DOI 10.29254/2077-4214-2022-4-167-131-137
UDC 616.36-002.2:577.118:612.017.1

Derbak M. A., Sitkar A. D.

CYTOKINE IMBALANCE AND COLLAGEN IV LEVEL IN CHRONIC HEPATITIS C PATIENTS WITH DIFFERENT ZINC CONTENTS

Uzhhorod National University (Uzhhorod, Ukraine)

morika1415@gmail.com

Patients with chronic hepatitis C (CHC) develop zinc (Zn) deficiency as a result of oxidative stress, liver fibrosis and exposure to pro-inflammatory cytokines. The aim was to evaluate the levels of interleukins IL-1β, IL-4 and IL-6, TNF-α and collagen IV depending on the Zn content in blood serum in patients with CHC. The study included 88 patients with CHC, who were divided into 2 groups: Group I – patients with a reduced level of Zn (n=42), Group II – patients with a normal level of Zn (n=46). The levels of IL-1β, IL-4 and IL-6, TNF-α and collagen IV were determined in all patients. It was established that in patients of the I group, the activity of the inflammatory process according to the levels of ALT and AST is significantly higher (by 1,8 (p=0,019) and 1,6 times (p=0,024)) compared to the patients of the II group, and is positively correlated with the levels pro-inflammatory cytokines. In group I patients, the levels of IL-1β and IL-6 are significantly higher (by 1,5 and 1,4 times, respectively, p<0,001), and the level of IL-4 is lower (by 33,0%, p<0,001), compared to patients of the II group. A negative correlation of Zn with the levels of IL-1β (ρ=–0,542, p<0,001), IL-6 (ρ=–0,556, p<0,001) and TNF-α (ρ=–0,476, p<0,001) was revealed, and a positive correlation (ρ=0,485, p<0,001) between Zn and IL-4 levels. A moderate negative correlation was found between serum Zn and collagen IV levels (ρ=–0,379, p<0,001). In CHC patients with a reduced Zn content, the activity of the inflammatory process is significantly higher compared to patients with a normal Zn level, and is positively correlated with the levels of pro-inflammatory cytokines and the degree of liver fibrosis.

Key words: *chronic hepatitis C, zinc, inflammation, cytokines, fibrosis.*

Connection of the publication with the planned scientific research.

The scientific work is a part of the research topic of the Department of Faculty Therapy of Uzhhorod National University: "Combined pathology and correction of homeostasis disorders of Carpathian region residents, taking into account the action of adverse factors", state registration number 0121U110808.

Introduction.

According to WHO data in 2019, 58 million people have chronic hepatitis C (CHC), which leads to about 400,000 deaths every year. HCV develops in approximately 70% of people after acute HCV infection and is characterized by persistent viremia and liver inflammation [1]. The persistence of HCV is primarily due to its ability to interfere with the host's immune response to infection and inhibit apoptotic pathways, thus allowing continuous viral replication [2]. HCV induces hepatocyte oxidative stress and mitochondrial dysfunction, leading to a strong antioxidant response, including induction of metallothionein genes [3]. Continued viral replication also leads to a strong inflammatory response, characterized by a large number of activated immune cells in the liver, as well as elevated levels of serum aminotransferases and pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α). As a result, chronic hepatocyte damage mediated by ineffective innate and adaptive immune responses promotes liver fibrosis, ultimately leading to cirrhosis and hepatocellular carcinoma [2]. In general, studies show that chronic zinc (Zn) deficiency, which is one of the essential microelements, occurs with CHC [4, 5, 6]. It is believed that as a result of HCV-mediated mitochondrial dysfunction, the presence of oxidative stress disrupts Zn homeostasis, as it is a signaling molecule and second messenger in redox reactions [5]. It is important that after the elimination of the virus after treatment with interferons or antiviral drugs of direct action, the level of Zn in blood serum increases significantly, probably due to the reduction of the inflammatory process in the liver and improvement of its absorption in the intestine [7]. In CHC, a decrease in the level of Zn can also be a consequence of liver fibrosis, which includes various mechanisms, in particular, hypoalbuminemia, damage to the intestinal mucosa against the background of portal hypertension, and increased excretion of Zn in the urine through the portosystemic shunt [4, 7, 8]. However, the concentration of Zn in blood serum can decrease under the influence of inflammation even in the absence of hypoalbuminemia due to the influence of pro-inflammatory cytokines, which have been proven to play an important role in the homeostasis of this trace element [9]. Zn deficiency leads to an increase in the recovery time for many infectious diseases, as it plays a significant role in immunity [10]. Zn inhibits the replication of viruses in vitro, in particular human immunodeficiency virus, rhinovirus, herpes simplex virus, respiratory syncytial virus, and HCV [11]. Therefore, the question of the association of Zn content in blood serum with the severity of inflammation and liver fibrosis in patients with CHC remains open.

The aim of the study.

To assess the levels of interleukins IL-1β, IL-4 and IL-6, TNF-α and collagen IV depending on the Zn content in blood serum in patients with chronic hepatitis C.

Object and research methods.

88 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, cirrhosis of the liver, myocardial infarction in the first 6 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. The control group consisted of 30 practically healthy people.

All patients underwent clinical and laboratory examinations according to the standard of medical care for hepatitis C in adults. 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 HCV + RNA in the blood by the PCR method with viral load and genotyping. Testing was performed on a thermal cycler with a real-time PCR product detection system "iQ 5", Vio-Rad, USA. General clinical, biochemical, serological and molecular genetic studies were performed in accredited laboratories: clinical and diagnostic laboratories of the Regional Clinical Infectious Diseases Hospital in Uzhhorod and private laboratories "Dila" and "Astra-Dia". The level of Zn in the blood serum of patients was also determined, where a level of 0,553-1,046 mg/L was considered the norm. Depending on the serum Zn level, the patients were divided into 2 groups: the first group included patients with a reduced level of Zn (n=42) and the second group included patients whose serum Zn level was within the normal range (n=46). The studied groups were homogeneous in terms of sex ($\chi2$=0,386, df=1, p=0,534) and age (U=888, p=0,514).

The following biochemical blood parameters were analyzed in all patients: activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), total bilirubin (TB), total protein (TP), albumin (Alb). The degree of liver fibrosis in 52,3% (46/88) of patients was determined by the non-invasive diagnostic method Fibro/ActiTest (conducted in the accredited laboratory "Dila" according to the criteria proposed by the developers of the method), and in 47,7% (42/88) of patients by by the results of the method of indirect fibroelastometry of the liver (diagnostic device "FibroScan" 502 F01261 with sensor M 7 70129, (Echosens, France), which was carried out on the basis of the Regional Clinical Infectious Diseases Hospital in Uzhhorod). Liver fibrosis was assessed using the METAVIR scale, and the degree of activity of the pathological process was determined by the level of ALT elevation according to the International Classification of Liver Diseases (Los Angeles, 1994). In the study, Enzyme-Immuno-Sorbent-Assay (ELISA) was used to determine the levels of cytokines (IL-1β, IL-4, IL-6, TNF-α) in blood serum on the automatic immunoenzyme analyzer "STATFAX" according to the recommended protocols to test systems DRG (USA), according to the instructions included in the kits with reagents. The level of collagen IV (ColIV) in blood serum was investigated by ELISA using Argutus Collagen IV kits (Germany) for its quantitative determination, according to the instructions included in the kits with reagents.

The duration of CHC in 76,4% of patients was 9,8±0,3 years on average, and in 23,6% of patients it was detected for the first time. HCV 1b genotype was detected in all patients.

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.

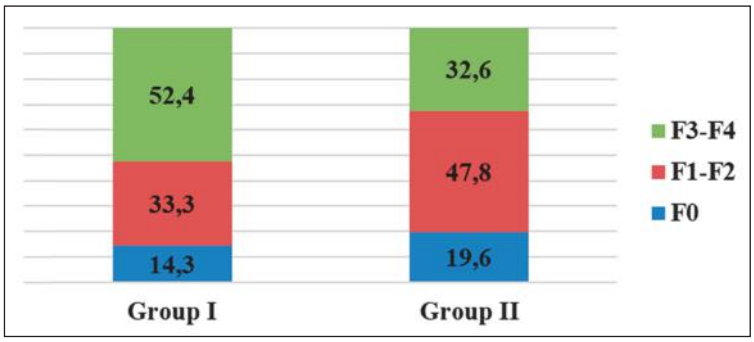

Figure 1 – Distribution of patients (in %) depending on the liver fibrosis degree.

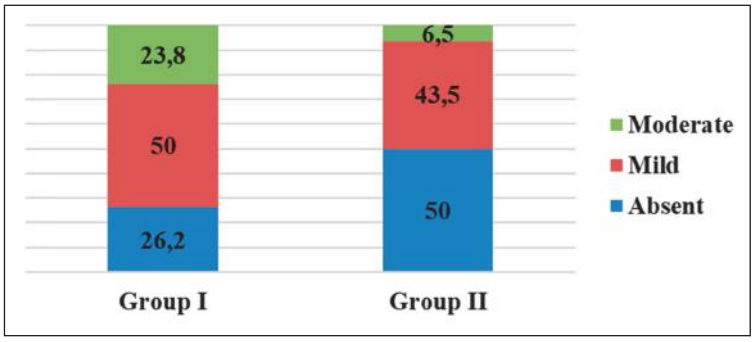

Figure 2 – Distribution of patients (in %) depending on the inflammatory process activity.

Statistical analysis was performed in the Jamovi 2.2.5 program using the Mann-Whitney U test, the Pearson's chi-squared test with Yates's correction for continuity 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 M±SD and Me (Q1; Q3) depending on the data distribution. The critical level of significance was α=0,05.

Research results and their discussion.

The examined patients had a latent course of CHC with the following clinical syndromes and symptoms: asthenovegetative, dyspeptic, arthralgia, general weakness, decreased work capacity, periodic heaviness in the right hypochondrium, skin itching, abdominal distension, and with varying degrees of liver enzyme activity. Also, patients, especially women, who had low Zn levels were more likely to complain of dry and scaly skin, acne, hair loss, and brittle nails,

compared to patients who had normal Zn levels.

The distribution of patients depending on the liver fibrosis degree and the inflammatory process activity is shown in **figures 1 and 2**, respectively.

A higher frequency of higher degree of fibrosis (F3-F4) was observed in the group of patients with a reduced serum Zn level, compared to patients whose Zn level was within the normal range (52,5% vs. 32,6%). However, when constructing the conjugation tables, it was established that patients in the studied groups did not statistically significantly differ in the degree of liver fibrosis ($\chi2=3,53$, df=2, p=0,171).

In 50.0% of patients of the II group (with a normal level of serum Zn), the activity of the inflammatory process according to the levels of ALT was absent, against 26,2% of the patients of the I group. Between the compared groups, a statistically significant difference was found in the distribution regarding the activity of the inflammatory process ($\chi2=7,86$, df=2, p=0,02). A higher frequency of moderate degree of inflammation (ALT above 5 norms) was observed in the group of patients with a reduced serum Zn level (23,8% vs. 6,5%).

In patients, the levels of interleukins (IL) 1β, 4, and 6, TNF-α, and collagen IV in blood serum were additionally determined. The data of laboratory studies are shown in **table 1.**

Table 1 – Average values of the studied laboratory indicators

Indicator	Group I (n=42)	Group II (n=46)	Control (n=30)
Zn	0,494 (0,440; 0,531)*#	0,710 (0,629; 0,839)	0,720 (0,645; 0,835)
TB	13,7 (11,1; 15,6)	12,8 (10,1; 19,5)	14,0 (9,4; 17,7)
GGT	46,3 (25,3; 85,3)	33,0 (20,3; 53,5)	34,0 (24,1; 41,1)
ALT	88,2 (48,4; 140,0)*#	49,7 (35,4; 98,2)^	25,4 (11,9; 32,2)
AST	72,1 (43,8; 120,0)*#	44,8 (34,9; 83,6)^	22,7 (14,8; 25,9)
TP	68,6 (65,4; 75,0)*	72,2 (67,8; 77,3)	69,2 (64,1; 76,0)
AL	37,3 (32,6; 44,3)	41,7 (37,5; 44,5)	40,7 (36,9; 43,4)
IL-1β	30,435 (25,29; 38,83)*#	20,32 (18,87; 22,715)^	0,49 (0,335; 0,945)
IL-4	6,045 (4,522; 7,092)*#	9,02 (7,68; 10,453)^	0,245 (0,172; 0,318)
IL-6	18,550 (16,440; 20,375)*#	13,625 (10,875; 15,380)^	0,855 (0,453; 1,100)
TNF-α	145,05 (133,525; 163,825)*#	128,45 (111,425; 127,025)^	3,3 (2,23; 4,57)
ColIV	182,90 (135,225; 227,00)*#	148,35 (127,00; 198,675)^	83,7 (74,5; 96,4)

Notes: ** – statistically significant difference between I and II groups, # – statistically significant difference between I and control groups, ^ – statistically significant difference between II and control groups.*

A statistically significant difference was found between the studied groups regarding the activity of ALT and AST, the level of total protein, cytokines and collagen IV (p<0,05).

ALT activity was 1,8 times higher in the group of patients with a reduced level of Zn, compared to the group with a normal level of Zn (88,2 (48,4; 140,0) U/L vs. 49,7 (35,4; 98,2) U/L, U=686, p=0,019), and the activity of AST is 1,6 times higher (72,1 (43,8; 120,0) U/L vs. 44,8 (34,9; 83,6) U/L, U=695, p=0,024). On the contrary, the level of total protein was 4,7% lower in group I compared to group II (68,6 (65,4; 75,0) g/L vs. 72,2 (67,8; 77,3) g/L, respectively, U=687, p=0,02).

In general, the levels of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) were higher in the group of patients with reduced serum Zn levels, and the level of IL-4, on the contrary, was lower. It was established that in the I group the level of IL-1β was 1,5 times higher (30,435 (25,29; 38,83) pg/mL vs. (18,87; 22,715) pg/mL, U=300, p<0,001), and the level of IL-6 by 1,4 times (18,550 (16,440; 20,375) pg/mL vs. 13,625 (10,875; 15,380) pg/mL, U=243, p<0,001). The level of TNF-α was higher by 11,7% in patients of group I compared to group II (145,05 (133,525; 163,825) pg/mL vs. 128,45 (111,425; 127,025) pg/mL, respectively, U=428, p<0,001). On the contrary, the level of IL-4 was lower in group I by 33,0% compared to group II (6,045 (4,522; 7,092) ng/mL vs. 9,02 (7,68; 10,453) ng/mL, U=293, p<0,001).

It was also established that the level of collagen IV in the serum of patients with a reduced level of Zn was higher by 18,9%, compared to the group of patients with a normal level of Zn (182,90 (135,225; 227,00) μg/L against 148,35 (127,00; 198,675) μg/L, U=714, p=0,036).

During the analysis of relationships between laboratory indicators, an average degree of negative correlation was found between the levels of Zn and IL-1β (ρ=–0,542, p<0,001), an average degree of negative correlation between the levels of Zn and IL-6 (ρ=–0,556, p<0,001) and a moderate degree of negative correlation between Zn and TNF-α levels (ρ=–0,476, p<0,001). On the contrary, a moderate positive correlation was established between the levels of Zn and IL-4 (ρ=0,485, p<0,001). Also, a moderate negative correlation was found between serum Zn and collagen IV levels (ρ=–0,379, p<0,001) **(table 2)**.

The investigated pro-inflammatory cytokines were positively correlated with the activity of aminotransferases. Thus, IL-1β was weakly correlated with ALT and AST activity (ρ=0,288, p=0,007 and ρ=0,219, p=0,04, respectively), and there was a moderate relationship between IL-6 and ALT and AST activity (ρ=0,348, p<0,001 and ρ=0,30, p=0,004 respectively). A moderate correlation was also found between the level of TNF-α and the activity of ALT and AST (ρ=0,456, p<0,001 and ρ=0,363, p<0,001, respectively). On the contrary, a weak negative correlation was established between the level of IL-4 and the activity of ALT and AST (ρ=–0,294, p=0,005 and ρ=–0,283, p=0,008, respectively). In addition, weak degrees of correlation were found between GGT activity and cytokine levels, namely positive with IL-6 (ρ=0,270, p<0,011) and TNF-α (ρ=0,295, p=0,005) and negative with IL-4 (ρ=–0,348, p=0,037).

Serum collagen IV was also positively correlated with GGT activity (ρ=0,374, p<0,001), ALT (ρ=0,213, p=0,046), AST (ρ=0,365, p<0,001) and total bilirubin level (ρ=0,408, p<0,001), and negatively with the albumin level (ρ=–0,483, p<0,001).

In our study, a significant difference was found between groups both in terms of aminotransferase activity and cytokine levels (p<0,05). It was found that the activity of ALT, AST and the levels of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) were higher in the group of patients with a reduced serum Zn level, and the level

Table 2 – Correlation matrix of the studied laboratory indicators

		Zn	IL-1β	IL-4	IL-6	TNF-α	Col IV	TB	GGT	ALT	AST	TP	Alb
Zn	ρ	—											
	p	—											
IL-1β	ρ	**–0,542**	—										
	p	**<0,001**	—										
IL-4	ρ	**0,485**	**–0,305**	—									
	p	**<0,001**	**0,004**	—									
IL-6	ρ	**–0,556**	**0,315**	**–0,447**	—								
	p	**<0,001**	**0,003**	**<0,001**	—								
TNF-α	ρ	**–0,476**	**0,385**	**–0,137**	**0,281**	—							
	p	**<0,001**	**<0,001**	**0,204**	**0,008**	—							
Col IV	ρ	**–0,379**	**0,115**	**–0,137**	**0,152**	**0,199**	—						
	p	**<0,001**	**0,287**	**0,203**	**0,157**	**0,063**	—						
TB	ρ	**–0,097**	**–0,052**	**0,007**	**0,152**	**0,143**	**0,408**	—					
	p	**0,369**	**0,630**	**0,951**	**0,159**	**0,184**	**<0,001**	—					
GGT	ρ	**–0,178**	**0,115**	**–0,222**	**0,270**	**0,295**	**0,374**	**0,381**	—				
	p	**0,097**	**0,286**	**0,037**	**0,011**	**0,005**	**<0,001**	**<0,001**	—				
ALT	ρ	**–0,217**	**0,288**	**–0,294**	**0,348**	**0,452**	**0,213**	**0,230**	**0,676**	—			
	p	**0,043**	**0,007**	**0,005**	**<0,001**	**<0,001**	**0,046**	**0,031**	**<0,001**	—			
AST	ρ	**–0,245**	**0,219**	**–0,283**	**0,300**	**0,363**	**0,365**	**0,393**	**0,688**	**0,835**	—		
	p	**0,021**	**0,040**	**0,008**	**0,004**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	—		
TP	ρ	**0,264**	**0,036**	**0,148**	**–0,189**	**–0,048**	**–0,080**	**–0,050**	**–0,013**	**0,111**	**–0,107**	—	
	p	**0,013**	**0,742**	**0,167**	**0,078**	**0,654**	**0,459**	**0,642**	**0,905**	**0,301**	**0,321**	—	
Alb	ρ	**0,343**	**–0,031**	**0,120**	**–0,142**	**–0,105**	**–0,483**	**–0,293**	**–0,101**	**–0,009**	**–0,201**	**0,439**	—
	p	**0,001**	**0,772**	**0,264**	**0,187**	**0,332**	**<0,001**	**0,006**	**0,351**	**0,935**	**0,061**	**<0,001**	—

of IL-4, on the contrary, was lower. It was also established that Zn was negatively correlated with the levels of IL-1β (ρ=–0,542, p<0,001), IL-6 (ρ=–0,556, p<0,001) and TNF-α (ρ=–0,476, p<0,001). On the contrary, there was a moderate positive correlation between Zn and IL-4 levels (ρ=0,485, p<0,001). In addition, the studied pro-inflammatory cytokines were positively correlated with the activity of aminotransferases, and the frequency of moderate degree of inflammation was higher in the group of patients with a reduced level of serum Zn, compared to patients with a normal level of Zn (23,8% vs. 6,5%).

The obtained data echo the studies of a number of scientists who proved that Zn deficiency contributes to a decrease in the population of cytotoxic T cells, a decrease in the activity of natural killer cells and a Th1 regulatory response [5, 12]. In particular, dietary Zn restriction leads to a decrease in the levels of IL-2 and interferon γ (γ-IFN), but does not affect the Th2 cytokines IL-4 and IL-10 [5, 13].

Our results complement the data of other scientists, who proved that Zn deficiency is associated with oxidative stress and activation of macrophage-monocytes, presumably due to the dysregulation of the Th1 clone, leading to an increase in the generation of pro-inflammatory cytokines, such as IL-1β, IL-6, IL-8 and TNF-α, and to decrease the generation of γ-IFN [14, 15].

It has been shown that disruption of Zn homeostasis associated with oxidative stress and inflammation can enhance HCV replication and liver fibrosis [16, 17], which is consistent with the data obtained in our previous study [18]. Other scientists have shown that serum Zn level is an independent prognostic factor of the overall survival of patients with CHC, an indicator of the functional state of the liver and the degree of fibrosis [19, 20]. The results of research by Omran et al. 2017 [21] showed that serum Zn levels were negatively correlated with the degree of liver fibrosis and were significantly lower as fibrosis progressed. The level of Zn also decreases with the progression of liver fibrosis in patients with CHC. Kang et al. 2015 [22] found that Zn at a concentration of 200 μM can significantly inhibit the proliferation and activity of the liver stellate cell line LX-2, which is responsible for the synthesis of collagen in the liver, including the processes of fibrogenesis. In the experiment, it was established that Zn deficiency leads to increased synthesis of liver collagen in rat models [23], and according to Attallah et al. 2021 [24] Zn deficiency may promote hepatic stellate cell activation and collagen production, exacerbating liver fibrosis.

According to the data of our study, it was established that the level of serum collagen IV in the group of patients with a reduced level of Zn was higher by 18,9%, compared to the group of patients with a normal level of Zn (182,90 (135,225; 227,00) μg/L vs. 148,35 (127,00; 198,675) μg/L, p=0,036). A higher frequency of higher degree of fibrosis (F3-F4) was observed in the group of patients with a reduced serum Zn level, compared to patients whose Zn level was within the normal range (52,5% vs. 32,6%). A moderate negative correlation was also found between serum Zn and collagen IV levels (ρ=–0,379, p<0,001). This indicates the association of serum Zn level with collagen IV as a direct marker of fibrosis [25] in the studied patients, and thus the association of Zn with the process of fibrogenesis in the liver, as shown in our previous study [18]. The obtained data agree with the data of Kang et al. 2015 [22], who showed that Zn inhibits the proliferation and synthesis of collagen in hepatic stellate cells, and also promotes apoptosis of hepatic stellate cells and reduces the level of type IV collagen [23].

Conclusions.

It was established that in patients with CHC with a reduced level of Zn, the activity of the inflammatory

process according to the levels of ALT and AST is significantly higher compared to patients with a normal level of Zn, and is positively correlated with the levels of pro-inflammatory cytokines. The content of IL-1β and IL-6 is significantly higher (by 1,5 and 1,4 times, respectively, p<0,001) in patients with CHC with reduced Zn content, and the level of IL-4 is lower (by 33,0%, p<0,001), compared to patients with a normal Zn level. A higher frequency of a higher degree of fibrosis (F3-F4) was found in patients with CHC with a reduced level of Zn, compared to patients in whom the level of Zn was within the normal range (52,5% vs. 32,6%).

The established moderate negative correlation between Zn and collagen IV serum levels (ρ=–0,379, p<0,001) confirms the importance of zinc as a direct sign of fibrosis in patients with CHC.

Prospects for further research.

An active inflammatory process and progression of liver fibrosis associated with Zn imbalance may aggravate the course of CHC, which requires further pathogenetic justification.

References

1. World Health Organization. Hepatitis C [Internet]. Geneva: WHO; 2021 [updated 5 Feb 2021]. Available from: https://www.who.int/news-room/fact-sheets/detaIЛ/hepatitis-c.
2. Read SA, Parnell G, Booth D, Douglas MW, George J, Ahlenstiel G. The antiviral role of zinc and metallothioneins in hepatitis C infection. Journal of viral hepatitis. 2018 May;25(5):491-501. DOI: 10.1111/jvh.12845.
3. Diamond DL, Jacobs JM, Paeper B, Proll SC, Gritsenko MA, Carithers Jr RL, et al. Proteomic profIЛing of human liver biopsies: Hepatitis C virus–induced fibrosis and mitochondrial dysfunction. Hepatology. 2007 Sep;46(3):649-57. DOI: 10.1002/hep.21751.
4. Ko YL, Morihara D, Shibata K, Yamauchi R, Fukuda H, Kunimoto H, et al. Factors attenuating zinc deficiency improvement in direct-acting antiviral agent-treated chronic hepatitis C virus infection. Nutrients. 2018 Nov 2;10(11):1620. DOI: 10.3390/nu10111620.
5. Gupta S, Read SA, Shackel NA, Hebbard L, George J, Ahlenstiel G. The role of micronutrients in the infection and subsequent response to hepatitis C virus. Cells. 2019 Jun 17;8(6):603. DOI: 10.3390/cells8060603.
6. Pourhassan A, Fouladi DF, Samani SM, Morshedi Asl S. Serum Zinc and Haptoglobin in Noncirrhotic Azeri Patients with Chronic Active Hepatitis C: a Case–Control Study. Biological trace element research. 2015 Oct;167(2):187-93. DOI: 10.1007/s12011-015-0309-4.
7. Gupta SHH, Read S, Wijaya R, George J, Ahlenstiel G. The effect of fibrosis and direct-acting antiviral therapy on serum zinc levels in chronic hepatitis C infection. J. Gastroenterol. Hepatol. 2018;33:34-81.
8. Mohommad M. Zhou Z, Cave M, Barve A, McClain CJ. Zinc and liver disease. Nutr Clin Pract. 2012;27(1):8-20. DOI: 10.1177/0884533611433534.
9. Reda R, Abbas AA, Mohammed M, El Fedawy SF, Ghareeb H, El Kabarity RH, et al. The interplay between zinc, vitamin D and, IL-17 in patients with chronic hepatitis C liver disease. Journal of immunology research. 2015;2015:846348. DOI: 10.1155/2015/846348.
10. Abbasinazari M, Alavian SM, Behnava B, Asgharinia M, Salimi S, Keshvari M, et al. Effect of zinc supplementation on viral response in patients with chronic hepatitis C and Beta thalassemia major, a pilot study. Journal of Clinical and Diagnostic Research: JCDR. 2014 Dec;8(12):HC16. DOI: 10.7860/JCDR/2014/10403.5305.
11. Yuasa K, Naganuma A, Sato K, Ikeda M, Kato N, Takagi H, et al. Zinc is a negative regulator of hepatitis C virus RNA replication. Liver International. 2006 Nov;26(9):1111-8. DOI: 10.1111/j.1478-3231.2006.01352.x.
12. Sumaily KM. The roles and pathogenesis mechanisms of a number of micronutrients in the prevention and/or treatment of chronic hepatitis, COVID-19 and type-2 diabetes mellitus. Nutrients. 2022 Jun 24;14(13):2632. DOI: 10.3390/nu14132632.
13. Kaltenberg J, Plum LM, Ober-Blöbaum JL, Hönscheid A, Rink L, Haase H. Zinc signals promote IL-2-dependent proliferation of T cells. European journal of immunology. 2010 May;40(5):1496-503. DOI: 10.1002/eji.2009 39574.
14. Sevastianos VA, Voulgaris TA, Dourakis SP. Hepatitis C, systemic inflammation and oxidative stress: correlations with metabolic diseases. Expert Review of Gastroenterology & amp. Hepatology. 2020 Jan 2;14(1):27-37. DOI: 10.1080/17474124.2020.1708191.
15. Grüngreiff K, Hebell T, Gutensohn K, Reinhold A, Reinhold D. Plasma concentrations of zinc, copper, interleukin-6 and interferon-γ, and plasma dipeptidyl peptidase IV activity in chronic hepatitis C. Molecular Medicine Reports. 2009 Jan 1;2(1):63-8. DOI: 10.3892/mmr_00000062.
16. Guo CH, Chen PC, Ko WS. Status of essential trace minerals and oxidative stress in viral hepatitis C patients with nonalcoholic fatty liver disease. International Journal of Medical Sciences. 2013;10(6):730. DOI: 10.7150/ijms.6104.
17. Ko WS, Guo CH, Yeh MS, Lin LY, Hsu GS, Chen PC, et al. Blood micronutrient, oxidative stress, and viral load in patients with chronic hepatitis C. World journal of gastroenterology: WJG. 2005 Aug 8;11(30):4697. DOI: 10.3748/wjg.v11.i30.4697.
18. Sitkar AD, Derbak MA, Rostoka LM, Hanych OT. Association between serum zinc, copper and selenium levels and the degree of liver damage in patients with chronic hepatitis C. Wiadomosci Lekarskie (Warsaw, Poland: 1960). 2022 Jan 1;75(10):2434-8. DOI: 10.36740/WLek202210122.
19. Imai K, Beppu T, Yamao T, Okabe H, Hayashi H, Nitta H, et al. Clinicopathological and prognostic significance of preoperative serum zinc status in patients with hepatocellular carcinoma after initial hepatectomy. Annals of surgical oncology. 2014 Nov;21(12):3817-26. DOI: 10.1245/s10434-014-3786-3.
20. Murakami Y, Koyabu T, Kawashima A, Kakibuchi N, Kawakami T, Takaguchi K, et al. Zinc supplementation prevents the increase of transaminase in chronic hepatitis C patients during combination therapy with pegylated interferon α-2b and ribavirin. Journal of nutritional science and vitaminology. 2007;53(3):213-8. DOI: 10.3177/jnsv.53.213.
21. Omran DA, Darweesh SK, Fouad H, Mahmoud M, Saif S, Fared A, et al. Serum zinc deficiency and its relation to liver fibrosis in chronic HCV: a real-life Egyptian study. Biological Trace Element Research. 2017 Sep;179(1):1-7. DOI: 10.1007/s12011-017-0938-x.
22. Kang M, Zhao L, Ren M, Deng M, Li C. Zinc mediated hepatic stellate cell collagen synthesis reduction through TGF-β signaling pathway inhibition. International Journal of Clinical and Experimental Medicine. 2015;8(11):20463.
23. Takahashi M, Saito H, Higashimoto M, Hibi T. Possible inhibitory effect of oral zinc supplementation on hepatic fibrosis through downregulation of TIMP-1: a pilot study. Hepatology Research. 2007 Jun;37(6):405-9. DOI: 10.1111/j.1872-034X.2007.00065.x.
24. Attallah AM, Omran D, Abdelrazek MA, Hassany M, Saif S, Farid A, et al. IL28B rs12979860 polymorphism and zinc supplementation affect treatment outcome and liver fibrosis after direct-acting antiviral hepatitis C therapy. Journal of Genetic Engineering and Biotechnology. 2021 Dec;19(1):1-0. DOI: 10.1186/s43141-021-00250-y.
25. Chen W, Rock JB, Yearsley MM, Ferrell LD, Frankel WL. Different collagen types show distinct rates of increase from early to late stages of hepatitis C–related liver fibrosis. Human Pathology. 2014 Jan 1;45(1):160-5. DOI: 10.1016/j.humpath.2013.08.015.

ЦИТОКІНОВИЙ ДИСБАЛАНС ТА РІВЕНЬ КОЛАГЕНУ IV У ХВОРИХ НА ХРОНІЧНИЙ ГЕПАТИТ С З РІЗНИМ ВМІСТОМ ЦИНКУ

Дербак М. А., Сіткар А. Д.

Резюме. *Вступ.* Постійна реплікація вірусу гепатиту С призводить до сильної запальної відповіді, що характеризується великою кількістю активованих імунних клітин у печінці, а також підвищеними рівнями сироваткових амінотрансфераз і прозапальних цитокінів, таких як інтерлейкін-6 (ІЛ-6) та фактор некрозу пухлин α (ФНП-α). Як наслідок, хронічне пошкодження гепатоцитів, опосередковане неефективною імунною відповід-

дю, сприяє розвитку фіброзу печінки. Загалом дослідження показують, що при ХГС виникає стійкий дефіцит цинку (Zn). Внаслідок опосередкованої HCV мітохондріальної дисфункції, наявність окислювального стресу порушує гомеостаз Zn. При ХГС зниження рівня Zn також може бути наслідком фіброзу печінки. Концентрація Zn у сироватці крові може знижуватися і через вплив прозапальних цитокінів, які, як доведено, відіграють важливу роль у гомеостазі даного мікроелемента. Тому питання асоціації вмісту Zn у сироватці крові із вираженістю запалення та фіброзом печінки у хворих на ХГС залишається відкритим.

Мета дослідження. Оцінити рівні інтерлейкінів ІЛ-1β, ІЛ-4 і ІЛ-6, ФНП-α та колагену IV залежно від вмісту Zn у сироватці крові у хворих на хронічний гепатит С.

Об`єкт і методи дослідження. Під спостереженням знаходились 88 пацієнтів з верифікованим діагнозом ХГС у яких визначали рівні Zn, інтерлейкінів ІЛ-1β, ІЛ-4, і ІЛ-6, ФНП-α та колагену IV у сироватці крові. Залежно від рівня Zn сироватки всі пацієнти були розподілені на 2 групи: I група – пацієнти зі зниженим рівнем Zn (n=42), II група – пацієнти, з нормальним рівнем Zn (n=46).

Результати. Вища частота помірного ступеню запалення (АлАТ вище 5 норм) спостерігалась у групі хворих зі зниженим рівнем Zn сироватки (23,8% проти 6,5%, p=0,02) Активність АлАТ була у 1,8 разів вища у групі пацієнтів із зниженим рівнем Zn, порівняно із групою з нормальним рівнем Zn (p=0,019), а активність АсАТ у 1,6 разів вища (p=0,024). Встановлено, що у I групі рівень ІЛ-1β був у 1,5 разів, а рівень ІЛ-6 у 1,4 рази вищим (p<0,001), а рівень ІЛ-4 – нижчим на 33,0%, порівняно з II групою (p<0,001). Показано, що Zn негативно корелював із рівнями ІЛ-1β (ρ=–0,542, p<0,001), ІЛ-6 (ρ=–0,556, p<0,001) та ФНП-α (ρ=–0,476, p<0,001). Напроти, між рівнями Zn та ІЛ-4 була позитивна кореляція помірного ступеня (ρ=0,485, p<0,001). Також встановлено, що рівень колагену IV у сироватці хворих I групи був вищим на 18,9%, порівняно із II групою (p=0,036). Знайдено помірну негативну кореляцію між рівнями Zn та колагену IV сироватки (ρ=–0,379, p<0,001). Більшу частоту вищого ступеня фіброзу (F3-F4) спостерігали у групі хворих зі зниженим рівнем Zn сироватки, порівняно з хворими, у яких рівень Zn був у межах норми (52,5% проти 32,6%).

Висновки. Встановлено, що рівень Zn сироватки у хворих на ХГС пов'язаний із активністю запального процесу та рівнями цитокінів. Показано кореляцію між рівнями Zn та колагену IV сироватки та більшу частоту вищого ступеня фіброзу (F3-F4) у хворих зі зниженим рівнем Zn сироватки, порівняно з хворими, у яких рівень Zn був у межах норми (52,5% проти 32,6%). Отже дисбаланс Zn та його асоціація з активним запальним процесом та прогресуванням фіброзу печінки потребує подальших патогенетичних обґрунтувань.

Ключові слова: хронічний гепатит С, цинк, запалення, цитокіни, фіброз.

CYTOKINE IMBALANCE AND COLLAGEN IV LEVEL IN CHRONIC HEPATITIS C PATIENTS WITH DIFFERENT ZINC CONTENTS

Derbak M. A., Sitkar A. D.

Abstract. *Introduction.* Continuous replication of hepatitis C virus leads to a strong inflammatory response characterized by a large number of activated immune cells in the liver, as well as elevated levels of serum aminotransferases and pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α). As a result, chronic damage to hepatocytes mediated by an ineffective immune response contributes to the development of liver fibrosis. In general, studies show that chronic zinc (Zn) deficiency occurs in chronic hepatitis C (CHC). As a result of HCV-mediated mitochondrial dysfunction, the presence of oxidative stress disrupts Zn homeostasis. With CHC, a decrease in the level of Zn can also be a consequence of liver fibrosis. The concentration of Zn in blood serum can also decrease due to the influence of pro-inflammatory cytokines, which have been proven to play an important role in the homeostasis of this trace element. Therefore, the question of the association of Zn content in blood serum with the severity of inflammation and liver fibrosis in patients with CHC remains open.

The aim. To evaluate the levels of interleukins IL-1β, IL-4 and IL-6, TNF-α and collagen IV depending on the content of Zn in blood serum in patients with chronic hepatitis C.

Materials and methods. 88 patients with a verified diagnosis of CHC were under observation, and the levels of Zn, interleukins IL-1β, IL-4, and IL-6, TNF-α, and collagen IV in blood serum were determined. Depending on the serum Zn level, all patients were divided into 2 groups: Group I – patients with a reduced level of Zn (n=42), Group II – patients with a normal level of Zn (n=46).

Results. A higher frequency of moderate degree of inflammation (ALT above 5 norms) was observed in the group of patients with a reduced serum Zn level (23,8% vs. 6,5%, p=0,02). ALT activity was 1,8 times higher in the group of patients with a reduced level of Zn, compared to the group with a normal level of Zn (p=0,019), and the activity of AST is 1,6 times higher (p=0,024). It was found that in group I, the level of IL-1β was 1,5 times higher, and the level of IL-6 was 1,4 times higher (p<0,001), and the level of IL-4 was lower by 33,0%, compared to II group (p<0,001). It was shown that Zn was negatively correlated with the levels of IL-1β (ρ=–0,542, p<0,001), IL-6 (ρ=–0,556, p<0,001) and TNF-α (ρ=–0,476, p<0,001). On the contrary, there was a moderate positive correlation between Zn and IL-4 levels (ρ=0,485, p<0,001). It was also established that the level of collagen IV in the serum of patients of the I group was higher by 18,9% compared to the II group (p=0,036). A moderate negative correlation was found between serum Zn and collagen IV levels (ρ=–0,379, p<0,001). A higher frequency of higher degree of fibrosis (F3-F4) was observed in the group of patients with a reduced serum Zn level, compared to patients whose Zn level was within the normal range (52,5% vs. 32,6%).

Conclusions. It was found that serum Zn level in patients with CHC is related to the activity of the inflammatory process and cytokine levels. A correlation between serum Zn and collagen IV levels and a higher frequency of a higher degree of fibrosis (F3-F4) in patients with a reduced serum Zn level compared to patients with a normal Zn level

(52,5% vs. 32,6%) were shown. Therefore, Zn imbalance and its association with an active inflammatory process and the progression of liver fibrosis require further pathogenetic justifications.

Key words: chronic hepatitis C, zinc, inflammation, cytokines, fibrosis.

ORCID and contributionship:
Derbak M. A.: 0000-0003-4791-4080 [EF]
Sitkar A. D.: 0000-0001-7890-5908 [ABCDF]

Conflict of interest:
There is no conflict of interest between the authors of this article**.**

Corresponding author
Derbak Mariya Antonivna
Uzhhorod National University
Ukraine, 88000, Uzhhorod, 20 Hryboiedova str.
Tel.: +380506275075
E-mail: morika1415@gmail.com

A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article.

Received 16.06.2022
Accepted 10.11.2022