Correlation of metal ions and liver enzymes in blood plasma

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Determination of different metals (K, Na, Mg, Ca, Sr, Cr, Mn, Fe, Cu, Zn, Cd, Ni, Co and Pb) by flame atomic absorption spectrometry (FAAS) and of liver enzymes (ALT, AST, ALP and GGT) by catalytic reactions in human blood plasma from non-infected patients, those infected with hepatitis C virus and from patients with viral C cirrhosis has been performed. The results demonstrated that the analytical procedures could be successfully applied for a rapid and accurate determination of metals and enzymes in different blood samples. A negligible correlation was found among the concentrations of macro- and microelements and liver enzymes in blood plasma samples for different patients. In the later stages of viral liver disease, the concentration of enzymes becomes lower. The aim of the present study was to investigate, to our knowledge for the first time, the correlation between the levels of different metals and the concentration of liver enzymes (ALT, AST, ALP and GGT) in blood plasma samples from patients with hepatitis C virus and patients with viral C cirrhosis.

Key words: metals, enzymes, blood plasma, determination, hepatitis C, cirrhosis

INTRODUCTION

The physiological importance of micro- and macroelements in the human organism, especially in blood, has been shown by many publications. For example, different metals present in the blood can form different complexes with amino acids, fatty acids, albumin, glucose, fibrinogen, salicylate, cholesterol and many other organic compounds and biomolecules found to be the main constituents of blood plasma [1–3]. Depending on the concentration of metals in the body parts, different metal–ligand equilibria could be established in the system. Consequently, these changes could invoke changes in the global bioprocesses and different clinical symptoms and metabolic stress in the human organism. Also, the variation of microelements in body fluids, such as blood, plasma and saliva, could provide information about the genetic health possibilities [4]. At low concentrations, microelements play an important role in the metabolism and biological processes as enzyme activators, stabilizers of the functional component of proteins, etc. Above trace levels, however, these elements play other roles. It has been stated that for all trace elements considered to be essential, there exists a fairly narrow “concentration window” between the essential and the toxic levels. The toxic doses of microelements and their compounds can lead to serious health problems [5–7].

Recently, we have shown that different metals in the blood and liver samples from patients infected with hepatitis C virus could be successfully determined by flame atomic absorption
The determination of metals was performed directly without any preconcentration. From the results presented in [8], it was concluded that the concentrations of transition metals (Cr, Mn, Cu and Zn) are higher in blood samples (plasma and cells) of persons infected with hepatitis C virus in comparison with non-infected persons. The distribution of metals between blood plasma and blood cells could present a very important clinical information as well [9, 10]. The distribution of Na, K, Mg, Ca and Sr in blood samples from the patients with hepatitis C was also investigated [11, 12]. Interestingly, strontium was detected neither in the blood plasma nor blood cells of the samples. Also, magnesium and calcium were differently distributed between plasma and cells of different patients. Magnesium levels in the blood plasma infected with hepatitis C virus increased with the increasing duration of the disease. However, the calcium levels were independent of the duration of infection. Besides, no tendency in magnesium distribution in different components of blood (plasma and cells) between female and male patients was detected [11, 12]. The preliminary observations showed that Cu, Cr and Mn concentration in blood cells and in plasma could be a signal of the seriousness and a later stage of the disease [13]. Fe, Zn and Pb concentrations, however, did not vary significantly in the blood cells of infected and non-infected persons. The importance of calcium levels in the identification of hypothermia as the cause of death was also investigated [14].

There is an increased interest in the role of liver enzymes (which are proteins, organic catalysts) such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT), in clinical or forensic medicine, nutrition and physiology [15–23]. For example, an elevated concentration of AST and ALT could be a biochemical marker, even of lymphoma [17]. Recently, it has been determined that older systemic sclerosis patients had a significantly higher concentration of ALP [18]. A significant liver injury is indicated by an elevated level of ALT and a reduced level of AST [19]. The same conclusion was made by other authors [20] who state that AST and ALP levels decreased significantly in patients with primary cirrhosis. It has been demonstrated [22, 23], that GGT regulates also a wide variety of cellular functions.

The aim of the present study was to investigate the distribution of different metals (K, Na, Mg, Ca, Sr, Cr, Mn, Fe, Cu, Zn, Cd, Ni, Co and Pb) and liver enzymes (ALT, AST, ALP and GGT) in blood plasma samples from patients infected with viral hepatitis C and from patients with C cirrhosis, and to compare the obtained results with data from non-infected samples.

EXPERIMENTAL

The level of metals in the blood samples was determined by the flame atomic absorption spectroscopic method (FAAS, Hitachi 170–50). The instrumental parameters were adjusted according to the manufacturer's recommendations. The following conditions of metal determination by the flame AAS method were used: (i) absorption line (766.5 nm (K), 589.0 nm (Na), 422.7 nm (Ca), 285.2 nm (Mg), 406.7 nm (Sr), 357.9 nm (Cr), 279.5 nm (Mn), 248.3 nm (Fe), 240.7 nm (Co), 232.0 nm (Ni), 324.8 nm (Cu), 213.8 nm (Zn), 228.8 nm (Cd), and 288.3 nm (Pb)); (ii) electric current of the lamp (15 mA (K, Ca, Cr, Co), and 10 mA (Na, Mg, Sr, Mn, Fe, Ni, Cu, Zn, Cd, Pb)); (iii) flame (propane-butane (K, Na, Mn, Fe, Co, Cu, Cd, Pb), and acetylene (Ca, Mg, Sr, Cr, Ni, Zn)); (iv) gas pressure (9.81 · 10⁻⁷ Pa (K, Fe, Co, Cu, Cd), 1.47 · 10⁻⁶ Pa (Na), 2.94 · 10⁻⁶ Pa (Ca, Sr, Cr, Mn), 2.45 · 10⁻⁶ Pa (Mg, Zn), 3.43 · 10⁻⁶ Pa (Ni), and 7.35 · 10⁻⁶ Pa (Pb)). Air pressure (1.47 · 10⁻⁷ Pa) was the same during FAAS determination of all elements.

Double-distilled water and analytical-grade reagents were used for the preparation of stock standard solutions of metals, which were used to obtain calibration solutions by dilution. The calibration graphs for metals were found to be linear within the concentration ranges from 0.05 to 0.60 µg mL⁻¹ for K, from 0.02 to 0.30 µg mL⁻¹ for Na, from 0.5 to 8.0 µg mL⁻¹ for Ca, from 0.05 to 0.4 µg mL⁻¹ for Mg, from 0.25 to 2.4 µg mL⁻¹ for Sr, from 0.25 to 6.0 µg mL⁻¹ for Cr, from 0.1 to 4.0 µg mL⁻¹ for Mn, from 0.1 to 4.0 µg mL⁻¹ for Fe, from 0.2 to 2.0 µg mL⁻¹ for Co, from 0.25 to 4.0 µg mL⁻¹ for Ni, from 0.05 to 3.0 µg mL⁻¹ for Cu, from 0.05 to 0.7 µg mL⁻¹ for Zn, from 0.025 to 0.4 µg mL⁻¹ for Cd, and from 0.1 to 4.0 µg mL⁻¹ for Pb, with the detection limits of 0.02 µg mL⁻¹ for K, 0.01 µg mL⁻¹ for Na, 0.2 µg mL⁻¹ for Ca, 0.02 µg mL⁻¹ for Mg, 0.2 µg mL⁻¹ for Sr, 0.1 µg mL⁻¹ for Cr, 0.05 µg mL⁻¹ for Mn, 0.05 µg mL⁻¹ for Fe, 0.06 µg mL⁻¹ for Co, 0.1 µg mL⁻¹ for Ni, 0.02 µg mL⁻¹ for Cu, 0.02 µg mL⁻¹ for Zn, 0.01 µg mL⁻¹ for Cd, and 0.04 µg mL⁻¹ for Pb.

Blood samples were taken from 20 volunteer non-infected persons, from patients infected with viral hepatitis C, and from patients with viral C cirrhosis. The determination of metals in blood specimens was performed directly without any preconcentration. For the separation of blood cells from blood plasma prior FAAS determination, the centrifugation method (spin speed 8000 min⁻¹) had been applied [11, 13]. For the determination of metals in blood plasma samples, the specimens to be analysed had been burnt in an ordinary furnace at 600 °C. The obtained residuals were dissolved in 10 mL of nitric acid (1 : 1), transferred into a 25-mL volumetric flask and diluted with double-distilled water.

Liver enzymes (ALT, AST, ALP and GGT) were determined in blood plasma after the blood had been taken from patients by standard venopuncture and centrifuged. The tests were made within three hours after the blood samples had been collected. The quantitative analysis of these four enzymes was made by using the CE ABBOTT Laboratories Aeroset and Architect c8000 systems with an automatic dilution protocol. Specific characteristics for each enzyme were presented: linear interval, limit of detection (LOD) and limit of quantity (LOQ).

Alanine aminotransferase (ALT), which is also called glutamat-piruvat transaminase (GPT), is an enzyme in-
volved in the metabolism of amino acids. It is found in many tissues, but its levels are highest in liver and kidneys. In a tissue injured ALT gets from the intracellular space into the blood flow. Particularly high ALT concentrations in the blood serum may be found in the presence of liver diseases (hepatitis, cirrhosis). Therefore, ALT is considered to be a very specific marker of liver diseases. The principle of the ALT determination test: ALT is a catalyst of transporting the amino group from L-alanine to α-ketoglutarate, so pyruvate and L-glutamate are formed. Pyruvate, with the participation of NADH and lactate dehydrogenase, is reduced to L-lactate. By this reaction, NADH is oxygenated to NAD. The reduction of the absorption is measured in the 340 nm wavelength. The kit for ALT detection contains two liquid reagents ready for use: R1 (β-NADH, concentration 0.16 mg/ml; lactate dehydrogenase, concentration 2.57 V/ml; L-alanine, concentration 392 mmol/l and R2 (α-ketoglutarate, concentration 77 mmol/l; L-alanine, concentration 1000 mmol/l). The ALT linear interval is up to 942 U/L. If the ALT value in a specimen is more than 942 U/L, a 1 : 5 dilution is made by the automatic protocol, and then the concentration is counted from the result multiplied by the dilution factor. The limit of ALT detection (LOD) is 1.3 U/l and the limit of quantity (LOQ) 5.1 U/l. The normal ALT value interval for adults is 0–35 U/L.

Aspartate aminotransferase (AST), also called glutamate oxaloacetate transaminase, is a group of enzymes which catalyze interconversion of amino acids and α-keto acids in the transportation of amino groups. The biggest concentration of AST is found in liver, heart, muscles and kidney tissues. In case of a lesion of these tissues, the content of AST in blood serum increases significantly. The test AST catalyses the amino group transport from L-aspartate to α-ketoglutarate and with the formation of oxaloacetate and L-glutamate. Oxaloacetate, with the participation of NADH and malate dehydrogenase (MDH), is reduced to L-malate. During this reaction, NADH is oxygenated to NAD. The reduction of absorption, caused by NADH oxygenation to NAD, is measured in the 340 nm wavelength. The set for AST detection contains two liquid reagents ready for use: R1 (β-NADH, concentration 0.16 mg/ml; malate dehydrogenase, concentration 0.64 V/ml; L-aspartate, concentration 232 mmol/l) and R2 (α-ketoglutarate, concentration 51.3 mmol/l; L-aspartate, concentration 100 mmol/l). The AST linear interval is up to 913 U/L. The LOD of AST is 0.9 U/l and the LOQ 2.2 U/l. The interval of the normal AST value for adults is 5–35 U/L.

Alkaline phosphatase (ALP) is a group of at least five isoenzymes specific to human tissues. This enzyme is mostly produced in liver, bones and guts. In case of a disease of these organs, the secretion of ALP to the blood increases. The ALP hydrolyses phosphate monoesters in an alkaline medium. The test ALP catalyses the hydrolysis of colourless p-nitrophenyl phosphate and composes p-nitrophenol and inorganic phosphate. In an alkaline medium of the test, p-nitrophenol is present in the form of yellow phenoxye. The increase of absorption in the 404 nm wavelength is directly proportional to ALP activity in the test. Magnesium and zinc ions are used to activate the test ALP. The set for ALP detection contains two liquid reagents ready for use: R1 (2-amino-2-methylpropanol, >1.2 mmol/l; magnesium, >7.2 mmol/l; zinc sulphate, >3.6 mmol/l; HADTA, >7.2 mmol/l) and R2 (4-nitrophenyl phosphate >171.6 mmol/l). The ALP linear interval is up to 2200 U/L. The LOD of ALP is 1.6 U/l and LOQ 5.0 U/l. The interval of the normal ALP value for adults is 40–120 U/L.

Gamma-glutamyl transferase (GGT) in the biggest concentration is found in kidneys. This enzyme gets into the blood serum first of all from the hepatobiliary system. GGT increases in the presence of different liver diseases, especially in case of biliary obstruction. In virus hepatitis, GGT increases 2–5 times on the average. The GGT catalyses the transport of gammaglutamyl group from the donor substrate (3-carboxy-4-nitroanilide) to the glycylglycine acceptor, and 3-carboxy-4-nitroaniline is produced. The increase of absorption is measured at a 412 nm wavelength. It is directly proportional to the test GGT activity. The set for GGT detection contains two liquid reagents ready for use: R1 (Glycylglycine, concentration 191 mmol/l) and R2 (ammonium saline of L-gama-glutamil-3-carboxy-4-nitroanilid, concentration 30.6 mmol/l; sodium azide, 0.1%). The GGT linear interval is up to 1543 U/L. The LOD of GGT is 1.0 U/l and LOQ 3.3 U/l. The interval of the normal ALP value for adults is 9–40 U/L.

RESULTS AND DISCUSSION

Blood samples were taken from 20 volunteer healthy people, from patients infected with hepatitis C, and from patients with viral C cirrhosis. In order to avoid psychological stress or an undesired controversial reaction people could have after reading this article, we do not elaborate here on the severity and peculiarities of infection caused by these diseases or how critical is the condition of each patient. The level of ten metals (K, Na, Mg, Ca, Cr, Mn, Fe, Cu, Zn and Pb) in the blood plasma specimens was found to be higher than the detection limits. Therefore, the determination of these metals was performed directly without any preconcentration. Interestingly, according to FAAS analysis data, the elements such as Sr, Cd, Ni and C, were not found in the blood plasma of healthy people, or those infected with hepatitis C, or of patients with cirrhosis, or their concentrations were below the detection limit (0.2 µg/g for Sr, 0.01 µg mL⁻¹ for Cd, 0.1 µg mL⁻¹ for Ni, 0.06 µg mL⁻¹ for Co). The levels of selected elements in the blood plasma of different patients are presented in Table 1.

The R. S. D. values obtained for metals in the blood plasma of patients infected with hepatitis C and from patients with viral C cirrhosis (6.7–11.8%) indicate a high degree of homogeneity, which could be expected for blood samples. Moreover, the obtained values are not unusual for such type
of analysis and can be considered as suitable for a routine analysis.

It is evident that the concentration of sodium and potassium in blood plasma is much higher in comparison with other elements. The level of sodium and potassium did not vary much in the blood plasma of different patients, implying that the content of alkaline metals in the blood plasma seems to be independent of the level of infection. An opposite situation was observed for the distribution of alkaline earth metals in the blood plasma from different patients. The concentration of magnesium in cirrhosis-infected blood plasma (80.02 µg/g) was higher than in hepatitis-C-infected blood samples (70.21 µg/g). Interestingly, the concentration of calcium in the hepatitis-C-infected blood plasma (105.33 µg/g) was almost half as low as in the blood plasma obtained from patients with cirrhosis (60.81 µg/g). Such a different behaviour of Mg and Ca is very interesting and rather unexpected, since the complex formation ability of these two metals with the chelating ligands or proteins should be very similar. Therefore, the determined extra concentration of Mg²⁺ and the hyper-low concentration of Ca²⁺ in the blood plasma of patients with cirrhosis could be probably caused by the duration of illness. Thus, the initial observations show that the concentration of Mg and Ca in the blood plasma could be a signal of the seriousness and depth of the disease. On the other hand, Na and K concentrations do not vary significantly in the blood plasma from differently infected patients.

As mentioned above, Sr, Co, Ni, Cd, and additionally Cr, were not present in blood samples of with hepatitis C nor of patients with cirrhosis, or their concentrations were detection limit. Moreover, a minor content of Pb (0.18 µg/g) was detected only in the blood plasma from patients with cirrhosis. Of course, the concentration of iron is much higher in comparison with other elements. The levels of very important two biological elements, Cu and Zn, in the blood samples were found to be significantly higher than of Mn. In general, the content of Zn rather than Cu in the blood plasma decreases with increasing the level of illness. Initial observations show a tendency that Fe, Cu, Zn and Pb concentration in the blood plasma could be as signal of the seriousness and depth of the mentioned diseases.

Data on the levels of the same elements in the blood plasma of non-infected patients are presented in Table 2.

As one can seen from Table 1, the distribution of potassium and sodium levels in blood plasma depending on the duration of infection is chaotic. Thus, these two elements do not offer any important information about hepatitis C and cirrhosis. Data on the distribution of Mg and Ca in the blood plasma of patients with a different background demonstrate that magnesium concentration is slightly lower and calcium higher in the blood plasma of healthy people. These results also support a hypothesis that the level of Mg and Ca in the blood plasma of infected patients could be related to the progression of infection. Sr, Co, Ni, Cd, as well as Mn and Pb, were

<table>
<thead>
<tr>
<th>Metal</th>
<th>Patients with viral hepatitis C (20 samples)</th>
<th>Patients with C cirrhosis (20 samples)</th>
<th>Healthy people (20 samples)</th>
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<tr>
<td></td>
<td>Concentration of metal, µg/g</td>
<td>R. S. D., %</td>
<td>Concentration of metal, µg/g</td>
</tr>
<tr>
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<td>798.25</td>
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<tr>
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<td>1385.83</td>
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</tr>
<tr>
<td>Ca</td>
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</tr>
<tr>
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<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cr</td>
<td>25.60</td>
<td>9.2</td>
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<td>2.56</td>
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<tr>
<td>Pb</td>
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<td>0.18</td>
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*Average of five independent determinations.
absent in the blood plasma of non-infected blood specimens. So, the occurrence of Mn and Pb only in the blood plasma of ill people could be an indicator of hepatitis C or cirrhosis. On the other hand, Cr was found only in blood plasma of healthy people. Absolutely no tendency, however, in the distribution of Fe among different patients was detected. One can also see that Cu concentration in the blood plasma of the infected samples slightly prevailed over its content in non-infected blood plasma. On the contrary, the content of Zn in blood plasma decreased monotonically from 7.73 µg/g (healthy people) to 2.56 µg/g (cirrhosis patients).

The obtained results show a various distribution of different metals in the blood plasma of differently ill people. We can conclude that data on the distribution of magnesium and calcium, manganese and lead, chromium, copper and zinc in the blood plasma of healthy people and patients infected with hepatitis C and cirrhosis are promising for the further medical observations. The changed concentrations of these metals in blood plasma, however, might be a sign of or a possible reason for the appearance of symptoms of the above-mentioned diseases.

The distribution of liver enzymes, such as ALT, AST, ALP and GGT, in the blood samples from patients with viral hepatitis C and those ill with C cirrhosis was also investigated. We did not include the enzymes of healthy volunteers in our analysis because all of them had normal levels of ALT, AST, ALP and GGT, and we made a comparison only between the two groups of patients. The data were processed with the SPSS statistic package (version 17.0 for Windows). The description statistics for quantitative variables was presented as mean and standard deviation values; for qualitative variables, rates were presented. In the comparison of the groups for qualitative variables, the chi-square test and for quantitative variables the non-parametric Mann–Whitney and Kruskal–Wallis tests were used. The level of significance was fixed and considered to be equal of 0.05. Two-sided values of p are presented everywhere. The results are presented in Table 2.

We found all liver enzymes to exceed the normal values (normally, ALT and AST should be ≤ 35 U/L, ALP ≤ 120 U/L, GGT ≤ 40 U/L). There were no statistically significant differences between increased levels of liver enzymes in both groups of patients (the p value of ALT – 0.95, AST – 0.079, ALP – 0.159, GGT – 0.012), although the GGT values are notably higher in patients with hepatitis C (the average value 122.73 ± 139.22) as compared with cirrhosis patients (56.81 ± 36.67). We noted the ALT concentration to be much more elevated from the normal level (112.91 ± 85.03 and 111.12 ± 73.88 instead of 35 U/L) in both groups of patients and of AST in patients with hepatitis C (103.41 ± 66.59); this shows an active inflammatory process in the liver, induced by virus C.

The correlation between the average content of liver enzymes (ALT, AST, ALP, GGT) and the metal ions in blood plasma was analysed in patients with hepatitis C and in patients with virus C cirrhosis groups. We present the correlation index c in Tables 3 and 4. No correlation was found between the values of liver enzymes and the previously mentioned metals, except those listed in Tables 3 and 4.

An increased AST concentration was found in patients with a higher Mn level in blood plasma of patients with viral hepatitis C (c = 0.5; p < 0.05). In the group with C cirrhosis, elevation of ALP was noted in patients with higher Mn (c = 0.89; p < 0.05) and Cr (c = 0.63; p < 0.05) concentrations, and elevated AST was found in patients with a higher Zn concentration (c = 0.55; p < 0.05) in blood plasma. No visible correlation was found between GGT values and metal concentrations. These metals are absorbed through the small intestine and transported to the liver. They occur as a component of enzymes (example, alkaline phosphatase includes zinc) and take part in different metabolic reactions.

The levels of 14 metals in blood plasma samples from 20 volunteer healthy people, patients infected with hepatitis C and patients with cirrhosis were checked by flame atomic absorption spectrometry (FAAS). The determination of metals was performed directly without any preconcentration. Traces of Sr, Co, Ni, and Cd were found in none of the blood samples. The distribution of potassium, sodium and iron levels in blood plasma depending on the duration of infection was chaotic. However, data concerning Mg, Ca, Cr, Mn, Cu, Zn, and Pb show an interesting distribution of different metals in blood plasma of differently ill people. The change of these metal concentrations in blood plasma might be a sign or a possible reason for the appearance of hepatitis C and cirrhosis symptoms. The content of enzymes, such as ALT, AST, ALP and GGT, in the blood samples of patients with viral C hepatitis and C cirrhosis has been performed. ALT concentration significantly exceeded the normal level in both groups of patients, while AST was significantly higher in patients with hepatitis C. This shows a more intensive inflammatory activity in patients with an earlier (hepatitis) stage of the viral

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<tr>
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<td>Cr</td>
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CONCLUSIONS

The levels of 14 metals in blood plasma samples from 20 volunteer healthy people, patients infected with hepatitis C and patients with cirrhosis were checked by flame atomic absorption spectrometry (FAAS). The determination of metals was performed directly without any preconcentration. Traces of Sr, Co, Ni, and Cd were found in none of the blood samples. The distribution of potassium, sodium and iron levels in blood plasma depending on the duration of infection was chaotic. However, data concerning Mg, Ca, Cr, Mn, Cu, Zn, and Pb show an interesting distribution of different metals in blood plasma of differently ill people. The change of these metal concentrations in blood plasma might be a sign or a possible reason for the appearance of hepatitis C and cirrhosis symptoms. The content of enzymes, such as ALT, AST, ALP and GGT, in the blood samples of patients with viral C hepatitis and C cirrhosis has been performed. ALT concentration significantly exceeded the normal level in both groups of patients, while AST was significantly higher in patients with hepatitis C. This shows a more intensive inflammatory activity in patients with an earlier (hepatitis) stage of the viral
disease. The analysis of a correlation between the mean values of metal concentration and enzymes revealed that only several elements have shown such a correlation. Further investigations are needed to prove the impact of metals on the activity of liver enzymes and their relationship with the stage of the viral C liver disease.

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