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Original Article

Role of Total Carnitine to Free Carnitine Ratio (TC/FC) in Monitoring Outcome of Haemodialysis

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ABSTRACT

Carnitine, a quaternary ammonium compound actively involved in beta peroxidation of fatty acids is documented to be lost in excess in patients of chronic kidney disease (CKD) especially in those undergoing MHD, this loss is attributable to malfunctioning of kidneys and being washed away by dialytic sessions. This decrease is attributable to most of the complications encountered in these patients.

Objective:

- To measure carnitine levels (total and free) in patients on dialysis in comparison with healthy controls
- To determine total versus free carnitine ratio (TC/FC), for detecting accumulation of carnitine metabolites.

Materials and Methods: This Observational Cross-sectional study was conducted in the department of chemical pathology (AFIP), in collaboration with Armed forces institute of Urology (AFIU) from August 2020 to January 2021. Non probability convenient sampling technique was employed for sampling. All individuals undergoing dialysis for duration ≥6 months were enrolled in case cohorts. Carnitine (free and total) levels were determined by using human bioassay ELISA kits and their ratios were calculated for comparison**.**

Results: 160 participants were enrolled in study subdivided as diabetic and non-diabetic groups with 1:1 male to female ratio. TC/FC ratio was found to be high in case group, in comparison with control group. A rising trend of ratios was observed with increasing duration of dialysis.

Conclusion: TC/FC ratio was high in dialysis patients suggestive of carnitine metabolite accumulation, suggesting that efficacy of dialysis should be promptly monitored on the basis of TC/FC ratio for early detection of complications secondary to carnitine metabolites accumulation.

Key words: Total Carnitine, Free carnitine, CKD-chronic kidney disease, haemodialysis.

Introduction

Carnitine (3-hydroxy-4-N-trimethylammonio butanoate) is a small, organic, hydrophilic molecule. Biologically, it exists as free carnitine and acyl carnitine, which account for carnitine system. Carnitine plays a vital role in energy metabolism through the transposition of long-chain fatty acids across the inner mitochondrial membrane. It also affects the rate of beta oxidation of long-chain fatty acids which are later involved in energy production.[1] Plasma carnitine accounts for approximately 1% of the total body carnitine pool, the majority (> 98%) being present in the skeletal and cardiac muscles. A small a-mount is also present in the kidney, liver and brain.[2] In humans, the main source of carnitine is from diet.

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After absorption from small intestine, free carnitine (FC) is transformed into its active form i.e. acetyl carnitine, which is involved in carnitine shuttle system in mitochondria. This system is responsible for transfer of fatty acid chains into the mitochondria used for energy production by the process of peroxidation, resulting in the formation of acyl carnitine (AC) upto 80%, along with malonyl carnitine (10%) and other carnitine metabolites (10%).[3] Carnitine (free carnitine) a tertiary ammonium compound is a small, negatively charged molecule been passively filtered and reabsorbed along healthy renal tubules. Almost 97% of carnitine is effectively reabsorbed, any condition affecting renal tubular function will result in secondary carnitine deficiency, owing to decreased reuptake.[4] Carnitine deficiency predispose to many of the complications observed in patients of renal malfunction, especially tin CKD undergoing maintenance haemodialysis (MHD) as their treatment.[5]

In patients of End Stage Renal disease, not being on haemodialysis (HD), plasma acyl carnitine along with other metabolites assemble; due to a decreased renal clearance of esterified carnitine moieties. Pa-tients of CKD maintained on HD, usually present with secondary plasma carnitine insufficiency; at-tributed to increased removal via HD, reduced protein intake, accumulation of metabolic intermediates in addition to impaired carnitine biosynthesis. Here a high AC/FC ratio is usually found in plasma. In patients of CKD, who are on MHD results in rapid fall of carnitine concentrations, as low as 40% of the base line, with a slow restoration of the carnitine concentration during the inter-dialytic period, mainly from organs of storage (skeletal muscle).[6] This restoration is the main cause of decreased levels of free bioavailable carnitine with advancing duration of MHD. Dialysis-related carnitine deficiency has been associated with several symptoms frequently encountered in uremic patients including anaemia, car-diomyopathy, skeletal muscle weakness, and fatigability.[7] Maintaining the tissue or serum level of car-nitine can alleviate some of these disorders.[8] As shown in different studies, carnitine might also have a role in controlling the serum levels (cholesterol and triglycerides) in normal range, improving anaemia and nutritional factors. Although routine carnitine supplementation is not a part of routine treatment of CKD patients undergoing MHD, they may show some beneficial effects from adding carnitine into their routine medication regimen.[9-10]

This study is designed for the assessment of carnitine levels (FC and TC) and calculation of their ratios in CKD patients. Furthermore, their comparison was done with healthy controls of same age and sex, among CKD patients undergoing MHD. The ratios are employed in this to overcome the age and sex dependent differences in carnitine concentrations.[11]

Table-I. Anthropometric parameters of participants

Parameters	Cases (mean±SD) n=80	Controls (mean±SD) n=80	p-value
Age (Years)	56.2±10.7	51.3±15.3	
BMI (kg/m^2)	26.20±1.02	24.12±1.02	<0.001

Table-II. Biochemical parameters of study participants

Parameters	Cases (mean±SD)	Controls (mean±SD)	p-value
Urea (umol/L)	11.8±5.37	5.38±1.2	
Creatinine (mmol/L)	411±178.9	121.4±17.68	
Duration on MHD (months)	13.2±6.2	-	
Total carnitine (nmol/L)	98.85±75.55	39.5±6.2	<0.001
Free carnitine (nmol/L)	22.35±3.21	24.12±1.02	<0.001
TC/FC ratio	4.53±2.01	1.2±0.10	
eGFR (ml/min/1.73m2)	11.8±3.6	53.0±4.2	
HbA1c	7.2±1.24	5.2±1.01	

Pearson correlation and linear regression analysis signifies a strong correlation between carnitine levels (both total and free) and duration of MHD, thus predicting that duration of MHD is attributable to both of increased total carnitine (p = 0.009) on one end and decreased FC levels (p = 0.01) on other end in patients on MHD as shown in fig 1-2.

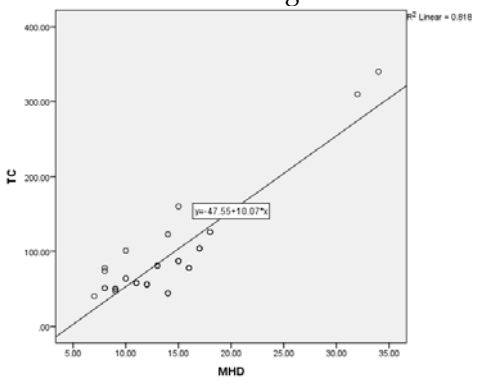

Fig 1. Correlation between MHD and total carnitine (TC) levels

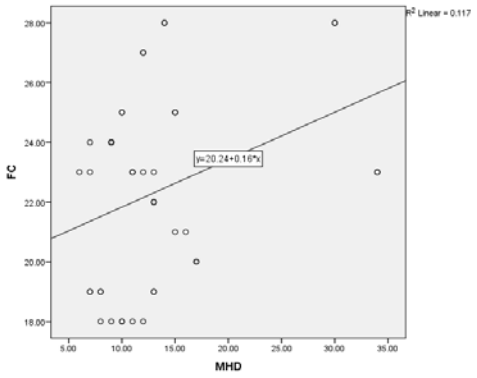

Fig 2. Correlation between MHD and free carnitine (FC) levels

Materials and Methods

This was an observational cross sectional study conducted at the department of Chemical Pathology and Endocrinology, AFIP, Rawalpindi with sample collection from Armed Forces Institute of Urology (AFIU), Rawalpindi. WHO calculator was used for sample size calculation, based on prevalence of CKD at 12.5%.[12.5] The sample size was 40. A total of 160 individuals were enrolled in the study; 80 in case groups - 40 in diabetic and 40 in non-diabetic CKD case group. Their corresponding control group consisted of 80 individuals of same age and sex, 40 diabetics not having any renal complications and 40 healthy non-diabetic participants. After taking consent, patients' weight and height were noted on pre-designed proforma and their respective BMI were calculated. 2ml blood samples were drawn in plain gel tubes, centrifuged at 1500 rpm for 3 min and analysed for urea and creatinine. eGFR were calculated by using standard MDRD formula. 2ml sample was taken in K-EDTA tubes for glycosylated haemoglobin (HbA1c). Another 3 ml sample was taken in pre-chilled sodium heparin tubes for TC and FC analysis. Plasma was separated within 20 min of collection by centrifugation at 1500 rpm for 10 mints and samples were preserved as aliquots at -20c till analysis. Previous studies have demonstrated that L-carnitine and its derivatives were stable for four weeks under standard collection and storage environment.[13] Human Bioassay Technology Laboratory ® ELISA kit having sensitivity up to 0.05 mmol/L, was used for analysis of total carnitine. Commercially provided control materials were used for quality control. In case of diabetics, HbA1c was assayed by capillary electrophoresis on Sebia ® Octa3 system. Serum urea and creatinine were assayed on fully automated chemistry analyser ADVIA 1800 by Siemens ®.

Statistical Analysis

Kolmogorov-Smirnov (K-S) test was used for the determination of normality of data by both visual and through normality test, which showed that the data had parametric distribution. Descriptive statistics were calculated as mean ± SD for lab results of quantitative variables, while frequency and percentages were calculated for qualitative variables. Student 't' test was applied for assessment of significance of difference of carnitine levels among both diabetics and non-diabetic participants (both cases and controls). Level of significance was defined as significant for $p<0.05$. Pearson correlation technique was used for determination of correlation between carnitine levels and duration of dialysis. Univariate Regression analysis was applied for prediction of dependency of altered carnitine levels (raised TC and decreased FC) on increasing duration of MHD.

Results

A total of 160 individuals were enrolled in the study; 80 in case groups (40 in diabetic and 40 in non-diabetic CKD case group) and 80 individuals in control group of same age and sex (40 diabetics not having any renal complications and 40 healthy non-diabetic participants). Anthropometric measures of study participants are shown in **Table I**. Student 't' test analysis of BMI between cases and control cohorts indicated that BMI was significantly higher among the cases than controls ($p <0.001$). Preliminary biochemical investigations along with mean carnitine levels (TC and FC) among case and control cohorts are shown in **Table II**. Mean duration of MHD among case population was 13.2±6.2 months. The ratio between mean total carnitine and free carnitine was much higher (4.53±2.01) as compared to control cohorts (1.2±0.10).

Discussion

In this cross sectional study, total carnitine levels were significantly high in both diabetic and non-diabetic case groups in comparison with their counter healthy control groups while free carnitine or acetyl carnitine was found to much low in case groups. No significant differences were observed among diabetic and non-diabetic case cohorts for both total carnitine and free carnitine. It has been documented that low carnitine levels are likely to occur following long-term dialysis in CKD patients.[14] Clinical features include severe and continuous muscle cramps and episodic hypotension during or following dialysis session, low energy levels affecting quality of life, skeletal muscle myopathy, Cardiovascular complications, and anaemias secondary to uraemia often not responding to or requiring large doses of EPO,. Levo carnitine supplementation might improve cardiac function, BA (Branchial ankle) pulse wave velocity, Erythropoietin - resistant anaemia, and preservation of physical function in dialysis patients.[15-16] Levo carnitine supplementation may results indicative improvement due to carnitine deficiency in patients on PD as well as in patients on HD. However, small scale clinical evidence/literature is available regarding L-carnitine supplementation in patients on HD. The results of a 90 day course of L-carnitine therapy on several indices

have been shown to be stipulate renal anaemia.[17] In current study it was found that increasing dialysis duration were related to the level of carnitine deficiency among patients on MHD. The molecular weight of carnitine is 161 Da and it circulates as non-protein bound molecule in the circulation. Therefore, plasma carnitine levels are found to be significantly decreased in patients of HD as most of the carnitine (approximately 68%) being washed out of the body following dialysis session.[18] As observed in our study total carnitine level was found to be much higher in case cohorts as compared to controls because carnitine metabolites, most common being the acyl carnitine, are large sized charged molecules not being filtered by dialyzing membranes resulting in accumulation of these pro inflammatory metabolites in the body.[19] Studies suggested that under normal circumstances kidneys maintain a constant ratio of TC/FC with free carnitine almost entirely reabsorbed by the kidney thereby maintaining a normal carnitine levels in plasma, while removing the toxic metabolites out of body.[20] In this observational study carnitine levels (total and free) were found to be in average ratios of 1.0 with the lowest being 0.6 while highest calculated to be 1.3. Ratios were found to be slightly higher among diabetic population attributing to significant lower free carnitine levels among diabetics, suggestive of a constant reabsorption and excretion equilibrium as suggested by previous studies.

While in case of case cohorts, a successive rise in total carnitine concentrations was observed with increasing dialysis sessions along with proportional fall of free carnitine levels (FC) with their highest ratio calculated to be 13.4 with corresponding dialysis duration of 34 months while lowest being 2.2 with dialysis duration of 10 months, suggesting inability of failing kidneys to remove toxic metabolites out of body.[21] These observations are suggestive of low carnitine levels as being involved in most of the complications encountered by patients of HD, especially muscle cramps and decreased muscle strength observed following dialysis session.[22] Cardiovascular (CV) complications are one of the common causes of mortality in patients undergoing MHD, with increased events of atherosclerosis and PEW contributing most towards associated cardiovascular complications.[23] Although obesity, increased cholesterol levels and elevated BP levels were established as risk factors for Cardio Vascular complications and necessitous results in general population, it seems that higher rather than lower values for these risk factors act protective in CKD patients.[24] This is reverse epidemiology phenomenon or dialysis-risk-paradox.[25-26] Approximately 70% of HD patients die secondary to cardiovascular events, as per literature.[27] Although little evidence is available regarding role of carnitine in HD patients but there are documented clinical effects of carnitine supplementation in HD population in terms of improved status of EPO resistant anaemia, compromised muscle activity in addition to other routinely encountered complications in HD patients.[28] Thus in addition to creatinine being monitored for the efficiency of dialysis carnitine levels should also be monitored for early prediction of complications associated with HD and prompt administration of L-carnitine should be done for improvement in overall state of patient's wellbeing ratios (TC/FC) are preferred for monitoring as ratio is maintained constant irrespective of patient age, sex or ethnicity or any other factor affecting carnitine plasma status.

Conclusion

In patients of chronic kidney disease undergoing haemodialysis, it was observed that with progressive dialysis, the free carnitine is washed away while the metabolites accumulate. But the levels of total carnitine remain unchanged owing to accumulation of metabolites of carnitine. TC/FC ratio well correlates with the duration of haemodialysis. Presently, the efficacy of haemodialysis is being monitored on the basis of pre and post dialysis serum urea and creatinine levels. However, lack in free carnitine and accumulation of carnitine metabolites have huge impact on dialysis related complications. Hence TC/FC ratio need to be included in parameters for a successful haemodialysis.

Conflict of Interest

This study has no conflict of interest to declare by any author.

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A. Conception/Study/Designing/Planning
B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion