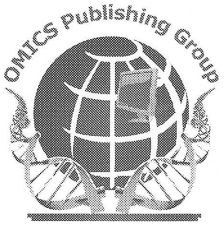

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Metabolic Indexes State Studing in Patients with Chronic Viral Hepatitis C

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Abstract

Endogen intoxication trigger in acute hepatitis C is hepatic cells cytolysis caused by viral reproduction with subsequent immune system reaction. All above mentioned needs further study of formation mechanisms in Chronic Hepatitis C (CHC) and motivation of immunosuppressive therapy. So this research aim was to determine the monitoring metabolic indexes state in patients with chronic viral hepatitis C and motivate such diagnostic criteria as significant tests for controlling the endogen intoxication.

Materials and methods: For this purpose, in serum of patients with hepatitis C and conventionally healthy people from control group the activity of enzymes were detected. To study the bioenergetics changes in serum lactate/pyruvate and NAD/NADH$^+$ correlation were investigated. Mentioned indexes detection was performed using photometric method with a set of chemicals manufactured by firm "Filisit-Diagnosticum", Ukraine. For determination of lactate and pyruvate test kits produced by "Olveks", Ukraine, were used according to manufacturer's recommendations.

Results and discussion: Thus, the results of monitoring indexes study in patients with CHC have found multiple organ disorders of metabolic processes based on membrane pathology. Inhibition of reduction synthesis bioenergetics in human body and catabolic processes activation which are connected with the tension of many organs and tissues that in turn leads to endogenous intoxication. That's why monitoring indexes state determination is important in evaluation of the course severity degree at CHC according to the level of endogen intoxication.

Keywords: Endogen intoxication; NAD/NADH$^+$; Chronic hepatitis C

Introduction

By frequency lesion population hepatitis C ranks second after influenza and other ARI as it is characterized by long latent course with the high probability of developing cirrhosis and hepatocellular carcinoma according to greater duration and severity of the process, the tendency to chronicity and prognosis of adverse effects [1-5].

Hepatitis C virus is recognized as a major factor in fibrosis and cirrhosis developing. Parenchymal damage of cell membranes may lead to metabolic disorders, which plays a major role of liver fibrosis formation in hepatitis C [6].

A wide range of possible consequences of HCV-infection from spontaneous recovery to decompensated Liver Cirrhosis (LC) and Hepatocellular Carcinoma (HCC) involves complex multifaceted system of relations between human body and virus. Among them there are factors associated with virus (genotype, mutations, population heterogeneity, viremia level) and host (sex, age, infection route, infection course duration, genetic predisposition, immune system function state, comorbid background, etc.) [5-8].

Endogenic Intoxication (EI) trigger in acute hepatitis C is hepatic cells cytolysis caused by viral reproduction with subsequent immune system reaction. EI syndrome in acute viral hepatitis is accompanied by increasing of lipid peroxidation processes, disorders in antioxidant and immune system activity, blood cells damage, nervous system involvement. Gromashevska [9] approves that the metabolic disturbances that have universal significance in the development of pathology, such as lipid peroxidation activation, antioxidant system shifts, energy potential decreasing, hypoxia, are described in various pathological processes [1,9-11].

All above mentioned needs further study of formation mechanisms in Chronic Hepatitis C (CHC) and motivation of immunosuppressive therapy. So this research aim was to determine the monitoring metabolic indexes state in patients with chronic viral hepatitis C and motivate such diagnostic criteria as significant tests for controlling the endogenic intoxication.

Materials and Methods

Structural metabolic homeostasis study in patients with chronic hepatitis C was carried out by monitoring clinical and biochemical parameters determination. For this purpose, in serum of patients with hepatitis C (men and women aged from 15 to 60 years) and conventionally healthy people from control group the activity of enzymes (ALT, AST, γ-GTP, Alkaline Phosphatase (AP), and the content of total bilirubin, glucose, urease, kreatinine, total protein, cholesterol, High Density Lipoproteins (HDL), triglycerides, albumin were detected. To study the bioenergetic changes in serum lactate/pyruvate and NAD/NADH$^+$ correlation were investigated. Mentioned indexes detection was performed using photometric method with a set of chemicals manufactured by firm "Filisit-Diagnosticum", Ukraine. For determination of lactate and pyruvate test kits produced by "Olveks", Ukraine, were used according to manufacturer's recommendations.

Method for determining lactate and pyruvate is based on enzymatic oxidation of lactic acid into pyruvic acid by lactate dehydrogenase while restoring NAD^+ in $NADH_2$. Equilibrium reaction is shifted toward the formation of lactic acid, but when you add hydrazine formed pyruvic

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acid can be bind completely. Lactic acid quantity is determined by the concentration of formed NADH after measuring optical density at 340 nm. Pyruvic acid quantity is expected according to decreasing in NADH concentration. NADH concentration (in micromole) in the sample is calculated according to formula: $X = (\Delta A_{340} \times V)/6.22$, were (in which) ΔA_{340} changing in reaction mixture optical density, V-volume of sample in ml. NADH molar absorption coefficient at 340 nm is 6.22×10^3 $M^{-1}cm^{-1}$ [12].

110 patients with chronic viral hepatitis C we examined. Among them 50 patients were with manifestation form (MF CHC), 55 had latent form (LF CHC), 9 patients were with cirrhosis and 3 had hepatocellular carcinoma with a positive PCR result for HCV. In 63 patients were distinguished virus genotypes: 1b in 30 patients, 3a in 15, genotype 2 in 14 and 1a in 4 patients accordingly. Patient's average age was 39.4 ± 3.2 years. Control group included 25 healthy people who had no history of liver disease. Statistical analysis of the results was performed by Student-Fischer indexes.

Results and Discussion

Structural metabolic homeostasis study in patients with chronic hepatitis C determined the increasing in average values of AST, ALT, γ-GTP, AP in comparison with control group. AST indexes were increased in 2.1, 2.0 and 1.5 accordingly at MF CHC, LF CHC and liver cirrhosis. Despite the fact that the average activity of AST increased in 1.5 times in CHC in comparison with control group the reliability of these differences has not been established. ALT increased in 2.3, 1.8 and 2.0 accordingly at MF CHC, LF CHC and liver cirrhosis. Similar dynamics was observed for γ-GTP activity which was increased in 2.3, 2.0 and 2.1 accordingly at MF CHC, LF CHC and liver cirrhosis. It should be noted that 2% of patients experienced a decrease and 21% an increase of alkaline phosphatase activity, which represented a significant number of patients with impaired function of this enzyme activity, but according to all studied groups the average index of alkaline phosphatase activity was also within the physiological norm. An increase of indirect bilirubin fraction was detected in 11.7% of patients with cirrhosis, in 8.3% with the LF CHC and in 8.7% with the MF CHC. These data indicate metabolic reorganization and tension of liver functions in patients with CHC (Table 1).

The analysis of enzyme activity dynamics, their organic specificity, structural and metabolic role is allowed to determine the tension and involvement in pathological process of all internal organs, systems and body functions in the development of CHC. The appearance of membrane-structural enzymes in higher values in patients serum may indicate and reflect the development of membrane pathology in which dysfunction of nuclear-cytoplasmic interactions is formed, intracellular metabolism and bioenergetics processes are disturbed and it is important in the pathogenesis of hepatitis.

Study of major carbohydrates, protein and fat exchange metabolites in patients with CHC revealed disorders in serum content of urease, creatinine, total protein, albumin, cholesterol, HDL and triglycerides. There were no changes in glucose content at different forms of chronic hepatitis C. Urease was increased in 43%, 50% and 53% and creatinine was decreased in 32.4%, 42% and 36.2% accordingly at MF CHC, LF CHC and cirrhosis. In all cases there was an increasing in total protein and albumin contents. Thus, total protein was increased in 14.8%, 9.3%, and 18,9% in patients with MF CHC, LF CHC and cirrhosis. The same situation was with albumin concentration: at CHC it content was increased in 16%. Increasing in triglycerides content (264 ± 13.4 mg/dl) and decreasing in HDL quantity (28.2 ± 1.2 mg/dl) indicated lipid metabolism disorder and risk of liver fatty dystrophy formation. There was shown no differences in cholesterol indexes at patients with CHC and control group. Research has found disorders of protein and fat metabolism in patients with CHC at which it should be considered that catabolic processes predominate over anabolic (Table 2).

To detect bioenergetics changes we examined the ratio of pyruvic acid and lactate as markers of carbohydrate metabolism oxidative stage (the ratio of aerobic and anaerobic phases), and NAD^+ and $NADH_2$ levels as mandatory participants of oxidation-reduction reactions and regulators of cell metabolism. Decreased $NADH_2$ index (0.002 ± 0.0001 mmol/l) was determined in comparison with control group (0.01+0.0005 mmol/l). The NAD^+ concentration (0.494 ± 0.03 mmol/l) was significantly ($P<0.05$) increased at patients with CHC in comparison with normal content (Table 3). An increased content of oxidized nicotinamide coenzymes was detected in patients with CHC.

Increasing in the ratio of $NAD^+/NADH_2$ reduces the activity of NAD^+ dependent enzymes in the cytosol and mitochondria. Restoration of dehydroxyacetonphosphate which is an intermediate metabolite of glycolysis and glyconeogenesis leads to inhibition of the

Indexes	MF CHC (n=50)	LF CHC (n=55)	Cirrhosis (n=9)	HCC (n=3)	Control group (n=25)
AST (u/L)	38.6 ± 6.7*	29.3 ± 8.4	40.8 ± 9.2*	43.5 ± 8.7*	19.3 ± 4.6
ALT (u/L)	40.4 ± 8.5*	27.8 ± 7.2	39.6 ± 8.1*	37.3 ± 9.6*	22.7 ± 4.6
γ-GTP (u/L)	54.2 ± 18.3*	45.3 ± 16.7	47.5 ± 14,9*	56.3 ± 12.6*	29.4 ± 7.2
AP (u/L)	1.92 ± 0.4*	1.86 ± 0.2*	2.6 ± 1.2*	2.4 ± 0.8*	2.2 ± 1.04
Bilirubin	25.1 ± 3.77	15.5 ± 0.63	32.6 ± 2.1*	34.3 ± 1.6*	16.5 ± 2.63

Note: *differences are significant P<0.05.

Table 1: State of marker organspecific enzymes in patients with CHC depending on the form of the disease (M ± m).

Indexes	Research group (M ± m)				
	MF CHC (n=50)	LF CHC (n=55)	Cirrhosis (n=9)	HCC (n=3)	Control group (n=25)
Glucose (mmol/l)	4.1 ± 0.7	3.8 ± 0.5	3.9 ± 0.4	4.2 ± 0.8	4.4 ± 0.6
Urease (mmol/l)	8.9 ± 0.5*	9.3 ± 0.6*	9.7 ± 0.8*	9.5 ± 0.7*	6.2 ± 0.8*
Creatinine (mcmol/l)	51.6 ± 12.4*	44.5 ± 9.3*	48.7 ± 10.5*	46.8 ± 13.4*	76.3 ± 7.2
Total protein (g/l)	67.3 ± 5.2*	70.6 ± 3.7*	56.4 ± 2.2*	76.5 ± 2.8*	72.5 ± 6.4
Albumin (g/l)	32.4 ± 4.4*	36.3 ± 2.8*	30.5 ± 5.2*	64.4 ± 1.45*	48.4 ± 3.5
Cholesterol (mmol/l)	3.1 ± 0.17	2.7 ± 0.22*	2.5 ± 0.35	2.4 ± 0.40*	4.6 ± 0.25
Triglycerides (mmol/l)	2.5 ± 0.22*	2.7 ± 0.33*	2.6 ± 0.18*	2.8 ± 0.43*	1.7 ± 0.26

Note: *differences are significant P<0.05

Table 2: State of major monitoring proteins and carbohydrates exchange metabolites in patients with CHC.

	MF CHC	LF CHC	Cirrhosis	HCC	Normal content
NAD^+	0.48 ± 0.4*	0.36 ± 0.08*	0.58 ± 0.12**	0.65 ± 0,3**	0.126 ± 0.03
$NADH_2$	0.008 ± 0.004*	0.002 ± 0.001*	0.003 ± 0.001**	0.0007 ± 0.0002**	0.01 ± 0.0005
$NAD^+/NADH_2$	0.15 ± 0.028	0.12 ± 0.035	0.18 ± 0.04	0.21 ± 0.03	0.09 ± 0.02

Note: *the difference is reliable in comparison with normal content,
**the difference between the groups is significant

Table 3: NAD^+ and $NADH_2$ serum concentration in patients with CHC (mmol/l).

last. Increased concentration of $NADH_2$ compared with NAD^+ slows lactate oxidation and increases the ratio of lactate/pyruvate resulting in even more slowdown of glyconeogenesis. Lactate concentration increases in the blood.

Pyruvate oxidative decarboxylation is accompanied by formation of NADH, which brings electrons to the respiratory chain and provides ATP synthesis. As the ratio of $NAD^+/NADH_2$ is relatively constant in cells so the increase in NADH concentration reduces the rate of pyruvate decarboxylation. Therefore, $NAD^+/NADH_2$ ratio change is an important indicator which reflects the energy needs of cells that regulate the rate of pyruvate oxidation decarboxylation. Catalytic activity of pyruvate dehydrogenase complex decreases when cells have enough fatty acids quantity that we observed in our study. Determined decrease of triglycerides and HDL contents in patients with CHC explains the accelerated use of fatty acids in terms of lipids peroxide oxidation.

Lactate and pyruvate parameters study has found the following. In patients of all groups lactate indexes exceeded the parameters of control group and amounted 2.12 ± 0.23 and 1.89 ± 0.45 mmol/l for MF CHC and LF CHC accordingly in comparison with control value (1.56 ± 0.235 mmol/l). Pyruvate serum indexes were significantly lower than in the control group (0.056 ± 0.011 mmol/l) and composed accordingly for MF CHC and LF CHC 0.031 ± 0.012 and 0.174 ± 0.01 mmol/l.

The increase of lactate concentration may be used like a stimulus for collagen synthesis in fibroblasts, which leads to accumulation of connective tissue in liver and cirrhosis development, and on it basis turns to tumor growth.

Thus, the results of monitoring indexes study in patients with CHC have found multiple organ disorders of metabolic processes based on membrane pathology.

Inhibition of reduction synthesis bioenergetics in human body and catabolic processes activation which are connected with the tension of many organs and tissues that in turn leads to endogenous intoxication. That's why monitoring indexes state determination is important in evaluation of the course severity degree at CHC according to the level of EI.

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