The study of hemodialysis effectiveness on the change rate of lipid peroxidation and L-Carnitine level in hemodialysis patients

Mohammad-Reza Safari¹, Maryam Isfahani², Nasrin Sheikh³

1. Department of Laboratory Medicine, Faculty of Paramedicine, Hamadan University of Medical Sciences & Health Services, Hamadan, Iran
2. Department of Clinical Biochemistry, Faculty of Medicine, Kermanshah University of Medical Sciences & Health Services, Kermanshah, Iran
3. Department of Biochemistry & Nutrition, Faculty of Medicine, Hamadan University of Medical Sciences & Health Services, Hamadan, Iran

Correspondence
Mohammad-Reza Safari
Department of Laboratory Medicine
Hamadan University of Medical Sciences & Health Services
Hamadan, Iran
Phone: +98 811 8282801; Fax: +98 811 8281442
Email: safari@umsha.ac.ir

Abstract
Carnitine is a small molecule widely present in all cells from prokaryotic to eukaryotic. It is an important element in β-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 severly 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±3.6 mg/l and 2.07±1.6 mg/l, respectively (P<0.001). The weight mean of pre-hemodialysis MDA was 4.17±1.24 μmol/l, following hemodialysis -4.98±1.2 μmol/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 pro-motion 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 ef-fectiveness on the change rate of serum L-carnitine and lipid peroxidation.

**MATERIALS AND METHODS**

**Chemicals:** Thiobarbituric acid (TBA), Buthylated 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. gave 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 ± 0.82 mmol/L [16].

**Statistical analysis:** All results were presented as mean ±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±3.6 and 2.07±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±1.24 μmol/l and 4.98±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
**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 μmol/l, where the post dialysis level was 5.6 μmol/l [4]. Also, Alhomida's research indicated that the average of serum L-carnitine before and after hemodialysis was 42±6.3 μmol/l and 17.1±6.3 μmol/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±0.38 nmol/ml) in comparison to prehemodialysis state (0.83±0.22 nmol/ml) [24], but Nand and Surri showed that MDA was decreased after hemodialysis (pre hemodialysis 2.96±0.89 μmol/l and post hemodialysis 2.32±0.84 μmol/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.

References

1. Chazot C.: Carnitine supplementation in Hemodialysis patients. Clin Nephrol 2003, 59: 24-30.
2. Rapport, L., Harm, B.P., MR.: Carnitine. Pharmacol J 2000, 265): 270-73.
3. Enomoto, A.: J Biol Chem 2002, 277 : 36262-36271.
4. Evans, A.: Dialysis- related carnitine disorder and levo-carnitine pharmacology. Am J Kidney Dis 2003, 41 (4 Suppl 4) : S 13-S26.
5. Bellinighieri, G., Santoro, D., Galvani, M.: Carnitine and hemodialysis. Am J Kidney Dis 2003, 41(1 Suppl 1): S116-S122.
6. Harper, H.A. Harper’s biochemistry. 2003, 139-141.
7. Schreiber, B.: Levo carnitine and dialysis: a review. Nutr Clin Pract 2005, 20: 218-243.
8. Ronco, C., La-Grec, A.: Pathophysiology of oxidative stress and its implication in uremia and dialysis. Contrib Nephrol Basel 1999, 127: 1-37.
9. Shackelford, R.E., Kaufmann, W.K., Paules, R.S.: Oxidative stress and cell cycle check point function. Free Radic Biol Me 2000, 28: 1387-1404.
10. Galli, F., Floridi, A., Buoncristiani, V.: Oxidative stress in hemodialysis patients. Contrib Nephrol Basel Karger 2002, 137: 371-378.
11. Galli, F., Canestrari, F., Buoncristiani, V.: Biological effects of oxidative stress in haemodialysis: the possible role of vitamin E. Blood Purif 1999, 17: 79-94.
12. Malliaraki, N., Mpiamplias, D., Kampa, M.: Total and corrected antioxidant capacity in haemodialyzed patients. BMC Nephrology 2003, 4: 1-8.
13. Dursun, E., Ozben, T., Suleymanlar, G.: Effect of hemodialysis on the oxidative stress and antioxidant. Clin Chem Lab Me 2002, 40: 1009-1013.
14. Jentzsch, A.M.: Improved analysis of malondiadehyde in human body fluids. Free Rad Biol Me 1996, 20: 251-256.
15. Safari, M.R.: Free radicals in biological system. Hamadan: Hamadan University of Medical Sciences Publication 2002, 154-170.
16. Eskandar, G.C., Kandemir, O., Polat, G., Tamer, L., Ersöz, G., Attik, U.: Serum L-carnitine levels and lipoproteins composition in chronic viral hepatitis patients. Clin Biochem 2001, 34: 431-433.
17. Bent, M., Suhail, A.: A review of impact of L-carnitine therapy on patient functionality in maintenance hemodialysis. Am J Kidney Dis 2003, 41(4 Suppl 4): S44-S48.
18. Alhomida, A.S.: Effect of chronic renal hemodialysis on serum total free and acyl carnitine concentrations. Arch Med Res 1997, 28: 101-107.
19. Hoppel, C.: The role of carnitine in normal and altered fatty acid metabolism. Am J Kidney Dis 2003, 41 (4 Suppl 4) : S4-S12.
20. Furuno, T., Kanno, T., Arita, K.: Roles of long chain fatty acids and carnitine in mitochondrial membrane permeability transition. Biochem Pharmacol 2001, 2: 1037-1046.
21. McCallum, C.D., Epar, R.M.: Insulin receptor autophosphorylation and signaling is altered by modulation of membrane physical properties. Biochemistry 1995, 34: 1815-1824.
22. Sener, G., Paskaloglu, K., Satiroglu, H.: L-carnitine ameliorates oxidative damage due to chronic renal failure in rats. J Cardiovasc Pharmacol 2004, 43: 698-705.
23. Samouilidou, E., Grapsa, E.: Effect of dialysis on plasma total antioxidant capacity and lipid peroxidation products in patients with end stage renal failure. Blood Purif 2003, 21: 209-212.
24. Ozden, M., Maral, H., Akaydin, D.: Erythrocyte glutathione peroxidase levels in hemodialysis and CAPD patients. Clin Biochem 2002, 35: 269-273.
25. Nandi, N., Suri, S., Aggarwal, H.K.: Lipid peroxidation before and after hemodialysis in chronic renal failure. Indian J Nephrol 1997, 7(2): 58-62.
26. Aymelek, G., Yesim, A., Mehmen, N.: Lipid peroxidation and antioxidant systems in hemodialyzed patients. Dialysis Transplant 2002, 31: 88-98.
27. Locatelli, F., Canaud, B.: Oxidative stress in ESRD: an emerging threat to patient outcome. Nephrol Dial Transplant 2003, 18: 1272-1280.
28. Boaz, M., Matas, Z., Biro, A.: Serum malondialdehyde and prevalent cardiovascular disease in hemodialysis. Kidney Int 1999, 56: 1073-1083.
29. Sommerburg, O., Grune, T., Hampl, H.: Does long term treatment of renal anemia with recombinant erythropoietin influence oxidative stress in HD patient? Nephrol Dial Transplant 1998, 73: 2583-2587.
30. Schreiber, B.D.: Congestive heart failure in patients with chronic kidney disease and on dialysis. J Med Sci 2000, 325: 179-193.
31. Goral, S.: Levocarnitine and muscle metabolism in patients with end-stage renal disease. J Ren Nutr 1998, 3: 118-121.
32. Rhajehdehi, P., Mojerlou, M., Behzadi, S.: A randomized double-blind placebo-controlled trial of supplementary vitamins E,C and their combination for treatment of hemodialysis cramps. Nephrol Dial Transplant 2001, 16: 1448-1451.
33. Lim, P.S., Cheng, Y.M., Wei, Y.H.: Increase in oxidative damage to lipids and proteins in skeletal muscle of uremic patients. Free Radic Res 2002, 36: 295-301.
34. Hae-Hyuk, J., Du-Hee, C.: Serum malondialdehyde and coronary artery disease in hemodialysis patients. Am J Nephrol 2004, 24: 537-542.
35. Galli, F., Roviadati, S., Benedetti, S.: Lipid peroxidation, leukocyte function and apoptosis in hemodialysis patients treated with vitamin E-modified filters. Contrib Nephrol Basel Karger 1999, 127: 156-171.
36. Tarng, D.C., Huang, T.P., Liu, T.Y.: Effect of vitamin E-bonded membrane on the 8-hydroxy 2′-deoxyguanosine level in leukocyte DNA of hemodialysis patients. Kidney Int 2000, 58: 790-799.
37. Dypbukt, J.M., Ankarcrona, M., Burkitt, M.: Different prooxidant levels stimulate growth, trigger apoptosis, or produce necrosis of insulin secreting RINm5F cells. J Biol Chem 1994, 269: 30553-30560.
38. Deng, G., Vaziri, N.D., Jabbari, B.: Increased tyrosine nitration of the brain in chronic renal insufficiency: Reversal by antioxidant therapy and angiotensin-converting enzyme inhibition. J Am Soc Nephrol 200, 12: 1892-1899.