitivity in skeletal muscles by promoting glucose utilization [8]. Carnitine also suppresses oxidative stress by chelating free Fe^{2+} ion and activating antioxidants [9]. The antioxidant efficacy of carnitine treatment has been reported in various conditions such as hypertension, hypercholesterolemia, atherosclerosis, or aging that increase systemic oxidative stress [10–13]. Thus, carnitine can prevent AGE accumulation in tissues and organs by reducing oxidative stress and improving glucose metabolism. The muscle content of carnitine is substantially reduced in HD patients due to decreased carnitine synthesis in kidney, removal from blood during HD, and insufficient dietary intake, which is reflected in serum carnitine level [14]. Based on this, we hypothesized that decreased level of serum carnitine would enhance AGE accumulation by exacerbating glucose metabolism and increasing oxidative stress, thereby promoting the progression of arteriosclerosis. However, it remains unclear whether decreased level of serum carnitine has this effect in HD patients. To this end, this study aimed to determine whether decreased level of serum carnitine serves as a factor that accelerates arteriosclerosis and to clarify the relationships between carnitine, AGEs, and arteriosclerosis in HD patients.

Methods

This study was approved by the Bioethics Committee for Clinical Research A of Jichi Medical University Hospital. Written informed consent was obtained from all patients after they received a detailed explanation of the study protocol.

One hundred and thirty patients with end-stage renal disease who received outpatient care for regular maintenance HD three times a week at Sohbudai Nieren Clinic in August 2015 were recruited. Exclusion criteria were as follows: patients with malignancy or dementia or those regularly taking a carnitine-based drug. That is, 8, 3, and 3 patients were excluded for malignancy, dementia, and taking a carnitine-based drug, respectively. Consequently, the final study population consisted of 116 patients on maintenance HD.

Study design

After clinical characteristics of the patients were obtained from medical records or by interview, we performed blood examinations and AGE measurements and assessed carotid intima-media thickness (cIMT) as an indicator of arteriosclerosis.

Clinical characteristics and blood examination

The following clinical characteristics were assessed: gender, age, height, dry body weight, body mass index, systolic and diastolic blood pressure, heart rate, duration of HD, prevalence of diabetes mellitus, treatment, and left ventricular ejection fraction. Body mass index was calculated as dry body weight in kilograms divided by height in meters squared. Systolic and diastolic blood pressure and heart rate were measured in the supine position using a vascular profile device (BP-203RPE, Omron Colin, Kyoto, Japan) before the HD session. Left ventricular ejection fraction was measured with an echocardiogram (SSD-5500 system, Aloka, Tokyo, Japan) as an indicator of left ventricular function when patients had stable hemodynamic condition under the optimal dry weight after the HD session.

Blood samples were collected just before the HD session after a 2-day HD interval to measure blood hemoglobin A1c and serum levels of glycated albumin (GA), triglyceride, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol as indicators of glycolipid metabolism. We also measured serum levels of albumin, creatinine, uric acid, urea nitrogen, β2-microglobumin, C-reactive protein, corrected calcium [15], and phosphate. Serum levels of free carnitine, acyl carnitine, and total carnitine were measured with the enzyme cycling method [16].

Fractional clearance of urea (Kt/V) was used to evaluate the HD adequacy, which was given by urea clearance (K, mL/min), HD treatment time (t, min), and urea distribution volume (V, mL) [17].

Advanced glycation end products

We measured skin autofluorescence (SAF) as an indicator of AGEs. SAF was determined using an AGE reader with an ultraviolet source of specific wavelengths (DiagOptics

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BV, Groningen, The Netherlands), which was reported to reflect AGE accumulation in biopsied skin [18]. The forearm was placed on the AGE reader to measure SAF at three sites, 1 cm away from each other, where neither bruises nor obvious pigmentation was present. SAF was automatically analyzed with a device connected to a computer that had analysis software installed [19].

Arteriosclerosis

The carotid intima-media thickness (cIMT) was measured in the supine position with an ultrasonogram (SSD-5500 system, Aloka) by a skilled technician who had no prior knowledge of patients' clinical characteristics. The far and near walls of the right and left common carotid arteries, carotid bulbs, and internal carotid arteries were scanned in both short-axis and long-axis views using a 11-MHz linear probe. The thickest site of the intima-media complex including plaque lesions was measured as the maximum cIMT in increments of 0.1 mm to assess the severity of arteriosclerosis.

Statistical analysis

All data are expressed as mean ± standard deviation. We analyzed relationships between free carnitine, GA, SAF, and cIMT using Spearman's correlation coefficient. $P < 0.050$ was considered statistically significant. Univariate and multivariate regression analyses were performed to clarify the relationship between free carnitine and cIMT. In the analyses, we used cIMT as a dependent variable, and factors related to arteriosclerosis progression as independent variables, after assessing the multicollinearity among independent variables [20]. All statistical analyses were performed using the Statistical Package for Social Sciences, version 23.0 for Windows (IBM Corporation, NY, USA).

Results

Clinical characteristics of the patients are summarized in Table 1. The mean age of patients was 62 ± 13 years, and 54.9% were male. Patients with a history of type 2 diabetes mellitus comprised 41.4% of the study population. Serum level of free carnitine ranged from 14.9 to 53.3 μmol/L (mean, 28.6 ± 8.1 μmol/L), and cIMT from 0.6 to 3.3 mm (mean, 1.6 ± 0.6 mm).

Relationships between free carnitine, GA, and SAF are shown in Fig. 1. No significant relationship was found between free carnitine and GA. SAF was negatively correlated with free carnitine ($r = -0.223$, $P = 0.017$).

Relationships between free carnitine, SAF, and cIMT are shown in Fig. 2. The cIMT was negatively correlated with free carnitine ($r = -0.252$, $P = 0.006$) and positively correlated with SAF ($r = 0.263$, $P = 0.005$).

Table 1 Clinical characteristics

Number of patients (male/female) (*n*)	116 (65/51)
Age (years)	62 ± 13
Body mass index (kg/m^2)	21.3 ± 3.3
Type 2 diabetes mellitus (+/−)(*n*)	48/68
Treatment (%)	
ARB/ACE inhibitor	31.0
Iron therapy	37.1
Systolic blood pressure (mmHg)	149 ± 24
Diastolic blood pressure (mmHg)	88 ± 16
Heart rate (/min)	77 ± 12
Duration of hemodialysis (years)	7.6 ± .2
Kt/V (range)	1.10 ± 0.31
Hemoglobin A1c (%)	5.4 ± 0.7
Glycated albumin (%)	16.7 ± 3.8
Triglyceride (mg/dL)	139 ± 110
Total cholesterol (mg/dL)	169 ± 32
Low-density lipoprotein cholesterol (mg/dL)	94 ± 26
High-density lipoprotein cholesterol (mg/dL)	51 ± 19
Albumin (g/dL)	3.8 ± 0.3
Creatinine (mg/dL)	11.26 ± 3.08
Uric acid (mg/dL)	6.9 ± 1.4
Urea nitrogen (mg/dL)	62.9 ± 13.5
Corrected calcium (mg/dL)	9.0 ± 0.8
Phosphate (mg/dL)	5.7 ± 1.3
C-reactive protein (mg/dL)	0.27 ± 0.79
β2-microglobumin (mg/L)	24.9 ± 5.4
Total carnitine (μmol/L)	44.5 ± 11.7
Free carnitine (μmol/L)	28.6 ± 8.1
Acyl carnitine (μmol/L)	15.9 ± 4.7
Acyl carnitine/free carnitine	0.6 ± 0.1
Skin autofluorescence (arbitrary unit)	3.4 ± 0.7
Left ventricular ejection fraction (%)	66.9 ± 8.9

Data are presented as mean ± standard deviation. Kt/V is used to evaluate the hemodialysis adequacy
ARB angiotensin II receptor blocker, *ACE inhibitor* angiotensin-converting enzyme inhibitor

The results of univariate and multivariate regression analyses for cIMT are shown in Table 2. There was no significant multicollinearity in independent variables. Age, prevalence of diabetes mellitus, creatinine, albumin, and free carnitine were significantly correlated with cIMT ($\beta = 0.548$, $P = 0.001$; $\beta = 0.225$, $P = 0.015$; $\beta = -0.364$, $P = 0.001$; $\beta = -0.336$, $P = 0.001$; $\beta = -0.252$, $P = 0.006$, respectively). Age, albumin, and free carnitine were identified as significant independent limiting factors for cIMT ($\beta = 0.310$, $P = 0.006$; $\beta = -0.176$, $P = 0.044$; $\beta = -0.194$, $P = 0.037$, respectively).

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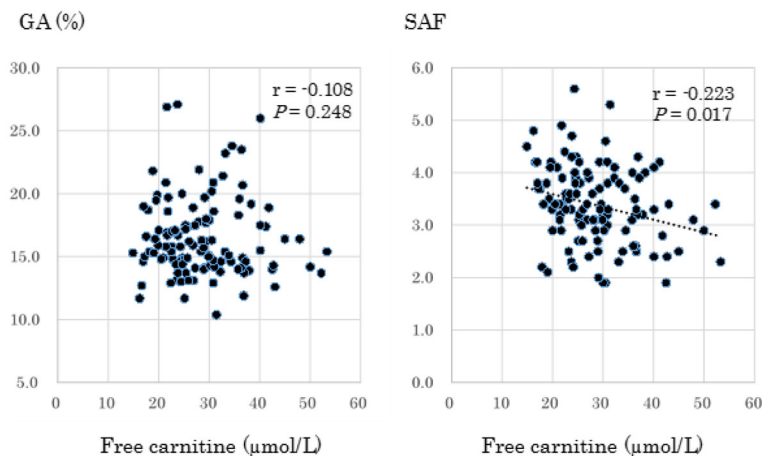
Fig. 1 Relationships between free carnitine, GA, and SAF. GA glycated albumin, SAF skin autoflourescence

Discussion

In the present study, we found that a decreased level of serum carnitine was a significant independent determinant of the arteriosclerosis progression in HD patients. That is, multivariate regression analysis revealed that free carnitine was identified as one of significant factors increasing cIMT. In addition, we found that free carnitine was negatively correlated with SAF and that SAF was positively correlated with cIMT.

A previous study showed that decreased serum level of carnitine was independently correlated with increased tissue accumulation of AGEs in HD patients [21]. It has been also reported that AGE accumulation accelerates the progression of arteriosclerosis in HD patients [4]. Therefore, our findings showed that decreased level of serum carnitine might enhance AGE accumulation, thereby promoting the progression of arteriosclerosis in them.

Carnitine has been known to reduce AGE accumulation in tissues and organs by reducing oxidative stress and activating glucose metabolism [22]. However, the present study showed no significant relationship between free carnitine and GA. Because we recruited HD patients whose glucose metabolism was well controlled, most of their GA values were under the target value for glycemic control [23]. That may be the reason why we could not find the significant relationship between them.

Systolic blood pressure and low-density lipoprotein cholesterol are generally known to accelerate arteriosclerosis [24]. However, there were no significant relationships between systolic blood pressure or low-density lipoprotein cholesterol and cIMT in the present study. Savage et al. reported that no significant relationship between systolic blood pressure and cIMT was found in HD patients [25]. As the reason, they stated that the values of systolic blood pressure well controlled with antihypertensives were used for the data analysis. Furthermore, it was reported that hypertension or dyslipidemia was correlated with the progression of arteriosclerosis,
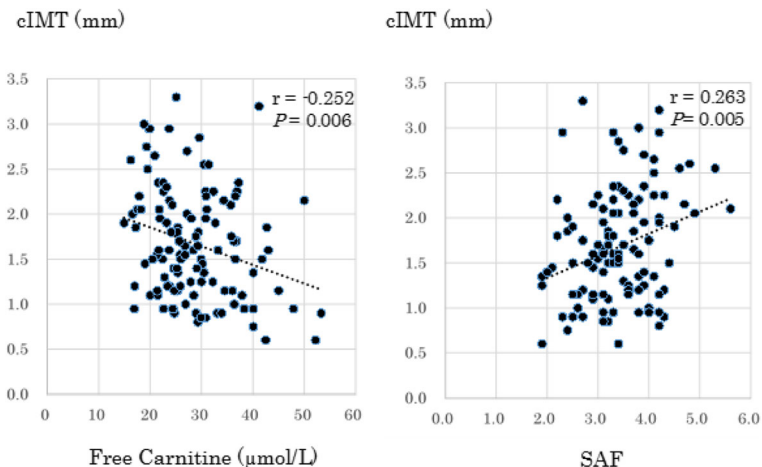
Fig. 2 Relationships between free carnitine, SAF, and cIMT. SAF skin autoflourescence, cIMT carotid intima-media thickness

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Table 2 Results of univariate and multivariate regression analyses for carotid intima-media thickness

	Univariate regression		Multivariate regression	
	β	P	β	P
Gender	0.132	0.158	0.159	0.108
Age (years)	0.548	0.001	0.310	0.006
Type 2 diabetes mellitus	0.225	0.015	0.106	0.235
Body mass index (kg/m^2)	− 0.162	0.082	− 0.037	0.685
Systolic blood pressure (mmHg)	− 0.089	0.344	− 0.065	0.432
Low-density lipoprotein cholesterol (mg/dL)	− 0.143	0.124	− 0.017	0.833
Creatinine (mg/dL)	− 0.364	0.001	− 0.091	0.411
Albumin (g/dL)	− 0.336	0.001	− 0.176	0.044
Corrected calcium (mg/dL)	0.055	0.556	0.040	0.628
Phosphorus (mg/dL)	− 0.092	0.327	0.063	0.480
Free carnitine (μmol/mL)	− 0.252	0.006	− 0.194	0.037

when arteriosclerosis was assessed by the mean cIMT that reflected the arterial wall thickness [26]. However, the maximum cIMT was used to assess arteriosclerosis in the present study, which was known to reflect the arterial wall thickness and atheroma formation [26]. The reason why no significant relationships were found between them is to adopt the values of systolic blood pressure and low-density lipoprotein cholesterol that were well controlled with medications and also to adopt the maximum cIMT for data analysis.

There are some limitations worth noting. First, although we speculated that reduced oxidative stress was an underlying mechanism by which carnitine reduces AGEs, we did not evaluate the relationship between carnitine and oxidative stress. In future studies, free radical and lipid peroxide production should be assessed in order to examine the relationship between carnitine and oxidative stress. Second, we could not demonstrate the significant relationship between carnitine and glucose metabolism in the present study. In order to clarify whether carnitine reduces AGEs through the improvement of glucose metabolism, we should perform the reanalysis using large sample sizes in diabetic HD patients and non-diabetic HD patients taking into consideration their condition such as diabetes treatment. Finally, the study design was cross-sectional, and we did not directly examine the effects of carnitine on the progression of arteriosclerosis. Future prospective clinical studies should be planned to elucidate the relationship between carnitine administration, AGEs, and arteriosclerosis.

Because arteriosclerosis is the primary cause of death in HD patients [27], there is a need for promising strategies to prevent its progression. Previous studies showed that carnitine administration attenuated the progression of atherosclerosis and improved cardiovascular dysfunction in HD patients [28, 29]. Given that serum level of carnitine is significantly reduced in most HD patients

[14], administering carnitine to this population could potentially prevent the progression of arteriosclerosis and improve prognosis.

Conclusions
Decreased level of serum carnitine might lead to the progression of arteriosclerosis via the AGE accumulation in maintenance HD patients.

Availability of data and materials
Please contact the corresponding author for data requests.

Authors' contributions
YK designed the study, collected the clinical data, performed the data analysis, and wrote the manuscript. TM designed the study and wrote the manuscript. KK designed the study and performed the data analysis. TN collected the clinical data. ST, NH, YI, IM, NK, MO, KT, SN, and YT provided substantial revisions to the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
This study was approved by the Bioethics Committee for Clinical Research A of Jichi Medical University Hospital (RIN No. 13–09).

Consent for publication
Not applicable.

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