



## THE STUDY OF HEMODIALYSIS EFFECTIVENESS ON THE CHANGE RATE OF LIPID PEROXIDATION AND L-CARNITINE LEVEL IN HEMODIALYSIS PATIENTS

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### Abstract

Carnitine is a small molecule widely present in all cells from prokaryotic to eukaryotic. It is an important element in  $\beta$ -oxidation of fatty acids. Carnitine is a scavenger of oxygen free radicals in mammalian tissues. Lack of carnitine in a hemodialysis patient can lead to carnitine deficiency. Oxidation of fatty acids and lipid metabolism are severely affected by carnitine deficiency. Oxidative stress is defined as imbalance between formation of free radicals and antioxidative defense mechanisms. It has been proposed to play a role in many disease states. In hemodialysis patients multiple factors can lead to a high susceptibility to oxidative stress. The aim of this study was to determine hemodialysis effectiveness on the change rate of serum L-carnitine and lipid peroxidation.

27 patients with chronic renal failure (24-80 yrs) who undergo hemodialysis for 6-12 months were selected (M= 17, F= 10). Malondialdehyde (MDA), as an indicator of lipid peroxidation was measured colorimetrically with a standard thiobarbituric acid (TBA) method. L-carnitine was measured with enzymatic UV method (ROCHE, Spectronic Genesis 2, 340 nm).

The weight mean of L-carnitine before and after hemodialysis was  $7.67 \pm 3.6$  mg/l and  $2.07 \pm 1.6$  mg/l, respectively ( $P < 0.001$ ). The weight mean of pre-hemodialysis MDA was  $4.17 \pm 1.24$   $\mu$ mol/l, following hemodialysis  $4.98 \pm 1.2$   $\mu$ mol/l ( $P < 0.001$ ). Results showed that 55.6% of patients suffered from carnitine deficiency. Serum carnitine was found to be decreased markedly after hemodialysis ( $P < 0.001$ ).

Our findings indicated that oxidative stress in these patients is further exacerbated by hemodialysis, as evidenced by increased lipid peroxidation. The relationship between serum L-carnitine and MDA before and after hemodialysis was observed ( $r = 0.82$ ;  $p < 0.001$ ;  $r = 0.75$ ;  $p < 0.001$ ).

### INTRODUCTION

L-carnitine is an essential factor for the membrane transport of acyl-CoA compounds, particularly for the intramitochondrial transport of long-chain fatty acids [1]. L-carnitine also helps to remove by-products of fatty acid metabolism and other toxic compounds from the cells [2].

The liver and kidney represent the main sources of endogenous carnitine synthesis [3]. Also among the homeostatic processes controlling the endogenous L-carnitine pool in humans, the kidney has a vital role through extensive and adaptive tubular reabsorption [4]. Kidney disease can lead to disturbances in L-carnitine homeostasis, and carnitine deficiency may occur in hemodialysis patients. Bellinghieri et al reported that hemodialysis may promote excessive losses of L-carnitine [5].

Oxidation of fatty acids and lipid metabolism are severely affected in carnitine deficiency [6]. Aberrant fatty acid metabolism has been associated with the promotion of free radical production, insulin resistance and cellular apoptosis [7]. Oxidative stress, by definition, is a biochemical condition in which oxidant species overwhelm antioxidant defense ultimately leading to a given biological damage [8,9]. Galli F, et al indicated the evidence for the presence of oxidative stress in hemodialysis patients [10]. In these patients multiple factors can lead to a high susceptibility to oxidative stress [11,12].

One of the most investigated biological effects of the oxidative stress in hemodialysis patients is lipid peroxidation [13].

In the view of the above, the aim of this study was to determine the hemodialysis effectiveness on the change rate of serum L-carnitine and lipid peroxidation.

## MATERIALS AND METHODS

**Chemicals:** Thiobarbituric acid (TBA), Butylated Hydroxy Toluene (BHT), and other reagents are purchased from Sigma (Deisenhofen, Germany) or Merck (Darmstadt, Germany). L-carnitine was assayed by the ready to use kit from ROCHE.

**Subjects:** 27 patients with chronic renal failure on hemodialysis were included in the study. All patients were dialyzed 3 times/wk (4 hrs sessions). The duration of dialysis was 6-12 months. The age of patients was 24-48 yrs. The patients were not taking any antioxidants. Diabetic patients were excluded from the study. The demographic information was written in a check-list form. The informed consent was obtained from all hemodialysis subjects included in the study.

Venous blood samples were taken into EDTA-tubes (1 g/l) after an overnight fast immediately before and after the dialysis session. Plasma was separated by centrifugation at 3000 g for 5 min at 4° C.

### Methods

Malondialdehyde (MDA), as an indicator of lipid peroxidation was measured colorimetrically with standard TBA method at 532 nm wavelength [14,15]. L-carnitine concentration was measured with enzymatic UV-method (Genesis, 340 nm). The reference range for L-carnitine in humans was  $3.85 \pm 0.82$  mmol/L [16].

**Statistical analysis:** All results were presented as mean  $\pm$ SD. Levene test, paired t-test, independent t-test were used for rejection or acceptance of the assumption. Pearson's correlation coefficient was calculated.

## RESULTS

The weight mean of serum L-carnitine, before and after hemodialysis was  $7.67 \pm 3.6$  and  $2.07 \pm 1.6$  mg/l, respectively. In this study 55.6% of patients suffered from carnitine deficiency ( $P < 0.001$ ). Pearson's correlation coefficient indicated the relationship between the value of serum L-carnitine before and after hemodialysis ( $r = 0.4$ ,  $P = 0.045$ ).

The significant difference between the value of serum L-carnitine before and after hemodialysis was observed ( $P < 0.001$ ) (Table 1).

Pre-hemodialysis and post-hemodialysis serum MDA was  $4.17 \pm 1.24$   $\mu$ mol/l and  $4.98 \pm 1.2$ , respectively. Pearson correlation coefficient indicated that there is a strong relationship between serum MDA before and after hemodialysis ( $r = 0.96$ ,  $P < 0.01$ ) (Table 2).

The relationship was found between average of serum L-carnitine and serum MDA before and after hemodialysis ( $r=0.82$ ;  $p<0.001$  ;  $r=0.75$ ;  $p<0.001$ ).

The confidence interval of percentage of change in average of L-carnitine and MDA is shown in Fig.1.

Table 1 : Mean and SD of L-carnitine concentration in females and males.

Parameter	Sex	Number	Mean±SD	Weight mean	P-value
L-carnitine (before hemodialysis)	Males	17	8.96±3.66	7.67±3.6	0.053
	Females	10	5.93±2.88		
L-carnitine (after hemodialysis)	Males	17	2.66±1.67	2.07±1.6	0.01*
	Females	10	1.06±0.85		
L-carnitine difference	Males	17	6.3±3.69	--	0.045
	Females	10	4.87±2.6		

\* difference between males and females; statistical tests: independent t-test and Levene test

Table 2 : Mean and SD of MDA concentration in females and males

Parameter	Sex	Number	Mean±SD	Weight mean	P-value
MDA (before hemodialysis)	Male	17	3.75±0.91	4.17±1.24	0.048*
	Female	10	4.87±1.45		
MDA (after hemodialysis)	Male	17	4.63±1.11	4.98±1.2	0.05*
	Female	10	5.57±1.16		
MDA difference	Male	17	0.88±0.28	--	0.05
	Female	10	0.7±0.4		

\* difference between males and females; statistical tests: independent t-test and Levene test

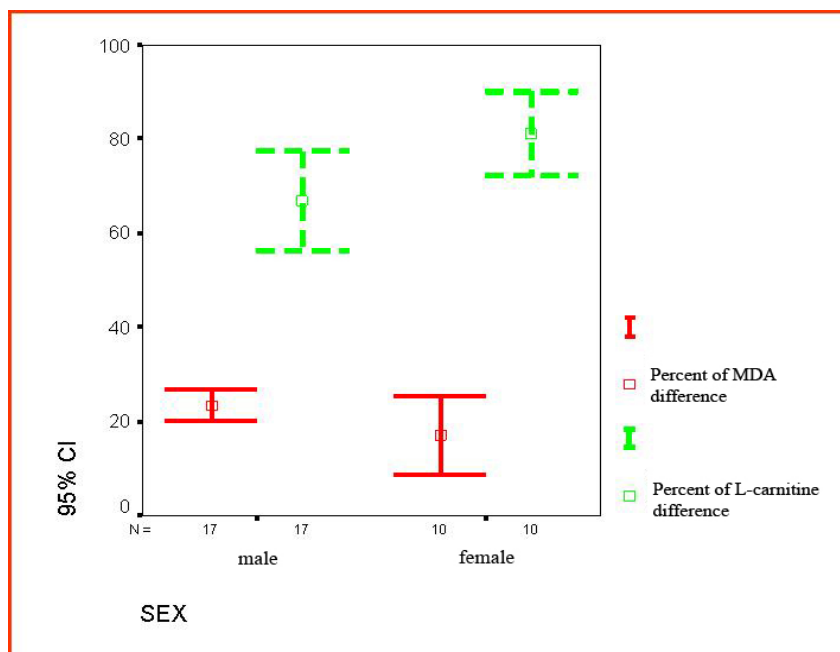


Fig. 1 : The confidence interval of percentage of change of average serum L-carnitine and serum MDA in females and males

## DISCUSSION

Existing evidence showed that despite advances in dialysis therapy, a high percentage of patients on maintenance dialysis therapy, suffered from complication of hemodialysis [17]. The study of Evans et al showed, that the average of predialysis serum L-carnitine concentration was 19.5  $\mu\text{mol/l}$ , where the post dialysis level was 5.6  $\mu\text{mol/l}$  [4]. Also, Alhomida’s research indicated that the average of serum L-carnitine before and after hemodialysis was  $42\pm 6.3 \mu\text{mol/l}$  and  $17.1\pm 6.3 \mu\text{mol/l}$ , respectively [18]. The results of this study were consistent with the studies of others and showed a decrease of L-carnitine after hemodialysis.

L-carnitine is a small water-soluble molecule, therefore it is freely dialyzed because of a molecular weight gradient; the acyl-carnitine moieties are less likely to be filtered by the membrane than free carnitine [4,19]. Accumulation of acyl-carnitine is believed to contribute directly to arrhythmogenesis [20]. This accumulation also alters mitochondrial membrane permeability and has been suggested to promote apoptosis. Altered membrane permeability has been implicated to modify the activity of various hormone receptors, including insulin receptors [21].

The susceptibility for oxidative stress is mainly correlated with MDA concentration [22,23]. It has been reported that the hemodialysis procedure altered lipid peroxidation. Ozden et al found that MDA was elevated in post-hemodialysis ( $1.39\pm 0.38 \text{ nmol/ml}$ ) in comparison to prehemodialysis state ( $0.83\pm 0.22 \text{ nmol/ml}$ ) [24], but Nand and Surri showed that MDA was decreased after hemodialysis (pre hemodialysis  $2.96\pm 0.89 \mu\text{mol/l}$  and post hemodialysis  $2.32\pm 0.84 \mu\text{mol/l}$ ) [25].

In this study we observed an increase of MDA, which possibly was the result of free radical reactions during the hemodialysis procedure. The absence of a complete correction of the uremic toxicity together with the untoward effect of the dialysis, malnutrition and the progressive worsening of the clinical condition can lead to a high susceptibility to oxidative stress [26,27].

Increased lipid peroxidation in hemodialysis patients is largely a result of anemia of kidney failure, therefore anemia management can improve this condition [28,29]. Chronic heart failure in hemodialysis patients is of high prevalence, it was indicated that lipid peroxidation and carnitine deficiency are main risk factors in some patients [30].

In this study 55.6% of patients suffered from carnitine deficiency before hemodialysis and serum L-carnitine decreased markedly after hemodialysis ( $P<0.001$ ). In these patients, oxidative stress is further exacerbated by hemodialysis, as evidenced by increased lipid peroxidation ( $P<0.001$ ).

Multiple pathogenic factors are responsible for intra-dialysis muscle cramping. Carnitine deficiency is a potential cause of intra-dialysis muscle cramping [31,32]. Also oxidative damage may play a role in the pathogenesis of skeletal myopathy in hemodialysis patients [33]. Oxidative stress has been proposed to play a role in many diseases states, including cardiovascular and infectious diseases [34,35], cancer [36], diabetes [37] and neurodegenerative diseases [38]. Therefore oxidative stress management and supplementation with L-carnitine, parallel to other therapeutic agents, may improve condition of hemodialysis patients.

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