ORIGINAL ARTICLE

ASSOCIATION BETWEEN SERUM ZINC, COPPER AND SELENIUM LEVELS AND THE DEGREE OF LIVER DAMAGE IN PATIENTS WITH CHRONIC HEPATITIS C

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ABSTRACT

The aim: To evaluate the content of trace elements Zn, Cu and Se in blood serum and their relationship with viral load and the degree of liver fibrosis according to the results of the FibroMax test in patients with CHC.

Materials and methods: 62 outpatients with a verified diagnosis of CHC were under observation, in which serum Zn, Cu and Se levels, viral load and degree of liver fibrosis were determined according to the FibroMax test.

Results: HCV 1b genotype was detected in all patients. The proportion of patients with a high viral load was 32%, with a low viral load – 68%. In 19% of patients, the level of Zn was below normal, and the levels of Cu and Se were within the reference values. The proportion of patients without fibrosis was 32%, 16% had minimal fibrosis, 40% had moderate fibrosis, 8% had progressive fibrosis, and 3% had severe fibrosis. 68% of patients had active inflammation of various degrees, liver steatosis – 65%, non-alcoholic steatohepatitis – 48%, inflammation caused by alcohol consumption was absent. No statistically significant difference was found in serum trace element levels and viral load ($p>0.05$). A weak negative correlation between the level of Zn and the degree of fibrosis ($\rho=-0.340$, $p=0.007$) and a negligible negative correlation between the level of Zn and inflammation activity ($\rho=-0.286$, $p=0.024$) were revealed. Patients with fibrosis grade ≥F2 had lower Zn levels compared to patients with fibrosis ≤F1 (0.607 (0.540, 0.691) mg/l vs. 0.716 (0.593, 0.875) mg/l, $p=0.01$), and when comparing there was no difference in Cu and Se levels ($p>0.05$).

Conclusions: Thus, there is a relationship between the level of Zn in blood serum and the degree of liver damage in patients with CHC, which indicates the prospects for further research.

KEY WORDS: chronic hepatitis C, trace elements, zinc, liver fibrosis, FibroMax

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INTRODUCTION

According to the latest global estimates published in the Global Hepatitis Report (2017), more than 71 million people had chronic HCV infection in 2015, extrapolating to 1% of the population. The main clinical form of HCV infection is chronic hepatitis C (CHC), which develops in an average of 70% of infected individuals, for 15-30% of whom there is a risk of liver cirrhosis within 20 years [1]. The chronic course of the disease definitely leads to changes in the entire metabolism, including the exchange of trace elements. Zinc (Zn), copper (Cu) and selenium (Se) are essential for the normal functioning of the whole human organism, including the immune system and antioxidant defense, and their basic metabolism takes place in the liver [2]. In general, researches show that in HCV, as a result of HCV-mediated mitochondrial dysfunction, Zn deficiency occurs [3]. A decrease in the level of Zn can also be a consequence of liver fibrosis, which includes various mechanisms. The metabolism of Cu is also disturbed in patients with CHC at various stages of the disease, which usually leads to an increase in its level in the blood [4]. HCV infection is associated with low levels of antioxidants, including Se, and increased levels of oxidative stress [5]. Disruption of Zn, Se, and Cu homeostasis associated with oxidative stress and inflammation may enhance HCV replication and liver fibrosis and reduce the effectiveness of antiviral treatment [6]. Therefore, the question of the association of the content of Zn and other trace elements in the blood with the course of CHC remains relevant.

THE AIM

To evaluate the content of trace elements Zn, Cu and Se in blood serum and their relationship with viral load and the degree of liver fibrosis according to the results of the FibroMax test in patients with CHC.

MATERIALS AND METHODS

62 outpatients with a diagnosis of CHC were under observation. Criteria for inclusion in the study: patients with a verified diagnosis of CHC who agreed to follow-up. Exclusion criteria were: alcoholic, autoimmune, and toxic liver damage, liver cirrhosis, myocardial infarction in the first 4 months, diseases

of the respiratory and gastrointestinal tract in the acute phase, decompensated diseases, diseases of the nervous system, psycho-emotional and mental disorders that prevent conducting this study and the patient's decision to stop participating in the study. The studied patients had no markers of infection with other hepatitis viruses (A, B, D, G, TT), highly specific markers of autoimmune hepatitis/ cross syndrome (anti-LKM-1, anti-SLA and anti-LC-1) and HIV infection. All patients denied the use of corticosteroids, non-steroidal anti-inflammatory and immunosuppressive drugs.

All patients underwent clinical and laboratory examinations according to the standard of medical care for hepatitis C in adults (2021). HCV was performed according to the 10th revision of the ICD and verified by the detection of total antibodies of the IgG class to the structural and non-structural proteins of HCV (antiHCV IgG +) by the serological ELISA method, as well as by the indication of the investigated RNA HCV + in the blood by the PCR method with viral load (VL) and genotyping. Testing was performed on a thermal cycler with a real-time PCR product detection system "iQ 5", Bio-Rad, USA. Laboratory studies were performed in the accredited private laboratory "Dila". The level of Zn, Cu and Se in the blood serum of the patients was determined. The degree of liver fibrosis was analyzed according to the data of the non-invasive diagnostic method FibroMax according to the criteria proposed by the developers of the method. Data included: FibroTest, ActiTest, SteatoTest, NashTest, AshTest. FibroMax is manufactured by BioPredictive (Paris, France). FibroMax results are calculated using a special patented algorithm depending on the patient's sex, age, height and body weight and 10 biochemical indicators: haptoglobin, α-2-macroglobulin, apolipoprotein-A1, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), total bilirubin, glucose, total cholesterol and triglycerides.

To determine the duration of CHC, a thorough collection and analysis of the epidemiological history was carried out, taking into account the ways and factors contributing to infection. The most frequent ways of infection were surgical interventions and dental manipulations (23.5% and 21.4%, respectively), parenteral manipulations and blood transfusions in the anamnesis were indicated by 19.2% and 17.8%, respectively. The number of persons who indicated infection during the performance of their professional duties was 4.3% and 3.7% of patients believed that they were infected during unprotected sexual acts. In 10.1% of patients, it was not possible to establish the ways of infection. The duration of CHC in 78.0% of patients was an average of 10.5±0.3 years, and in 22.0% of patients it was detected for the first time.

The assessment of body shape was carried out according to generally accepted anthropometric indicators. Body mass index (BMI) was considered anthropometric criteria of obesity. BMI in the range of 18.5-24.9 kg/m^2 was considered as normal body weight, 25.0-29.9 kg/m^2 as overweight, ≥30.0 kg/m^2 as obesity.

Statistical analysis was performed in the jamovi 1.6 program using the Mann-Whitney U test, the Kruskal-Wallis test, and the Spearman correlation coefficient. The normality of the distribution of interval variables was assessed by the Shapiro-Wilk test. The assessment of the strength of the relationship between the variables was evaluated according to the Chaddock scale. Mean values were described as Me (Q_1; Q_3). The critical level of significance was α=0.05.

The research was carried out with the personal signed consent of the patients and in accordance with the methodological recommendations of the Declaration of Helsinki (1975) with redrafting, the International Code of Medical Ethics (1983), the laws of Ukraine, the relevant provisions of the WHO, and was approved by the local ethics commission of the Uzhhorod National University (protocol №6/4 dated 09/07/2021), and all those who participated were informed and, as a result, gave their consent in the consent letter, the structure of which corresponded to the officially agreed one.

RESULTS

Among the examined patients, the proportion of men was 56%, women – 44%, the average age was 41.7±10.9 years. The distribution depending on BMI was as follows: 31% had normal weight, 45% had overweight, and 24% had varying degrees of obesity.

The examined patients had a latent course of CHC with the following clinical syndromes and symptoms: asthenovegetative, dyspeptic, arthralgias, general weakness, reduced work capacity, periodic heaviness in the right hypochondrium and itching of the skin, and with varying degrees of activity of liver enzymes.

HCV 1b genotype was detected in all patients. The criteria for dividing patients depending on the VL were: with a high viral load – HCV RNA ≥6x10^5 IU/ml and with a low VL – HCV RNA ≤5x10^5 IU/ml. The proportion of patients with high VL was 32%, with low VL – 68%. Laboratory data are shown in Table I.

The proportion of patients in whom the Zn level was below normal was 19% (the lowest value was 0.405 mg/l), Cu and Se levels in all patients were within the reference values.

The distribution of patients according to FibroMax results is shown in Figure 1. The proportion of patients without fibrosis (FibroTest) was 32%, minimal fibrosis was 16%, moderate fibrosis was 40%, progressive fibrosis was 8% and severe fibrosis was 3%. 68% of patients had active inflammation of various degrees (ActiTest), liver steatosis (SteatoTest) – 65%, non-alcoholic steatohepatitis (NashTest) – 48%, inflammation caused by alcohol consumption (AshTest) was absent.

No statistically significant difference was found in the levels of trace elements in blood serum depending on sex, BMI and VL, and there was no correlation with age (p>0.05). During the analysis of the relationship between the level of trace elements and indicators of the components of the FibroMax test (Table II), a weak negative correlation between the level of Zn and the degree of fibrosis (ρ=-0.340, p=0.007) was revealed, a negligible negative correlation between the level of Zn and inflammation activity (ρ=-0.286, p=0.024) and a negligible negative correlation between Zn and Cu levels (ρ=-0.271, p=0.033). Also, the level of Zn was negatively correlated with the levels of α-2-macroglobulin (ρ=-0.273, p=0.032) and ALT (ρ=-0.251, p=0.049).

Additionally, patients were divided into two 2 groups depending on the degree of fibrosis: Group I (48% of pa-

Table I. Results of laboratory examination of patients

Indicator	Result	Reference values
Zn	0,649 (0,569; 0,739)	0,553-1,046 mg/l
Cu	1,04 (0,883; 1,23)	0,7-1,4 mg/l
Se	0,0775 (0,0622; 0,0958)	0,046-0,14 mg/l
α-2-macroglobulin	2,27 (1,89; 3,0)	1,3-3,0 g/l
Haptoglobin	1,03 (0,7; 1,33)	0,4-2,4 g/l
Apolipoprotein-A1	1,43 (1,27; 1,58)	0,79-1,69 g/l
Total bilirubin	12,0 (9,0; 15,0)	5,0-21,0 μmol/l
GGT	29,5 (20,3; 44,0)	8-61 U/l
ALT	42,0 (28,0; 80,8)	0-55 U/l
AST	31,5 (26,0; 44,0)	13-40 U/l
Glucose	5,3 (5,0; 5,9)	4,1-6,0 mmol/l
Total cholesterol	4,43 (3,91; 5,02)	<5 mmol/l
Triglycerides	1,05 (0,765; 1,45)	<1,7 mmol/l

Table II. Correlations between trace element levels and FibroMax data

		Zn	Cu	Se	FibroTest	ActiTest	SteatoTest	NashTest	AshTest
Zn	ρ	—							
	p	—							
Cu	ρ	-0.271	—						
	p	0.033	—						
Se	ρ	-0.016	0.077	—					
	p	0.899	0.551	—					
FibroTest	ρ	-0.340	0.140	-0.016	—				
	p	0.007	0.276	0.904	—				
ActiTest	ρ	-0.286	0.080	-0.080	0.479	—			
	p	0.024	0.535	0.538	<0.001	—			
SteatoTest	ρ	-0.158	0.065	-0.035	0.101	0.331	—		
	p	0.221	0.613	0.784	0.435	0.009	—		
NashTest	ρ	-0.133	0.132	0.023	0.050	0.351	0.780	—	
	p	0.303	0.307	0.856	0.699	0.005	<0.001	—	
AshTest	ρ	-0.113	-0.010	0.123	0.175	-0.211	0.147	0.039	—
	p	0.381	0.937	0.342	0.174	0.100	0.255	0.764	—

tients) – no fibrosis or minimal fibrosis (≤F1), Group II (52% of patients) – moderate, progressive or severe fibrosis (≥F2). A statistically significant difference was found in Zn levels depending on the degree of fibrosis (U=296, p=0.01), which was lower in the group of patients with more pronounced liver fibrosis (0.607 (0.540; 0.691) mg/l vs. 0.716 (0.593; 0.875) mg/l). Regarding the levels of Cu and Se, no difference was found (p>0.05).

DISCUSSION

There are conflicting data regarding the relationship of Zn level with HCV infection. Some studies indicate a decrease in its level in CHC [6, 7], while others do not confirm this connection [8]. This may be due to eating habits, antiviral therapy and other factors [7]. Liver disease affects the digestion, assimilation, storage and metabolism of nutrients, which can lead to vitamin and micronutrient deficiencies and protein-energy insufficiency. There is no gold standard for the diagnosis of nutritional deficiency in patients with chronic liver disease, including insufficient studies of the nutritional status of HCV patients without cirrhosis [9]. In a study by Gottschall et al. 2015 [9] insufficient intake of Zn and other nutrients (Ca, Na, K, vitamin C) was found in more than half of patients with CHC (but not cirrhosis). According to the results of the study by Pourhassan et al. 2015 [7], serum Zn and haptoglobin levels were significantly lower in patients with CHC (but not cirrhosis) compared to controls. In a study using bioelectrical impedance of body composition, the level of Zn was reduced in 6% of examined patients with HCV infection, however, the disease was in the stage of cirrhosis [8]. According

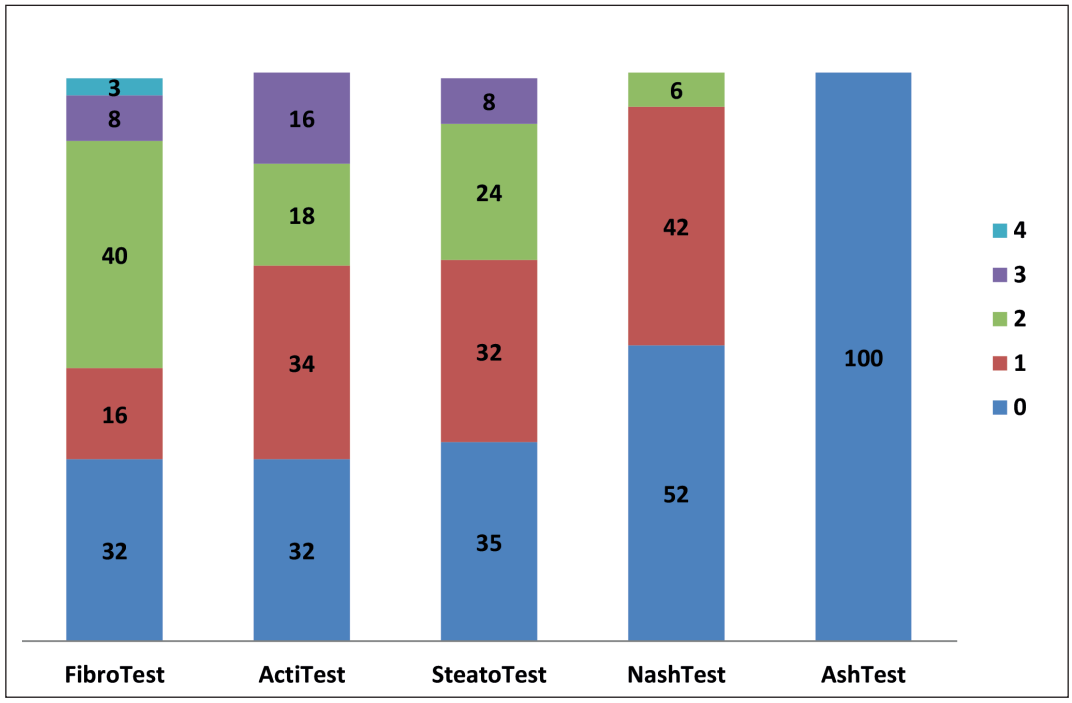

Fig. 1. Distribution of patients (in %) according to FibroMax data

to the results of other studies, the average level of Zn decreases with the progression of fibrosis, cirrhosis and was associated with the presence of varicose veins of the esophagus [10]. However, in a study by Suda et al. 2019 [11] Zn deficiency was observed in 27 (87.1%) patients with CHC, despite a good functional state of the liver. In our study, a decrease in the level of Zn was observed in 19% of patients. Such a small proportion of examined patients with hypozincemia may be due to the absence of pronounced cirrhotic liver changes and decompensation in them, as well as the relative compliance of patients with a healthy lifestyle, and probably a small sample size.

Persistent viral replication also results in a strong inflammatory response, characterized by an abundance of activated immune cells in the liver, as well as elevated levels of serum transaminases and proinflammatory cytokines such as IL-6 and TNF-α. As a result, chronic liver damage mediated by ineffective innate and adaptive immune responses promotes liver fibrosis, ultimately leading to cirrhosis and hepatocellular carcinoma [12]. It was shown that the serum Zn level is an independent prognostic factor of the overall survival of patients with CHC, as well as an indicator of the functional state of the liver and the degree of fibrosis. The concentration of Zn in serum and hepatocytes is reduced in patients with chronic liver diseases, and the depletion of Zn reserves is believed to accelerate the processes of fibrogenesis [13]. Zn deficiency can promote activation of hepatic stellate cells and collagen production, increasing fibrosis [14]. Zn inhibits the proliferation and synthesis of type IV collagen in hepatic stellate cells by increasing matrix metalloproteinase 13 [15]. Zn also promotes apoptosis of hepatic stellate cells. In addition, Zn can inhibit liver fibrosis by reducing the activity of lysyl oxidase [14].

A sufficient level of Zn in the liver stimulates the induction of metallothioneins, which are powerful antioxidants due to binding and release of Zn, but also have a moderate antiviral effect precisely due to the modulation of intracellular Zn homeostasis [2, 12]. Patients with CHC have low serum Zn levels and low metallothionein expression in the liver. Low metallothionein expression is associated with increased liver fibrosis, increased inflammation, and histological activity index (HAI), suggesting a protective role of metallothioneins in chronic inflammation [16].

In our study, a negative correlation was found between the level of Zn and the degree of liver fibrosis and the activity of the inflammatory process, as well as the levels of α-2-macroglobulin and ALT. Also, patients with fibrosis grade ≥F2 had lower Zn levels compared to patients with fibrosis ≤F1 (0.607 (0.540, 0.691) mg/l vs. 0.716 (0.593, 0.875) mg/l, p=0.01). Such data are consistent with the results of research by Omran et al. 2017 [13], where the level of serum Zn in patients with CHC was negatively correlated with the degree of liver fibrosis and was significantly lower as fibrosis progressed. This indicates the connection of Zn deficiency with the severity of liver damage.

The metabolism of Cu and Zn is closely related in the liver. Excessive Zn intake can lead to Cu deficiency due to metallothionein-mediated inhibition of intestinal Cu absorption [2]. In our study, a negative correlation of these trace elements was found, which indicates their antagonism in certain metabolic pathways both in normal and liver diseases. In addition, there is a certain indicative Cu/Zn ratio, the norm of which should be 0.8-1.2 μg/dL [5].

Regarding the trace elements Cu and Se, there are much fewer clinical studies, compared to Zn, regarding their association with chronic HCV infection [2]. In our study, no connection of these trace elements with liver damage was found, and their levels were within the reference values.

CONCLUSIONS

It was found that 19% of the examined patients had a Zn deficiency, while the levels of Cu and Se in all patients were within the reference values. There was no statistically significant differ-

ence in the levels of trace elements in blood serum depending on sex, BMI and VL, and there was no correlation with age (p>0.05). A negligible negative correlation was found between the levels of Zn and Cu (ρ=-0.271, p=0.033), as well as the level of Zn and α-2-macroglobulin (ρ=-0.273, p=0.032) and ALT (ρ=-0.251, p =0.049). It was established that the serum Zn level was negatively correlated with the degree of liver fibrosis (ρ=-0.340, p=0.007) and the activity of the inflammatory process (ρ=-0.286, p=0.024). A statistically significantly lower level of Zn was found in patients with a degree of fibrosis ≥F2, compared to patients with a degree of fibrosis ≤F1 (0.607 (0.540; 0.691) mg/l vs. 0.716 (0.593; 0.875) mg/l, p=0.01), and when comparing the levels of Cu and Se, no difference was found (p>0.05). Thus, there is a relationship between the level of Zn in blood serum and the degree of liver damage in patients with CHC.

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The Authors declare no conflict of interest.

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