ENGLISH VERSION: DYNAMICS OF INTRACELLULAR HOMEOSTASIS PARAMETERS IN PATIENTS WITH CHRONIC VIRUS HEPATITIS C*

Gramatyuk S.M.

Kharkiv Medical Academy of Postgraduate Education

In modern conditions, the attention of clinicians is attracted by the problems of disturbances in the system of homeostasis in patients with chronic viral hepatitis C, which is explained by the progress of knowledge in this area and new opportunities for detection and treatment of various types of this pathology. The aim of the research was to study the dynamics of intracellular homeostasis parameters in patients with chronic viral hepatitis C. Results of the study. The results of the conducted studies revealed profound disturbances in the metabolism of metal ions in patients with chronic viral hepatitis C. In serum, there was a significant ($p \leq 0.05$) increase in iron, copper, zinc, potassium, sodium, calcium and a decrease in magnesium ions; in erythrocytes – there was an increase in calcium, magnesium, sodium, copper and a decrease in potassium, zinc and iron. The established disturbances in patients with chronic hepatitis C indicate the polysystem structural and metabolic changes, intracellular homeostasis disorders and, as a consequence, the development of membrane pathology in chronic viral hepatitis C.

Keywords: homeostasis, chronic viral hepatitis C, metal ions

Viral hepatitis C (VHC) is one of the main causes of liver diseases worldwide and in Ukraine, including cirrhosis and hepatocellular carcinoma, for treatment of which organ transplantation is required [1].

Hepatitis C virus (HCV) was discovered in 1989. Since that time, it constantly encourages scientists for further research in this area. The widespread occurrence of chronic viral hepatitis C (CVHC) as one of the results of infection with HCV and the peculiarities of the virus itself give rise to a large number of medical and general clinical problems: the misdiagnosis of hepatitis C; lack of vaccination; treatment of patients with this pathology [2, 3]. Viral hepatitis C is a RNA-containing virus from the Flaviviridae family, it has 6 genotypes and over 50 subtypes. Nucleotide sequences of genotypes vary in the range of 30-50%. HCV has pronounced mutational properties. The absence of an active T cell immune response leads to a high frequency of chronic infection formation. Genetic heterogeneity of HCV creates diagnostic and clinical difficulties, complicates the development of vaccines and effective therapeutic drugs. Genotype 1, which in most cases is the cause of hepatitis C, both in Europe and in the United States, is characterized by a very low response to treatment [1-3].

In modern conditions, the attention of clinicians is attracted by issues of disorders in the homeostasis system in patients with chronic viral hepatitis C, due to some progress in knowledge in this area and new possibilities for detection and treatment of various types of this pathology in humans. According to O.V. Ataman, all indicators of intracellular homeostasis can be divided into three groups: 1. Indicators supported by the body systems (temperature, oxygen pressure and osmotic pressure) 2. Indicators supported by the system of the body and, partially, the cell itself (pH, glucose concentration and other energy substrates) 3. Indicators supported by the cell itself (concentration of ions Na, K, Ca content of macroergic compounds, metabolites) [12].

In modern clinical practice, the metabolic criteria for evaluation of endogenous intoxication in patients with chronic hepatitis C are insufficiently grounded, which involves dynamic changes and optimization of pathogenetic therapy. Thus, we have set an objective to expand understanding of the endogenous pathogenetic mechanisms of endogenous intoxication, to improve diagnostics and to predict the future state of patients with chronic hepatitis C disease. The purpose of the research is to study the dynamics of intracellular homeostasis in patients with chronic viral hepatitis C.

Materials and methods

A total of 73 patients with chronic viral hepatitis C were screened at the Mediciko Diagnostic Center ASK-Heles in Kharkiv. All patients were vaccinated with the virus genome, the presence of 3 genotypes of HCV infection was detected: 3a genotype was diagnosed in 13 patients, 2 genotypes – 11, the largest group was patients with 1b genotype of the virus – 49. The control group included 20 practically healthy persons who did not have the liver disease in anamnesis. The disease was established based on the detection of RNA HCV serum replicative activity in a qualitative and quantitative PCR method using a set of US ELISA kit test systems, according to the manufacturer's instructions on the BIO-rad analyzer.

The study included the determination of the content of Cu, Fe, Zn, and macrocells K, Na, Ca, Mg in blood serum and red blood cells, as well as the determination of the acute phase proteins of haptoglobin and ceruloplasmin.

The determination of micro- and macro elements was carried out on the biochemical analyzer A15, Spain, using the manufacturer's instructions.

Ceruloplasmin was determined via the generally accepted method by H.A. Ravin, the essence of which is based on the oxidation of p-phenylenediamine ceruloplasmin. An enzymatic reaction was stopped by the addition of sodium fluoride. The level of ceruloplasmin was determined based on the optical density of the reaction products. The content of haptoglobin in serum was investigated according to the method proposed by O.G. Archipelago, the essence of which is that haptoglobin forms a hemoglobin complex, which precipitates with the addition of rivanol. By the level of hemoglobin remaining in the solution, the content of haptoglobin in the serum was determined photometrically.

All studies were conducted in accordance with all norms of ethics and standard operating procedures approved at the Medical Diagnostic Center ASK-Hells. Statistical data processing was performed using the application package for Statistics for Windows 8.0. The following methods were used – descriptive statistics (the definition of numerical characteristics of variables – arithmetic

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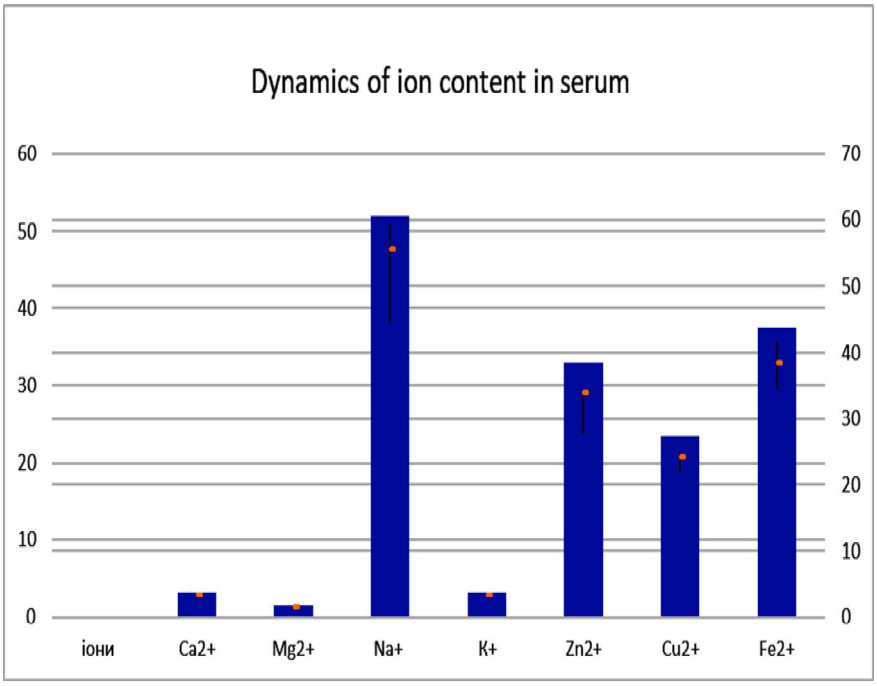
mean M, average sample error, determination of the reliability of differences p), checked by the criterion of Student-Fisher in representative samples, the method of correlation structures.

Results and discussion

The study of the dynamics of cell homeostasis in patients with chronic viral hepatitis C revealed changes in the exchange of Ca2 + ions, a significant increase in their serum concentration of 27%. In erythrocytes, a slight increase in Ca 2+ ions was noted (0.67 ± 0.11 mmol / L) as compared with the control group. Such dynamics of calcium ions in the investigated biological objects, blood serum and erythrocytes, can reflect the disruption of structural and metabolic processes, which are mainly associated with the mobilization, redistribution and withdrawal of this element from the body. The content of magnesium ions had the opposite orientation of the dynamics of exchange of calcium ions. Thus, the concentration of ions Mg2 + in serum decreased by 48.1% while in erythrocytes magnesium increased by 24.5%, respectively, which had a significant difference between the groups. Given the important cofactorial function of magnesium ions and their involvement in providing enzymatic reactions of anaerobic oxidation of glucose, it should be noted that their increase in erythrocytes may be due to an increase in the activity of an anaerobic pathway of energy in the form of ATP in chronic viral hepatitis C (Fig. 1).

a)



b)

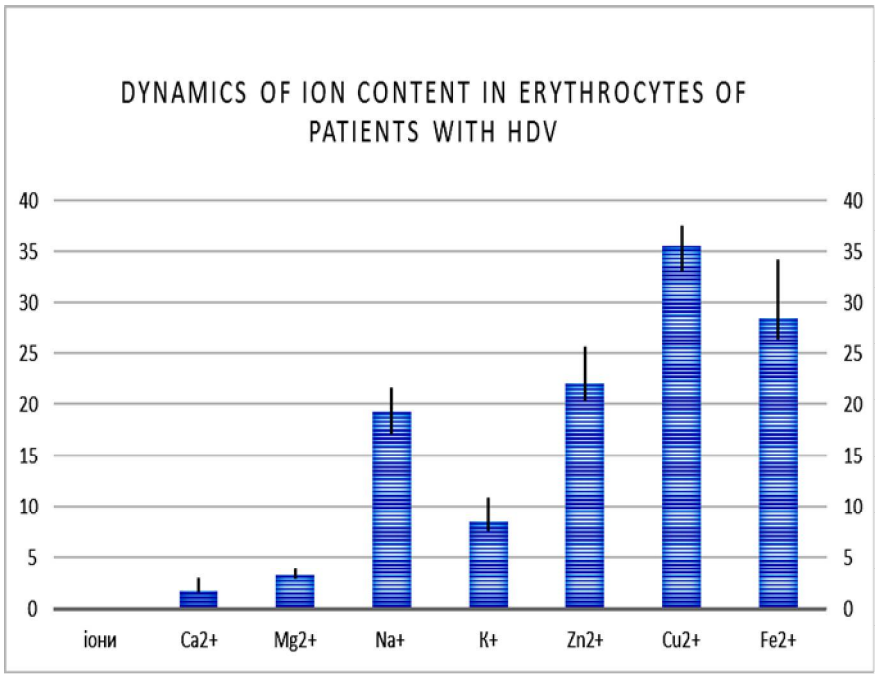


Figure 1. Dynamics of ion content in serum (a) and red blood cells (b) in patients with chronic viral hepatitis C.

Consequently, it can be assumed that in patients with chronic viral hepatitis C against the background of increase in the concentration of ATP in the cell, the work of potassium-sodium pump changes due to intracellular accumulation of magnesium, causing conformational changes in Na + – K + -ATPase. As a result of the study, the dynamics of calcium and magnesium ions, significant changes were detected that could be due to numerous structural and metabolic disorders and, in particular, membrane supramolecular cell structures, leading to intracellular homeostasis in patients with chronic viral hepatitis C.

Na + ions were elevated in blood serum and erythrocytes by 14.5% and 9.5%, as compared with the control group. The dynamics of exchange of potassium ions was characterized by an increase in blood serum of 25.3%, against a background of red blood cell counts of 20.7%. Increase in serum K + by 73.3% indicates a disruption of the stability of erythrocytes, which ensures the entry into the blood of K + and its withdrawal from the body of patients with chronic hepatitis C. Conjugation of Na + and K+ ions in erythrocytes was accompanied by an increase in the concentration of Na + and decrease K +. Such dynamics of these ions inevitably leads to depolarization of erythrocytic membranes, delayed Na+ ions and loss of K+, which characterizes the disruption of their physical, chemical and functional properties. These, the facts indicate a disruption of the transport function of plasma membranes in patients with chronic hepatitis C.

The study of the exchange of Zn2 + revealed a significant increase (p. 0.005) in its blood serum, 34.1%, and was (29.5 ± 0.43 mmol / l) in comparison with the control group. In red blood cells, on the contrary, a significant decrease in the content of zinc ions (16.8 ± 0.51 mmol / l) was observed. Studies show that patients with chronic viral hepatitis C have a significant loss of zinc ions. Increased level of zinc in the blood serum may be due to an increase in her globulins with which he forms strong complexes. A significant role in the development of the manifestation of zinc deficiency can belong to structural and metabolic disorders of biological membranes: plasma, mitochondrial, ribosomes, lysosomes, etc.

The study of the exchange of copper ions in patients with chronic viral hepatitis C showed an increase in their serum and red blood cells, respectively, by 32.1%, 41.6%, as compared with the control group. Copper is actively involved in the processes of hematopoiesis, immune and oxidative-reducing reactions. In serum, it is linked to α2-globulins and is part of the serum oxidase fraction – ceruloplasmin. An increase in the analyzed objects of copper ions, may be associated with the activation of hematopoiesis, the immune system and oxidative reactions, which should be considered as the phase of adaptation of the organism in the development of chronic hepatitis viral etiology.

The study of the dynamics of iron in organs and tissues revealed an increase in its concentration in serum on 119.4%. In erythrocytes there was a decrease in iron by 11.5%. Given the involvement of iron in oxygen transport and processes of cellular respiration, as well as the entry of hemoglobin, transferrin, ferritin and the analysis of the dynamics of iron ions in biological objects, one can judge the disruption of the stability of erythrocytic membranes, which is connected with the release of Fe2+ ions in the blood.

Many authors are convinced that an important role in providing the membranes with dynamic stability is given to the integration systems for controlling the homeostatic function of the organism (nervous, endocrine, immune, enzymatic), which is observed in patients with CSF [11 – 15, 17].

The main reason for the disruption of homeostasis may be the structural and metabolic lesion of membranes and, as a consequence, a change in the material, energy and information balance of cells, which underlie the formation of diseases and the development of endogenous intoxication [12].

Studying the condition of the systemic and antisystem interaction of the hepatitis C virus, which has membranotropic action, the disruption of the activity of enzymes of antiradical protection was detected against the background of reduction of transferrin (Table 1).

When studying the contents of ceruloplasmin, it is determined that they exceed the control values by 2.5 times in all groups of patients. However, there is a low level of transferrin and haptoglobin at the height of intoxication with the normalization of manifestation symptoms in the remission period. The low value of transferrin at the height of intoxication increases to the remission period, but it is significantly different from the control values (Table 1).

Table 1

Dynamics of the content of acute phase proteins – haptoglobin, transferrin and ceruloplasmin in patients with viral hepatitis C.

Groups of patients with hepatitis C	Ceruloplasmin mmol / l	Haptoglobin g / l	Transferrin mg / l
Chronic viral hepatitis C (n =73)	3.112±0.44	*0.211±0.026↓	*2.11±0.26 ↓
Control group (n =20)	2.335±0.65	0.335±0.065	3.5±0.5

*Note: * – a significant difference from the norm*

Practically no changes were found in the dynamics of the studied parameters, depending on the form of the disease. Analysis of the content of acute phase proteins – haptoglobin, transferrin and ceruloplasmin, revealed significant differences, depending on the genotype of the virus. So the biggest changes were observed, in patients with genotype 1b, haptoglobin in serum decreased by 26%, in patients with genotype 3a by 14%, from the values of the control group. The decrease in serum of hapthoglobin in patients with chronic viral hepatitis C can be considered as the strain of adaptive mechanisms for providing homeostasis of the body. Ceruloplasmin increased by 23.6%; 48% and 19.6%, respectively, in patients with genotype 3a, 1b and 2. Decreased levels of haptoglobin in patients with chronic hepatitis C, may indicate membrane pathology.

Thus, the results of the conducted research revealed deep disturbances in the metabolism of metal ions in patients with HBGS. In serum, there was an increase in iron, copper, zinc, potassium, sodium, calcium and magnesium ions; in erythrocytes – there was an increase in calcium, magnesium, sodium, copper, and a decrease in

potassium, zinc and iron, indicating polysystem structural and metabolic changes and intracellular homeostasis, as a consequence of development in chronic viral hepatitis C membrane pathology.

Prospects for further research consist in determining the laws of energy supply disturbance as a mechanism of damage to cells in patients with chronic viral hepatitis C.

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