RESEARCH ARTICLE

Impact of haemodialysis on plasma carnitine concentrations in haemodialysis patients

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Abstract

Objective: To evaluate the impact of haemodialysis on plasma carnitine levels.

Method: The cross-sectional study was conducted from April 20, 2020 to May 10, 2022, at the dialysis unit of the nephrology ward of Jinnah Postgraduate Medical Centre, Karachi, and the Pakistan Navy Ship Shifa Hospital, Karachi, in collaboration with the Department of Biochemistry, University of Karachi, and comprised patients of either gender aged >18 years. They were divided into chronic kidney disease group A and end-stage renal disease group B. Control group C included subjects from the general population. Free carnitine and total carnitine values were detected using enzyme-linked immunosorbent assay. Acyl carnitine was estimated by applying the standard formula, and the ratio between acyl carnitine and free carnitine was calculated for accurate assessment of the carnitine status. Data was analysed using SPSS 23.

Results: Of the 203 subjects, 143(70.44%) were cases and 60(29.55%) were controls. Among the cases, 120(84%) were recruited from Jinnah Postgraduate Medical Centre and 23(16%) from Pakistan Navy Ship Shifa Hospital. There were 60(29.55%) patients in group A, 83(40.88%) in group B and 60(29.55%) in group C. The mean age in group A was 47.90 5.±65 years, it was 44.10 ±8.92 years in group B and 40.90 ± 6.73 years in group C. There was a significant difference related to free carnitine, total carnitine, acyl carnitine values and the ratio between acyl carnitine and free carnitine values in groups A and B compared to control group C ($p<0.05$).

Conclusions: Patients on maintenance haemodialysis developed were found to have developed carnitine deficiency.

Key Words: Carnitine, Chronic kidney disease, Dialysis-related carnitine disorder, Haemodialysis.

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Introduction

The passage of long-chain fatty acids across the inner mitochondrial membrane, which facilitates beta (β) oxidation and energy production, requires endogenous substance L-carnitine. Healthy individuals maintain fairly narrow ranges of L-carnitine levels in their blood and tissues, which is evidence of the substance's importance in intermediary metabolism.[1] A healthy kidney is essential to maintaining this balance because it preferentially excretes short-chain carnitine esters, while protecting body L-carnitine reserves by considerable reabsorption of the filtered load.[2] Concentrations of L-carnitine in plasma are often greater in end-stage renal disease (ESRD) patients who are not receiving haemodialysis (HD) than in healthy people.[3] Contrarily, several investigations have discovered that ESRD patients receiving continuous HD have low levels of L-carnitine in their muscle and plasma. This L-carnitine depletion has been seen to correlate with the length of time a patient has been receiving HD, sometimes known as 'dialysis age' or 'dialysis vintage'. Chronic HD reduces endogenous L-carnitine concentrations mostly due to the substance's efficient removal via the dialysate, perhaps in combination with a reduction in L-carnitine intake from food ingestion and endogenous synthesis.[4]

Through extensive and adaptive tubular reabsorption, the kidney contributes significantly to the homeostatic processes that control the human endogenous L-carnitine pool.[5] In addition to causing significant decreases in tissue and plasma levels of L-carnitine as well as an increase in the ratio of acyl-L-carnitine (AC) to free L-carnitine (FC), long-term HD treatment can also alter the homeostasis of L-carnitine. Kidney disease can also cause these changes.[6] These modifications may prevent tissues from eliminating undesirable short-chain acyl groups and oxidising fatty acids.[6] When these biochemical anomalies are linked to clinical symptoms, including cardiomyopathy, anaemia refractory to erythropoietin treatment, intradialytic hypotension, or muscular weakness, the condition is known as a dialysis-related

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carnitine disease (DCD). In healthy people, intestinal absorption, endogenous synthesis and carrier-mediated renal tubular reabsorption work together to maintain plasma and tissue levels of L-carnitine.[7] The body's total carnitine (TC) level (FC and its esters) in healthy individuals has been determined to be 100mmol. Around 95% of the carnitine pool is found in the skeletal and cardiac muscles, 2-3% in the liver, and 0.5-1% in the extracellular fluids. TC concentrations in healthy persons range between 50nmol/mL and 60nmol/mL (TC = AC+FC).[8] The quantity of filtered FC is approximately 7.2mmol/day at a typical glomerular filtration rate (GFR) of 125mL/min. The average urine AC/FC ratio for daily TC excretion of 50% is 1. Plasma acylcarnitines build up in patients with chronic renal failure who are not yet receiving haemodialysis (HD), in part because of a reduced renal removal of esterified carnitine molecules. Plasma from these individuals often has a high AC/FC ratio.[9]

FC <20mol/L is regarded as deficient, 20-36mol/L as at high risk of becoming deficient, and a Ac/FC ratio >0.4 is regarded as indicative of carnitine insufficiency in Japanese standards.[10]

Despite the fact that serum FC level and AC/FC ratio are used to evaluate the condition of carnitine storage, this may not be the case in ESRD patients because of their toxic uraemic state and their disrupted metabolism.[11] Absolute carnitine insufficiency and excess acyl groups resulting from functional or relative carnitine shortage brought on by poor β-oxidation coexist in the majority of these people. In these conditions, a somewhat larger concentration of FC is necessary to buffer the extra acyl groups, transport the toxic acyl groups out of the mitochondria as AC, and maintain the ratio of acyl coenzyme A (CoA) to free coenzyme A, which raises the AC/FC ratio,[12] which is a more suitable metric than serum FC concentration to evaluate the state of carnitine reserves and fatty acid metabolism in these people.[13]

L-carnitine and related molecules may be found in plasma and other organs using a variety of procedures, like radio-enzymatic, enzymatic, mass spectrometry, high-performance liquid chromatography (HPLC). Issues like specificity, accuracy and precision have not been given much attention, and these approaches have not gone through a lot of cross-validation. The current study was planned to evaluate the impact of HD on plasma carnitine levels.

Patients and Methods

The cross-sectional study was conducted from April 20, 2020 to May 10, 2022, at the dialysis unit of the Nephrology ward of Jinnah Postgraduate Medical Centre (JPMC), Karachi, and the Pakistan Navy Ship (PNS) Shifa Hospital, Karachi, in collaboration with the Department of Biochemistry, University of Karachi, while enzyme-linked immunosorbent assay (ELISA) tests were performed at the Multi-Disciplinary Research Laboratory of the Bahria University of Health Sciences (BUHS), Karachi.

After approval from the institutional review boards of JPMC and PNS Shifa Hospital, the sample size was estimated using OpenEpi calculator in line with literature[14,15]. The sample was raised using convenience sampling technique from the JPMC and subsequently from PNS Shifa. haemodialysis. Those included were patients of either gender aged >18 years. The haemodialysis patients that were included who had been receiving maintenance HD for >6 months for at least 3 HD sessions per week using a low-efficiency dialyser and the chronic kidney disease (CKD) patients up to stage 4 16 of Chronic Kidney disease staging were included. Patients with any other chronic condition, such as tuberculosis (TB) or cancer, and subjects who had a heart disease diagnosis before HD initiation were excluded.

After taking informed consent, the individuals were divided into group A comprising chronic kidney disease (CKD) (up to stage 4 16) who were not receiving HD, and group B comprising ESRD patients receiving maintenance HD for >6 months. Control group C included subjects from the general population.

Data was collected using a predesigned proforma. Basic data included medical history, age, gender, HD duration, medication use, height, weight, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse, temperature and respiration rate. Levels of plasma FC were determined using ELISA kits for Human Total Carnitine (Bioassay Technology Laboratory China, Catalogue. No. BTB-E3315Hu), while plasma L-carnitine levels were determined using another ELISA kit (Bioassay Technology Laboratory China, Catalogue. No. BTB-E3426Hu). The method used in both the kits was Sandwitch ELISA, which is 2-5 times more sensitive than direct and/or indirect ELISA, and offers high specificity and sensitivity, allowing for the detection of low concentrations of antigens in complex samples. FC was estimated by applying the standard formula TC = AC + FC.

Data was analysed using SPSS 23. Independent samples t-test was used to describe continuous variables as mean +/- standard deviation, while frequencies and percentages were used to provide descriptive statistics for categorical data. P<0.05 was considered statistically significant.

Results

Of the 203 subjects, 143(70.44%) were cases and 60(29.55%) were controls. Among the cases, 120(84%) were recruited from JPMC and 23(16%) from PNS Shifa Hospital. There were 60(29.55%) patients in group A, 83(40.88%) in group B and 60(29.55%) in group C. The mean age in group A was 47.90 ± 5.65 years, it was 44.10 ±8.92 years in group B and 40.90 ±6.73 years in group C (Table 1), while gender distribution among the groups was also noted (Figure).

Table-1: Anthropometric data.

	Group					
	Control		Haemodialysis Group		CKD Patients	
	Mean	Standard Deviation)	Mean	Standard Deviation	Mean	Standard Deviation
Age (years)	40.90	6.73	44.10	8.92	47.90	5.65
weight (kg)	72.72	7.30	69.06	9.01	63.34	8.02
Height (Meters)	1.66	.12	1.66	.12	1.63	.11
SBP (mmHg)	110.00	9.80	112.00	21.00	130.00	12.80
DBP (mmHg)	62.00	6.50	60.00	13.60	70.00	9.40
BMI	26.75	4.38	25.52	4.69	24.17	3.99

CKD: Chronic kidney disease, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, BMI: Body mass index.

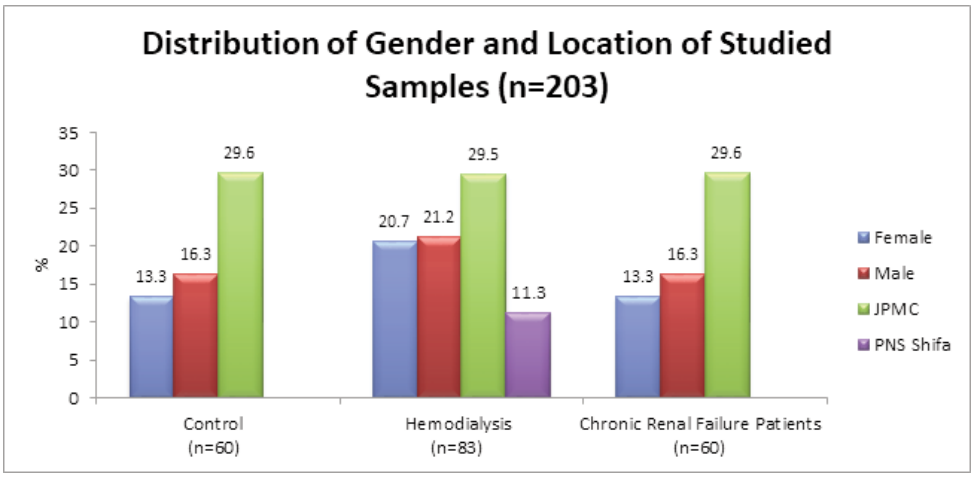

Figure: Distribution of gender and location of the samples.

Table-2: Carnitine levels in the study groups.

Parameters			Chronic Renal Failure (n=60)		Haemodialysis (n=83)		
	Control Group (n=60) Mean	Standard Deviation)	Mean	Standard Deviation	Mean	Standard Deviation	*p*-value
Plasma Acyl Carnitine (AC) (nmol/l)	23.57	4.1	20.4	5	22.8	3.6	<0.01*
Plasma Free Carnitine (FC) (umol/l)	55.32	7.3	48.8	8.4	17.6	4.7	<0.01*
Total Plasma Cartinine (umol/l) (PC)	83.8	8.5	80.1	8.4	40.4	7.2	<0.01*
AC/FC ratio	0.57	0.1	0.63	0.14	1.2	0.2	<0.01*

*p<0.05 was considered statistically significant using independent sample t-test.

There was a significant difference related to free carnitine, total carnitine, acyl carnitine values and the ratio between acyl carnitine and free carnitine values in groups A and B compared to control group C (p<0.05) (Table 2)

Discussion

Patients of ESRD suffer from a lot of metabolic abnormalities that directly affect their quality of life. In the current study, male patients were slightly more than females, which is line with literature.[14] The weight and BMI of the controls was more compared to the two patient groups. The uraemic condition, food restrictions, protein energy deficiency, and anorexia are the causes of the loss in weight in these patients.[15] L-carnitine levels in plasma and tissues are largely stable in healthy people due to homeostatic regulation. Through substantial and saturable tubular reabsorption, L-carnitine production, and the selective excretion of short-chain carnitine esters, a healthy human kidney is a key player in this regulation.[15] HD lacks the L-carnitine conservation-related homeostatic regulatory systems. Patients on HD vs nondialysis may not have the same mechanisms of decreased fatty acid metabolism. Patients receiving HD may develop a carnitine deficit due to the interaction of many variables. However, according to some studies, predialysis plasma carnitine levels are comparable to the average levels of healthy controls.[17] The same was observed in the current study. This can be explained by the fact that kidney is the main

organ responsible for the synthesis of carnitine, and is also the main organ leading to the excretion of carnitine. Decreased renal clearance in CKD patients leads to retention of carnitine in the body. In nondialysis CKD patients, free carnitine concentration does not decrease as renal function degrades, despite dietary protein limitation and possibly reduced carnitine production.[17]

In the current study, chronic HD patients had lower TC and FC levels, and their greater AC/FC ratio suggested aberrant mitochondrial oxidation. Studies done in the past have also demonstrated that when kidney function status advances from nondialysis CKD to long-term HD therapy, the main cause of poor fatty acid metabolism shifts from functional or relative carnitine shortage caused by defective oxidation to absolute carnitine deficiency. Calvani et al. showed that during HD, L-carnitine is effectively eliminated from the blood. Plasma L-carnitine levels decline by around 70-75% during a single HD session. During HD, the plasma clearance of L-carnitine is around 7.8L/h, or 130mL/min.[17] This exceeds the anticipated renal clearance of L-carnitine in a healthy person by at least 30 times (1-3mL/min). the increase in AC/FC ratio in the HD group can be explained by the fact that FC, a 161.2 Da unbound molecule, is eliminated during HD.[16] However, AC, with longer carbon chains, has less dialysance than molecules with shorter carbon chains, and, as a result, tends to accumulate.[18] Therefore, it is probable that these processes are root causes of the rise in AC/FC ratio and decline in serum carnitine concentration. The current results are consistent with earlier studies.[18,19]

The current study showed that HD patients were at a greater risk of developing DCD. This is a very important finding as carnitine deficiency contributes to many signs and symptoms in this population, including muscle fatigue, poor quality of life and cardiac comorbidities.[20] Therefore, treating these patients' carnitine deficit is essential to enhancing the overall standard of care. Clinical results suggest that carnitine supplementation may be advantageous for patients receiving continuous HD 20.

In the absence of solid clinical evidence, the doctor must decide if a trial of carnitine medication is suitable for each patient. All HD patients should take carnitine supplements, and their levels should be checked often. To learn more about the reasons and available treatment options for carnitine insufficiency in HD patients, larger-scale studies along with clinical correlations need to be conducted.

Conclusion

Patients on maintenance HD develop were found to have carnitine deficiency.

Limitations: The limitation of this study was small sample size..

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Conflict of Interest: None.

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Author's Contributions
SR: Principal investigator, conception and design.
MF: Supervision of work.
AN: Final approval, critical analysis, review of literature.
SM: Analysis of biochemical variables and laboratory work.
MIK: Clinical supervisor.