Original article

The Effect of L-Carnitine and Proinflammatory Cytokines in the Development of Nonalcoholic Steatohepatitis

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Abstract:

Objective: research is to study the role of L-carnitine, tumor necrosis factor α (TNF-α), and interleucine-6 (IL-6) in the development of NASH. ***Materials and Methods:*** examined 65 patients with NASH. Blood serum L-carnitine, TNF-α and IL-6 level was estimated using the enzyme-linked immunoassay method. ***Results:*** patients with NASH had a decreased blood serum level of L-carnitine. L-carnitine level was found to be in strong inverse correlation dependence on cellular damage markers, L-carnitine levels affect the performance TNF-α and IL-6. ***Conclusion:*** L-carnitine contributes to lipotoxic stress development and development of systemic liver inflammation.

Key words: Nonalcoholic steatohepatitis; L-carnitine; TNF-α; IL-6.

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Introduction:

The issue of nonalcoholic fatty liver disease (NAFLD) is of great topicality in contemporary clinical medicine due to its high occurrence and progredient course resulting in severe complications. In developed countries, NAFLD is the increased activity of liver ferments most widely spread chronic liver disease (its occurrence rate is from 17 to 33%), while 2-3% of general population [1] are diagnosed with nonalcoholic steatohepatitis (NASH). According to a number of authors, 30-40% patients with NASH are diagnosed with liver fibrosis [2, 3] during the primary survey. In 20-25% of patients, NASH can progress to hepatic cirrhosis, out of which amount 30-40% of patients die from its complication[4, 5]. NASH is currently considered to be one of the major reasons for with no clinical symptoms[6]. There is epidemiologic evidence to the fact that the growth of NASH rate is affected by the increased occurrence of metabolic syndrome, type 2 diabetes mellitus (DM), and obesity. In adult obese patients, the occurrence of NAFLD can be as high as 80-90%, being higher than average in patients with type 2 DM (30-50%) and as high as 92% [7-9] in patients with hyperlipidemia. Since the diseases have identical pathogenic mechanisms, concurrence and potentiation are common.

Nowadays, the evidence to mitochondrial damage and dysfunction playing a major role in the development and progress of NASH is becoming increasingly abundant [10, 11]. Abnormal structural and functional mitochondrial organization in patients with NASH has been proved to include abnormalities in the mitochondrial membrane apparatus, mitochondrial DNA structure, decreased activity of the respiratory chain complex and β-oxidation of free fatty acids (FFA). Lipid peroxides produced in fat depots contribute to the suppression of the mitochondrial respiratory function. Proinflammatory cytokines, which play an important role in NASH pathogenesis, are produced directly in mitochondria when exposed to free radicals[12].

It is a common fact that dyslipidemia plays a major role in the development and progress of NASH. FFA metabolism depends on the transport protein regulated with a carnitine-dependent ferment. Thus, deficient or abnormal L-carnitine metabolism causes abnormal transportation of long-chain fatty acids through the internal mitochondrial membrane and production of CoA [13-15].

L-carnitine activates lipid disintegration, stimulates

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oxidation of fatty acids, and contributes to their transportation to mitochondria, reducing fat storage in body tissues. Besides, L-carnitine helps reduce triglyceride, total cholesterol, and low-density lipoprotein (LDLP) cholesterol blood level [16, 17]. Under a different theory, FFA is a source of lipotoxic stress and mitochondrial damage [13]. There is evidence that there is a correlation between the severity of carnitine deficiency and that of hepatocellular damage and parenchymatous inflammation in patients with NAFLD[16].

Proinflammatory cytokines play an important role in the development and progress of NASH by stimulating liver inflammation, apoptosis, hepatocyte necrosis, and fibrosis induction[18].

However, the role of L-carnitine in the development and progress of NASH is insufficiently studied; the way in which carnitine deficiency affects proinflammatory cytokine rates in patients with NASH requires further research.

The objective of this research is to study the role of L-carnitine, TNF-α and IL-6 in the development of NASH.

Materials and methods:

We examined 65 patients with NASH. The patients' age was from 23 to 67 years; their mean age was 47.06±12.64 years. They included 36 (55.4%) females and 29 (44.6%) males. The control group consisted of 20 healthy individuals.

All the patients had their diagnosis verified using clinical laboratorial and instrumental (ultrasonography) methods. The patients were tested for hepatitis B and C markers using the PCR method to rule out viral etiology of the liver damage. The exception criteria were records of alcohol and hepatotrophic toxins consumption. The patients' liver functional status was estimated by the level of total bilirubin and its faction, alkaline phosphatase, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activity, thymol test, and lipid profile. Total cholesterol (TC), β-lipoprotein (β-LP), triglyceride (TG), high-density lipoprotein (HDLP), low-density lipoprotein (LDLP), and very low-density lipoprotein (VLDLP) level in blood serum were studies as markers of lipid metabolism. Atherogenic index was calculated by the following formula: (TC – HDLP) – 1.0.

Blood serum L-carnitine level was estimated using the enzyme-linked immunoassay method with the Human L-Carnitin ELISA Kit produced by Biotech (USA). Blood serum TNF-α and IL-6 level was estimated using the enzyme-linked immunoassay method.

The data were statistically processed using the standard Microsoft Excel software package. The statistical significance was estimated using the variation statistics method with Student's t-test; interparameter correlation was identified using Spearman correlation analysis. A difference was considered significant at $p < 0.05$. Ethical approval was taken from cthical Committee.

Results and Discussion:

All patients with NASH examined (Table 1) showed a significant increase in ALT and ACT level as compared to the control group ($p < 0.001$). The ALT rate was recorded 103.5±7.25 U/l, while the ACT rate was 96.0±6.3 U/l, which was an evidence of hepatocellular damage.

The analysis of lipid metabolism performed showed dyslipidemia, which was identified by a significant increase in TC ($p < 0.001$), β-LP ($p < 0,001$), TG ($p < 0.001$), LDLP ($p < 0.001$), VLDLP ($p < 0.001$), and atherogenic index ($p < 0.001$), as well as a decrease in HDLP ($p < 0.001$) in all patients as compared to the control group. It is coherent with the literary data on excessive income of fat and hydrocarbon, which are later transformed to fatty acids, serving as a substratum for the synthesis of TG and VLDLP, which accumulate in hepatocytes [7]. Due to increased triglyceride synthetase and triglyceride lipase activity, β-LP are synthesized based on the latter. Table 1 shows different biochemical parameters of liver.

Table 1. Liver Biochemical Parameters

Parameter, unit of measurement	NASH (n=65) M±m	Control group (n=20) M±n
ALT, u/l	103.5±7.25*	28.0 ±2.3
ACT, u/l	96.0±6.3*	29.5 ±2.0
TC, mmol/l	5.9±0.13*	4.6 ±0.25
β-LP, units	65.0±3.37*	41.5 ±2.0
TG, mmol/l	1.88±0.11*	1.12 ±0.27
HDLP, mmol/l	0.82±0.08*	1.47 ±0.26
LDLP, mmol/l	4.4±0.25*	2.73 ±0.32
VLDLP, mmol/l	0.43±0.06*	0.27 ±0.04
Atherogenic index, mmol/L	4.55±0.4*	2.25±0.45

Note: * – $p < 0.001$ significance of difference as compared to the control group.

All patients with NASH had a decreased blood serum level of L-carnitine (14.49±1.29 mcmol/l), ($p < 0.001$). A significant decrease in L-carnitine serum concentrations in the control group indicates

a deficiency of carnitine-associated mechanism of long-chain fatty acid transition to the mitochondrial matrix, resulting in abnormal fatty acid β-oxidation and progress in metabolic disorders, which is supported by our data concerning the change in TG and VLDLP level.

By assessing the level of L-carnitine as depending on the duration of the disease, it was found to decrease significantly with a disease duration of over 5 years (Table 2).

Table 2. Blood Serum Level of L-Carnitine in Patients with NASH Depending on the Duration of the Disease

Disease duration	L-carnitine, mmol/l (M±m)
under 5 years	13.4±0.81
6 – 10 years	15.66±1.41*
over 10 years	16.15±1.0*

Note: * – $p<0.05$ difference significance.

When compared to the group with a disease duration of under 5 years (13.4±0.81 mmol/l), patients with that of 6 to 10 years and over 10 years had a significant ($p<0.05$) decrease in the level of L-carnitine to 15.66±1.41 mmol/l and 16.15±1.0 mmol/l respectively.

Studying the patients' cytokine profile (Table 3) revealed a significant increase in TNF-α and IL-6 level in patients with NASH as compared to the control group ($p<0.001$).

Table 3. Cytokine Profile in Patients with NASH

Parameter, unit of measurement	NASH (n=65), M±m	Control group (n=20), M±m
TNF-α, pg/ml	8.5±0.51*	1.94±0.15
IL-6, pg/ml	19.66±1.18*	1.72±0.41

Note: * – $p<0.001$ difference significance as compared to the control group.

We have found a correlation relationship between blood serum L-carnitine level and blood lipids (Table 4).

We have found a reverse correlation between L-carnitine and TC ($r=-0.74$; $p<0.01$) and L-carnitine and β-LP ($r=-0.74$; $p<0.01$) in patients with NASH. Besides, a reverse correlation was between L-carnitine and TG ($r=-0.73$; $p<0.01$), between L-carnitine and LDLP ($r=-0.73$; $p<0.01$), between L-carnitine and VLDLP ($r=-0.67$; $p<0.01$), and between L-carnitine

Table 4. The Correlation between Blood Serum Level of L-Carnitine and Lipid Profile in Patients with NASH

Parameters	L-carnitine
TC	r =-0.74*
β-LP	r =-0.74*
TG	r =-0.73*
HDLP	r =0.74*
LDLP	r =-0.73*
VLDLP	r =-0.67*
Atherogenic index	r =-0.72*

Note: * – $p<0.01$.

and atherogenic index ($r=-0.72$; $p<0.01$) was discovered. L-carnitine and HDLP were found to be in direct correlation with a correlation ratio of $r = 0.74$ ($p<0.01$).

The findings of the study are indicative of the role which L-carnitine plays in lipid metabolism in patients with NASH.

Analyzing the interrelation between L-carnitine and proinflammatory cytokines, a strong feedback was discovered – the correlation ratio between L-carnitine and TNF-α was $r = 0.78$ ($p<0.01$), while that for L-carnitine and IL-6 was $r = 076$ ($p<0.01$).

L-carnitine level was found to be in strong inverse correlation dependence on cellular damage markers, including ALT and ACT. In patients with NASH, the correlation ratio for ALT and L-carnitine was $r=-0.76$ ($p<0.01$), and that for ACT and L-carnitine was $r=-0.79$ ($p<0.01$).

Conclusion:

decreased L-carnitine blood serum level in patients with NASH is indicative of carnitine deficiency, resulting in abnormal transportation of fatty acids to mitochondria, increased intensity of lipid accumulation in body tissues, in particular in liver, and progressive mitochondrial dysfunction. The correlation relations discovered is an evidence that L-carnitine contributes to lipotoxic stress development, resulting in lipid peroxidation activation and development of systemic liver inflammation, while cellular damage of hepatocytes aggravates the carnitine disbalance and the augmentation of metabolic disorders. The long duration of the disease causes compensatory mechanisms to deteriorate.

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