ENGLISH VERSION: THE PROGNOSTIC SIGNIFICANCE OF BIOMARKERS FOR THE IMBALANCE BETWEEN ENERGY AND PLASTIC POTENTIALS FOR LEARNING THE MECHANISM OF DEVELOPMENT OF HEPATIC ENCEPHALOPATHY IN CHRONIC VIRAL HEPATITIS C*

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We examined 73 patients with chronic viral hepatitis C who underwent a routine examination at the Medical Diagnostic Center ASK-Health in Kharkiv. All patients underwent virus genetyping, 3 genotypes of HCV-infection were detected: 3a genotype was diagnosed in 13 patients, 2 genotypes – in 11, the largest group was made up of patients with 1b genotype of the virus - 49. The control group included 20 practically healthy persons who did not have the history of liver disease. The activity of glyceraldehyde-3-phosphate dehydrogenase in lymphocytes of patients with chronic viral hepatitis C and the phenomena of hepatic encephalopathy (n = 11) is reliably (p≤0.01) higher - by 2.3 times - as compared with the control group. Establishing a change in the metabolism of phosphotriose in blood cells probably suggests the predominant use of phosphotriose and neuron-specific enolase for energy metabolism and ATP production, that is, it is possible to assume that there is an imbalance between the energy and plastic potentials in the blood cells of patients with chronic viral hepatitis C, especially in erythrocytes.

Key words: hepatic encephalopathy, viral hepatitis C, plastic potential.

Introduction

Chronic liver and liver cirrhosis are registered in 1.2 million people in Ukraine and hundreds of millions of people around the world. With a significant increase in the frequency of metabolic syndrome around the world, viral hepatitis is accompanied by a gradual increase in liver encephalopathy. The probability of developing complications in hepatitis C is 20% of cases [1]. Studies have indicated that overt hepatic encephalopathy affects 30 to 45% of patients with cirrhosis and a higher percentage may be affected by minimal degree of encephalopathy [2, 3].

Hepatic encephalopathy or portosystemic encephalopathy is a syndrome of largely reversible impairment of brain function occurring in patients with acute or chronic liver failure or when the liver is bypassed by portosystemic shunts. The mechanisms causing this brain dysfunction are still largely unclear [4, 5]. Hepatic encephalopathy is classified according to the data of the working group of the 11th World Congress of Gastroenterology, Vienna, in three types, based on liver disease; one of the groups is viral hepatitis.

One of the perspective directions that allow characterizing the pathophysiological mechanisms of the development of encephalopathy in viral hepatitis in the body is the study of the peculiarities of metabolic processes in cells. This is due to the fact that all changes in the cellular genetic program are realized, including through metabolic processes. In this case, of great interest is the study of metabolism not only hepatocytes, but also peripheral blood. This is due to the fact that dysmetabolic processes in blood cells cause development of their dysfunction [3 - 5].

It is known that one of the most powerful links in the development of hepatic encephalopathy with the further development of the hepatic coma is cirrhosis. Thus, we can assume that dysmetabolic processes in erythrocytes and their dysfunction can mediate microcirculation disorders, thereby contributing to the progression of cirrhosis and as a consequence of the development of hepatic encephalopathy [6 - 8].

In the works of some authors it was found that dysmetabolic processes in lymphocytes directly correlate with the development of their dysfunction, in particular, with the decrease of the blast-transformation index, and the reactions of the sockets [9-11].

Enzymes selected for this study catalyzed various ways of using glycolysis - both energy-forming and plastic (consumption of glycerol-3-phosphate for the synthesis of phospholipids that build cell membranes). For energy exchange of erythrocyte glycolysis has a central significance. It is known that the imbalance of the adenylate pool, the deficiency of ATP, leads to an increase in the aggregation capacity of erythrocytes, damage to plastic processes, reducing their deformation, and increasing the rigidity of membranes. In lymphocytes, T-lymphocyte function, such as proliferation, cytotoxicity [13-15], is disturbed.

One of the diagnostic controversies is the enzyme neuron-specific enolase (NSE). From the literature [16] it is known that NSE is one of the enzymes of glycolysis (2-phospho-D-glycerate-hydrolyzase), which exists in the form of several dimerized isoenzymes (aa, ab, ay, bb and yy), formed from three subunits - a, b and y. A great experimental and clinical material has been accumulated concerning the analysis of NSE in biological fluids at various pathological conditions. The neuron-specific enolase is the only commonly known marker for all differentiated neurons and refers to intracellular enzymes of the central nervous system [13-16].

However, today there is no comprehensive study of this enzyme in patients with chronic viral hepatitis C as a marker of liver encephalopathy.

The *aim* of the study is to determine the predictive and prognostic biomarkers of the imbalance between the energy and plastic potentials in the blood cells of patients with chronic viral hepatitis C.

Material and Methods

The 73 HCV+ patients and 20 healthy controls were enrolled in the present cross-sectional study. The patients were selected on the basis of their stable clinical condition over the past 3 months. HCV infection was diagnosed by the positivity of anti-HCV and HCV-RNA for at least 6 months of period.

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The study protocol was carried out in accordance with the Helsinki Declaration as revised in 1989. All subjects were informed about the study and the written consent was obtained from each one.

We investigated the activity of enzymes utilizing phosphotriose (glycerol-3-phosphate dehydrogenase (G3D) and glyceraldehyde-3-phosphate dehydrogenase (GAFD)). The activity of GAFD and G3D was determined by the rate of recovery of NAD+, which participates in the corresponding reactions as an acceptor of hydrogen atoms. The activity of these enzymes was expressed in nmol / min • l. Concentration of the protein was determined by Lowry's method.

The determination of the content of neuron-specific enolase in serum was carried out using the Human Neuron Specific Enolase ELISA Kit (Alpha Diagnostic, USA) according to the manufacturer's instructions.

The concentration of ATP in the erythrocyte suspension was determined by the PN method. Yaverbaum et al., Concentrations of ADP and AMF by H.U. Bergmeyer [7-10].

An independent (unpaired) Student's t-test (two-tailed) was chosen to test the significance of differences among means of small “n” sample sets.

Results and discussion

On the contrary, the activity of glyceraldehyde-3-phosphate dehydrogenase, which characterizes the intensity of glycolytic glucose splitting, was significantly increased (p≤0,01). This is due to the predominant consumption of glycolytic phosphometabolites in the process of energy production that is glycolytic synthesis of ATP. In addition, this may indicate an increase in the needs of the cell in 2,3-bisphosphoglycerate, which provides the process of transferring oxygen to the tissue.

The activity of glyceraldehyde-3-phosphate dehydrogenase in lymphocytes is higher compared with the control group (normal activity of glyceraldehyde-3-phosphate dehydrogenase was 0.69±0.02 nmol / min • l), however, these changes are not so pronounced as in erythrocytes.

Table 1

The activity of glycerol-3-phosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase in blood red blood cells in patients with chronic viral hepatitis C.

Index	HCV-infection Genotype 1b (n=49)	HCV-infection Genotype 2 (n=11)	HCV-infection Genotype 3a (n=13)	Control group (n=20)
glyceraldehyde-3-phosphate dehydrogenase v nmol / min • l	6,83±0,05*	5,32±0,45*	5,7±0,15*	12,25±1,13
activity of GAFD nmol / min • l	5,75±0,08*	8,95±0,6*	7,29±0,26*	5,16±0,27

*Note: * p <0.001 in comparison with the control group*

It was established that in erythrocytes of patients with chronic viral hepatitis C, the activity of GAFD in 2.3 level, compared with norm, was significant (p≤0.01). When comparing patients with different genotypes, there is no difference between the groups, but we have established a significant difference between patients with and without liver encephalopathy. Thus, during the study, it was found that in patients with chronic viral hepatitis C, increased use of phosphotriose in glycolysis.

There is an in the control group, the neuron-specific enolase values were 7.89±0.81 ng / ml while in patients with chronic viral hepatitis C it was 12.97±0.85 ng / ml, which is almost 65.3%. Relatively (p≤0.05) increased neuron-specific enolase content in more than 83% of patients was recorded in patients with liver encephalopathy, which may indicate a brain neuronal damage in patients with viral glandular encephalopathy.

In the analysis, it was found that in patients with manifestations of hepatic encephalopathy, a statistically significant decrease (p<0.001) in erythrocytes of ATP content was observed on average by 53.5% compared with control. Approximately, the same dynamics was observed in patients with the genotype 1b, namely a decrease of 39% when compared with control values. Groups of patients with genotypes 2 and 3a in the absence of manifestations of hepatic encephalopathy did not have a significant difference between them.

Table 2.

The activity of glycerol-3-phosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase in blood lymphocytes in patients with chronic viral hepatitis C.

Index	HCV-infection and hepatic encephalopathy (n=11)	HCV-infection and did not hepatic encephalopathy (n=21)	Control group (n=20)
glyceraldehyde-3-phosphate dehydrogenase in lymphocytes nmol / min • l	1,10±0,03*	1,16±0,05*	1,55±0,15
activity of GAFD in lymphocytes nmol / min • l	2,95±0,03*	3,29±0,02*	0,69±0,02

*Note: * p <0.001 in comparison with the control group*

The content of ATP in the group of patients with and without liver encephalopathy also significantly differed. It should be noted that in this context, the maintenance of ADP in patients with liver failure phenomena tended to decrease, and in patients with chronic viral hepatitis C in its absence, on the contrary, a statistically significant increase (p <0,001) was determined by a 22% increase compared with control . The level of AMP in the presence of hepatic encephalopathy compared with the control group, significantly decreased by 90%. In the absence of hepatic insufficiency there was a significant (p≤0.05) reduction by AMP - almost 2.5 times compared to control.

Table 3
The content of adenine nucleotides in the patients under examination, depending on the presence of hepatic encephalopathy (Me [25%; 75%])

Index / Group of patients	ATP mmol/l	ADP, mmol/l	AMP, mmol/l
HCV-infection and hepatic encephalopathy (n=11)	1,38 * [1,27; 1,50]	1,36*^ [1,24; 1,51]	1,64*^ [1,53; 1,70]
HCV-infection and did not hepatic encephalopathy (n=21)	1,42±0,13*	0,81 * [0,70;08]	1,10 * [1,03; 1,14]
Control group (n=20)	2,25 [2,10; 2,2]	1,01 [0,93; 1,08]	0,58 [0,52; 0,69]

*Note: * p <0.001 in comparison with the control group*

It is assumed that the disruption of bioenergetic processes is one of the triggers of the development of hepatic encephalopathy of viral etiology [13]. As a result of mitochondrial dysfunction, the lack of macroergues causes the deterioration of the work of energy-dependent membrane channels, in particular Na + -K + -ATP exchangers, Ca2 + -ATP exchangers, which leads to the deposit of Ca2 + ions in the cytosol, followed by activation of Ca2 + dependent enzyme effector stage apoptosis. The progressive energy deficit switches the energy metabolism to glycolysis, resulting in the accumulation of low oxidized fatty acid products - acyl CoA, acetyl CoA, NADH • N with inhibition of pyruvate dehydrogenase, decreased utilization of pyruvate and its transformation into lactate.

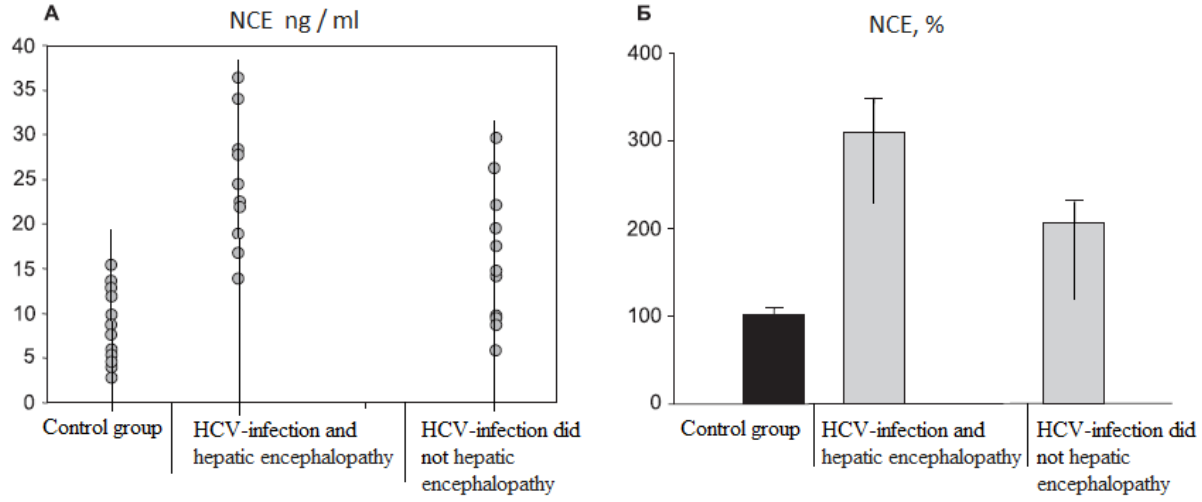

Figure 1. Levels of neuronspecific enolase in patients with chronic viral hepatitis C, depending on the presence of hepatic encephalopathy.

In the control group, the NCE was 7.89 ± 0.81 ng / ml, while in patients with chronic viral hepatitis C it was 12.97 ± 0.85 ng / ml, which is almost 65.3%. Relatively (p≤0.05) increased NCE content in more than 83% of patients was recorded in patients with liver encephalopathy, which may indicate a brain neuronal damage in patients with viral glandular encephalopathy.

Conclusions: The establishment of a change in the metabolism of phosphotriose in blood cells suggests the predominant use of phosphotriose and neuron-specific enolase for energy metabolism and ATP production, that is, it is possible to assume a possible imbalance between the energy and plastic potentials in blood cells of patients with chronic viral hepatitis C, especially in erythrocytes.

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