Abstract

Background. Hemodialysis (HD) patients are exposed to homeostasis change. Impaired lipid metabolism, heart failure, anemia, muscle cramps and other disturbances are common for HD patients. L-carnitine is an amino acid which regulates these symptoms. It is related to the energy production in the body and has important role in the glucose oxidation.

Methods. This study included 45 patients on HD (20 male and 25 female) at age of 42±14 years. The controls were 24 healthy subjects. Primary renal diseases of the patients were as following: nephroangiosclerosis (n=20); interstitiopyelonephritis (n=12); diabetes nephropathy (n=4); adult polycystic disease (n=4); Duration of the HD procedure lasted app. 4 hours. The patients were dialysed by both, hemophan and polysulphon membranes, with blood flow of 250 ml/min. Regarding ultrafiltration rate, it was related on certain water lost, determined from the previous HD. Patients were divided in 3 groups concerning HD duration in years: I group (≤ 5); II group (6-10); III group (≥ 11). Plasma L-carnitine (mg/L) was determined by the enzymatic UV method (Roche Diagnostic GmbH, Manheim, Germany). Triglyceride (TG) level was determined by enzymatic colorimetric tests Vitros 250 (dry chemistry - Ortho Diagnostic Johnson-Johnson, USA). Urea and creatinine (mmol/L) were examined by Bio Rad, US. Hematocrit (%) was determined by blood counter analyzer AABx Micros 60.

Results. L-carnitine level in HD patients after the HD session showed decreased level which compared to the control group showed significant difference (p<0.05). During the HD session the L-carnitine level was 16% decreased. The decreased levels of urea and creatinin, compared to their levels before the HD session, were also significant (p<0.0001). Hemoconcentration and the increased level of triglycerides were noticed after the HD session, due to the water lost. Proportionally dropped values of L-carnitine in the patients group with longer HD duration were found.

Conclusion. L-carnitine is a small molecule which probably passes through HD membrane, during the HD session. The longer duration age of HD, the lower L-carnitine level was noticed which may cause some HD symptoms.

Keywords: hematocrit; hemodialysis; L-carnitine; triglycerides

Introduction

Carnitine is a conditionally essential metabolite that plays a critical role in cell physiology. It is peptide, produced in liver, kidney, and brain from the essential amino acids: lysine and methionin. Carnitine is necessary for fatty acid transport to sites of beta-oxidation in the mitochondria, where it prevents organic acid accumulation. Because of these key regulatory functions, carnitine represents a crucial determinant of mitochondrial energy metabolism. Its deficiency may lead to metabolic and clinical disturbances. Loss of carnitine through dialytic membranes occurs in maintenance hemodialysis (HD), resulting in potential carnitine depletion and relative increments of esterified carnitine forms [1]. More than a half century L-carnitine has been known as an amino acid that regulate the lipid metabolism. Actually it is produced in the body and it is involved in fatty acid metabolism regarding energy production, realized in the mitochondria. L-carnitine (L-3-hydroxy-4-N-trimethylaminobutyric acid) is also known as vitamin Bt that regulate the entrance of long chain fatty acid into mitochondria, thus is involved in energy production as ATP. It has important role in glucose oxidation [2].

The mechanism of its role is related to the acyl-CoA synthetase with long chains that is located on the outer mitochondrial membrane, while β-oxidation enzymes are located in its matrix [3]. The inner mitochondrial membrane is not permeable to CoA esters, and therefore carnitmit palmitoiltransferase I and II as well as carnitine translocase are important for the transport of the activated fatty acids as carnitit esters in mitochondrion during the oxidation process. L-carnitine is normally eliminated through the urine, but in patients undergoing HD its excretion is impaired [4]. In end-stage renal failure,
erythropoietin lack is often combined by decreased L-carnitine level [5,6]. In the study of Reuter SE 2008, it has been suggested that these disturbances in carnitine homeostasis may be associated with a number of clinical problems common in this patient population, including erythropoietin-resistant anaemia, cardiac dysfunction, and dialytic complications such as hypotension, cramps and fatigue [7]. It causes anemia, when it is resistant to erythropoietin therapy at normal iron levels [8,9] due to its lack in red blood cell viability and osmotic resistance, respectively. [10,11]. Its lack causes impaired lipid metabolism by increased blood levels of both, cholesterol and triglycerides [12]. This condition may influence the chronic morbidity and mortality of these patients.

The aim of this study was to examine the L-carnitine level in hemodialysis patients before and after hemodialysis, and to examine its level related to the different HD duration.

Patients and methods

A number of 45 HD patients (20 male and 25 female) at age of 42 ± 14 years was examined and compared to a control group of healthy subjects (n=24), sex and age matched. Primary renal diseases of the patients were as following: nephroangiosclerosis due to hypertension provoked renal insufficiency (n=20); interstitial pyelonephritis (n=12); glomerulonephritis (n=5); diabetes nephropathy (n=4); adult polycystic disease (n=4); Duration of the HD procedure lasted app. 4 hours. The patients were dialysed by both, hemophan and polysulphon membranes, with blood flow of 250 ml/min. Regarding ultrafiltration rate, it was related on certain water lost, determined from the previous HD. Patients were divided in 3 groups concerning HD duration in years: I group (≤ 5) (n=15); II group (6-10) (n=22); III group (≥ 11) (n=8). The blood from the different duration group patients was taken after HD session. Supplement therapy like EPO, L-carnitine and iron was not given to any of the examined patients, in order not to have the influence on examined parameters. For the blood analysis, plasma samples were taken from the cubital veins from HD patients before and after the HD session. Plasma L-carnitine was determined by the enzymatic UV method (Roche Diagnostic GmbH, Manheim, Germany). Prior the assay, plasma samples were first deproteinised with 0.6 mol/L perchloric acid and with 1.2 M potassium carbonate. The quantity of NADH was measured by its absorption of 340nm. The results are expressed in mg/L. Urea and creatinine (mmol/L) were examined by Bio Rad, USA. Triglycerides (TG) was determined by enzymatic colorimetric tests Vitros 250 (dry chemistry - Ortho Diagnostic Johnson-Johnson, USA) and expressed in mmol/L. Hematocrit (%) was determined by blood counter analyzer AABx Micros 60.

Statistical analysis

For the statistical analysis, Student t-test was used and p value less than 0.05 was considered significant. The results are expressed as mean values ± standard deviations.

Results

<table>
<thead>
<tr>
<th>Table 1. L-carnitine level (mg/L) in HD patients: before and after the HD session compared to control group</th>
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<tbody>
<tr>
<td><strong>L-carnitine (mg/L)</strong></td>
</tr>
<tr>
<td>Before HD</td>
</tr>
<tr>
<td>After HD</td>
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<tr>
<td>N.S. – not significant</td>
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</table>

The L-carnitine level in HD patients before HD session showed no significant difference when compared to its level in healthy subjects. Nevertheless, L-carnitine level in HD patients after the HD session showed significant difference (p < 0.05) (Table 1). During the HD session the L-carnitine level decreased from 5.57 ± 1.6 mg/L to 4.72 ± 1.9 mg/L (p < 0.05) (Table 2).

<table>
<thead>
<tr>
<th>Table 2. Levels of urea, creatinine, hematocrit and triglycerides in HD patients before and after the HD session</th>
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<tbody>
<tr>
<td><strong>Urea (mmol/L)</strong></td>
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<tr>
<td>Before HD</td>
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<tr>
<td>After HD</td>
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<tr>
<td><strong>p</strong></td>
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The L-carnitine level in HD patients after HD session, concerning HD duration

<table>
<thead>
<tr>
<th>Table 3. L-carnitine level (mg/L) in HD patients after HD session, concerning HD duration</th>
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</thead>
<tbody>
<tr>
<td><strong>I group</strong></td>
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<tr>
<td><strong>≤ 5 years</strong></td>
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<tr>
<td>n = 15</td>
</tr>
<tr>
<td>L-Carnitine (mg/L)</td>
</tr>
<tr>
<td>N.S. – not significant</td>
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After the HD session, besides L-carnitine, the decreased levels of urea and creatinine were noticed (p<0.0001). Regarding the hematocrit and triglycerides level, hematocrit was noticed after the HD session (Figure 1). On the Table 3, no significant difference was found for L-carnitine level in different HD duration groups, although the longer duration, the lower L-carnitine level was noticed.

![Graph showing blood sample parameters before and after HD session](image)

**Fig. 1.** Examined blood sample parameters before and after HD session (in %)

### Discussion

L-carnitine is considered as "conditionally essential nutrient" or "conditional vitamin" which supraphysiological concentrations in plasma and target organs may exert beneficial effects on several metabolic parameters that have derangements of a common origin (e.g. insulin resistance, type 2 diabetes, dyslipidemia) and which are frequently present in end-stage renal disease patients undergoing dialysis. In our study, the primary renal diseases of HD patients were nephroangiosclerosis and interstitialpyelonephritis. Significantly decreased L-carnitine level after the HD session in HD patients emphasised the possible lost of this small molecule through the HD membrane. Therefore the structure of HD membrane may determine the quantity of lost L-carnitine. Nevertheless, L-carnitine level before HD rises either by the food intake or by extra renal production, e.g. liver, brain. Higher hematocrit and higher triglyceride level after HD session show hemococoncentration due to the water lost. According to this, L-carnitine is lost even more than our obtained results due to present blood concentration. However, in general, higher triglyceride level was found in HD patients before HD session, when compared to healthy subjects which implicate impaired lipid profile of these patients.

In the study of Vernez L et al., 2006, the kinetics of carnitine, individual acylcarnitines and butyrobetaine in patients on HD was investigated. During HD, the plasma concentrations dropped by approximately 80% for all compounds determined. In patients supplemented with 20 mg/kg carnitine, the amount of carnitine removed by haemodialysis equalled 42% of the dose administered. Due to our results, plasma concentration of L-carnitine level was dropped by approximately 16% after the HD session. Regarding the results we obtained, the L-carnitine level proportionally decreases with the HD duration. Although no statistical significance was found, probably because of small number of patient groups, in the group with the longest HD duration, lowest L-carnitine level was noticed, which probably elucidate the fact that longer the HD duration, harder it's renewed. This means that the capacity of L-carnitine production (endogenous plasma level) decreases with the HD duration.

Although the relationship between dialysis age and carnitine status is poorly understood, there are some studies that examined the relationship between duration of dialysis and plasma and skeletal muscle concentrations of L-carnitine and its esters in end stage renal disease patients. In the study of Evans AM et al., 2004, it is considered that long-term HD treatment is associated with a significant reduction in endogenous plasma, which is in accordance with the results we obtained. In their study, decreased muscle L-carnitine levels and a significant increase in plasma acylcarnitines were found. They have also noticed that the majority of the changes of plasma L-carnitine concentrations occur within the first few months of HD, while muscle levels continue to decline after 12 months of treatment.

Therefore, supplementation of L-carnitine is a treatment of choice in HD patients, particularly for those who are resistant to erythropoietin therapy, those who suffer from lipid disturbances, intradyalitic symptoms of cramps, hypotension, etc. In the study of Kazmi WH et al. 2005, patients with cardiovascular disease, defined as hospitalizations for angina, myocardial infarction, arrhythmia, congestive heart failure, cerebral vascular disease or peripheral vascular disease prior to receiving carnitine, and those with anemia and hypoalbuminemia derived the greatest benefit from carnitine therapy. Administration of L-carnitine to chronic HD patients is associated with lower hospital utilization. While the erythropoietin influence is focused on bone narrow pluripotentional cells stimulation, L-carnitine improves red blood viability and longevity and therefore low plasma L-carnitine in HD patients causes red blood cell osmotic fragility. L-carnitine supplementation is also registered in children who suffer from HD common symptoms. It is demonstrated that children with chronic renal failure on regular HD suffer from. In addition, L-carnitine plasma decreased level causes dyslipidemia, oxidative stress, and impairment of cardiac functions. Oral L-carnitine supplementation at a dose of 50 mg/kg within 2 months improved their condition. If other factors related to anemia are excluded, the postdialysis parenteral L-carnitine therapy can be considered in selected stable patients, which may improve anemia and may reduce the weekly requiring dose of the rHuEPO and also be cost-effective. In the study of Wanic-Kossowska M et al., 2007 the influence of combined therapy with L-carnitine and erythropoietin on selected blood morphology parameters in patients treated with HD was analyzed. They realized that combined therapy could decrease the requirement for exogenous erythropoietin. The correlation between serum carnitine concentration and erythrocyte osmotic resistance indicates indirectly the beneficial effect of L-carnitine administration on erythrocyte cell membrane stabilization. The review of Hurot JM et al., 2002 suggests a promising effect of L-carnitine on anemia management. However they suggest that the route
of L-carnitine administration should be evaluated because there is no evidence as to the most efficient method of administration in maintenance hemodialysis [21].

**Conclusions**

In summary, we could conclude that L-carnitine as an essential peptide is decreased in HD patients, especially after the HD session. It is probably lost through the HD membrane during the HD session. Although it is renewed during the period between the two HD sessions, his lack is proportional to the HD duration. This condition may cause some common dialytic symptoms that influence the morbidity and the mortality of HD patients.

L-carnitine is essential peptide that might be lost through the HD membrane which may cause some common dialytic symptoms that influence morbidity and mortality of HD patients. Although it is renewed between the two HD sessions, his lack is proportional to the HD duration. Therefore, L-carnitine substitution therapy might be beneficial improving the life quality and longevity of HD patients.

**Conflict of interest statement.** None declared.

**References**