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Features of cytokine status in patients with chronic hepatitis c and concomitant diabetes mellitus and its dynamics under the influence of treatment improvement using alpha-lipoic acid and lactulose

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Abstract
Features of chronic hepatitis C (CHC) course, it extrahepatic manifestations depend on immunopathological reactions. Immune system is unable to eliminate the virus, to prevent serious complications and consequences in case of CHC. However, the level of the main pro-inflammatory and anti-inflammatory cytokines in patients with CHC and concomitant diabetes mellitus (DM) has been scarcely studied. The article presents the data on the features of the immune response such as IL-2 and IL-4 content in the treatment of CHC with concomitant DM. The use of such drugs as Dialipon and Livolactum in the comprehensive basic treatment of CHC with concomitant DM promotes faster regression of clinical laboratory symptoms, the balance of cytokines towards normalization of IL-2 and IL-4 content in comparison with the patients who did not undergo this combined treatment.

Keywords: chronic hepatitis C, interleukin 2, interleukin 4, treatment

Introduction
The issue of viral hepatitis (VH) is one of the most urgent and unresolved issues in medical science and health care. According to the WHO data, the world's number of hepatitis B virus carriers reaches 350-400 million people, more than 1 milliard of people are infected with hepatitis C virus. The prevalence of this infection causes viral epidemic 4-5 times higher than the prevalence of HIV infection. According to the results of epidemiological studies Ukraine refers to the regions with high levels of viral hepatitis B, C, D contamination [1, 2].
In most cases, VH are registered in people on the background of concomitant and long lasting somatic diseases. CHC features associated with other diseases are understudied [1, 4, 5, 7]. Published results of the studies on this issue are not numerous and concern the course of CHC in HIV-infected patients with concomitant diseases of the thyroid gland, chronic diseases of the digestive and hepatobiliary systems (duodenal ulcer, cholecystitis, pancreatitis, etc.), chronic alcoholic liver disease [4, 6, 7, 8].
Meanwhile, CHC course is greatly influenced by comorbidity which causes pathological changes in the target organ, namely the liver.
An increase in the number of patients with diabetes mellitus (DM) is known to occur in Ukraine. Their number has currently exceeded 1 million people and tends to grow continually. The spread of diabetes mellitus in Ukraine has increased by more than 1.5 times over the past 10 years. Taking into account close functional interrelation between liver and pancreas [5, 6, 7]. consisting in liver participation in regulation of insulin activity (the main process providing glucose homeostasis, that is the synthesis and breakdown of endogenous glucose polymer glycogen, occurs in it) and the presence of HCV (namely genotypes 3a and 1b) in the body increasing the cells resistance to insulin, the mutual influence of chronic hepatitis C and diabetes is believed to be present [6, 8, 9]. Clinical studies indicate that the frequency of liver damage in patients with DM (the so-called "diabetic hepatopathy") constitutes 23-66%. The incidence of viral hepatitis is 8-10 times higher in case of insulin-dependent form of diseases than in healthy population [9].
Such patients respond to treatment worse, the course of infectious processes in them is more complicated due to changes in endocrine status, metabolism.
Thus, the above mentioned facts are aggravating in the course of infectious pathology, namely CHC. These issues are poorly presented in the available scientific literature. In addition, the

possibility of adverse effects and long-term complications in patients with CHC and DM, more severe course of the disease cause the need to improve conventional approaches to the treatment of this group of patients.

Literature data of recent years indicate that liver damage in case of CHC is caused both by the direct cytopathic effect of virus and by immune-mediated damage mechanisms with possible prevalence of any given at different stages of the disease [10, 12].

Peculiarities of CHC course are known to depend on immunopathological reactions [5, 12]. but unfortunately, immune system in the majority of patients with CHC is unable to eliminate the virus allowing it to replicate in hepatocytes for a long time [13].

Causative agent has the ability to "escape" from immunity effector mechanisms retaining its virulence and ability to cause chronic damage to the liver parenchyma and extrahepatic manifestations [5, 13].

In recent years the immune response as effector phase of immune reaction has been proved to be regulated by soluble mediators, namely cytokines. These low molecular weight proteins are produced mainly by activated immune system cells (macrophages, natural killer cells, T-lymphocytes) [10, 12]. Cytokines coordinate important processes in the liver: hepatocytes growth and their regeneration, inflammation processes, fibrosis and cirrhosis development [11]. Activation of Th-1 lymphocytes producing IL-2 leads to stimulation of cellular immune response that plays a crucial role in the virus elimination [11, 12]. On the other hand, activation of Th-2 lymphocytes producing IL-4 leads to the activation of humoral immune response that leads to long-term virus persistence with the development of process chronization and extrahepatic manifestations of CHC [10, 12].

Literature provides some data on the development of cytokine imbalance in case of diabetes mellitus as well. According to the researchers' data, anti-inflammatory interleukins induce intracellular proliferative changes in endothelial cells being one of the immediate causes of structural and functional damage to the vascular wall in patients with DM. They may also play a significant role in pathological processes of the liver in this group of patients [14, 15].

Therefore, we consider the study of the immune response nature in patients with CHC and concomitant DM to be important for better understanding of the pathological process severity, clinical symptoms and required treatment improvement.

The objective of the research was to study the level of interleukins 2 and 4 in patients with CHC and concomitant DM, their effect on the pathological process in case of this issue, efficacy of Dialipon and Lactuvite drugs affected by background therapy in this group of patients.

Materials and methods

104 patients with CHC were examined. The course of the disease associated with diabetes mellitus type II was observed in 84 patients. 20 patients with CHC without comorbidities constituted the experimental group. 84 patients with combined pathology underwent the specific combined antiviral therapy with pegylated interferon alpha-2b and alpha-2a in combination with ribavirin according to international recommendations for the treatment of chronic hepatitis C EASL (2006, 2009, 2012, 2013) and "Chronic Hepatitis C" (2014), unified clinical protocols of primary, secondary (specialized) care for adults and children. Treatment duration constituted 48 weeks according to international recommendations as the study included patients with genotype 1b. The dose of pegy lated interferon alpha-2b was determined in an amount of 1.5 μg/kg 1 time per week. Ribavirin dose required for combination therapy with pegy lated interferon alpha-2b was calculated according to patient body weight. In combination with antiviral therapy (AVT) the patients were prescribed alpha-lipoic acid in a dose of 300 mg/day intravenously by drop infusion per 200.0 ml of 0.9% sodium chloride solution for 10 days with subsequent transition to oral administration of the medication in a dose of 1 capsule (300 mg) in the morning for 60 days. Taking into account the impact of intestinal dysbacteriosis, the intensity of the imbalance of lipid peroxidation / antioxidant defense systems and cytokines, we used lactulose in the treatment regimen in a dose of 6.66 g once daily in the morning for two months.

Patients with CHC and concomitant DM type II were divided into 4 groups depending on the treatment. 20 patients (Group I) received only AVT. 21 patients (Group II) received alpha lipoic acid (ALA) in addition to AVT. 23 patients (Group III) received lactulose in addition to AVT. 20 patients (Group IV) received ALA and lactulose according to the proposed regimen along with AVT. The control group consisted of 20 apparently healthy individuals.

Monitoring of AVT effectiveness and safety was conducted on the basis of international recommendations on the management of patients with CHC undergoing standard AVT (EASL 2006, 2012-2014) on the 4th week of treatment (rapid viral response – RVR), 12-24th week (slow viral response – SLVR), 24th week after the therapy completion (sustained viral response – SVR).

Along with the assessment of viral response, the assessment of chemical responses achievement was conducted according to EASL (2006, 2012-2014) recommendations. Efficacy of the treatment was assessed according to the dynamics of virus reduction during the treatment and 24 weeks after the treatment.

Titers of cytokines were determined by ELISA test on the analyzer "Stat Fax 303 Plus" (USA) using standard reagents kit "Vektor Best" (Russia) in the Central laboratory for HIV infection, toxoplasmosis, venereal diseases and viral hepatitis diagnostics at the Regional Center of HIV Infection Prevention and AIDS Control at Regional Clinical Infectious Hospital in Ivano-Frankivsk.

All patients were included into the research after signing an informed consent.

Statistical processing of the research results was conducted on a PC using a standard package Statistica 5. Mean values (M), mean error (m), significance of differences according to Student's t-test were assessed. Pearson correlation coefficient was used to assess interrelation between the studied characteristics.

Results and discussion

Conducted research of blood serum established. As may be inferred from Table 1, cytokine profile was inadequate in all patients with CHC. Moreover, IL-2 level was not significantly different between the patients with CHC without comorbidity and the patients with CHC and MD ($p>0.05$) constituting 3.40±0.37 pg/ml and 3.11±0.16 pg/ml.

Table 1: Interleukin profile indices in patients with CHC and MD, M ± m

Indices	Groups			p_1	p_2
	Control (n=20)	Comparison (n=20)	Main (n=20)		
IL-2, pg/ml	10.11±0.33	3.40±0.37	3.12±0.15	<0.001	>0.05
IL-4, pg/ml	2.06±0.19	7.63±0.37	9.65±0.20	<0.001	<0.001

Notes.

p_1 – significance of difference in indices between the group of healthy people and patients with chronic hepatitis C and concomitant diabetes mellitus;

p_2 – significance especially if this group of patients suffeonic hepatitis C and the patients with chronic hepatitis C and concomitant diabetes mellitus.

According to the results of the research, IL-2 level in patients CHC and concomitant DM type II was significantly lower than in healthy individuals (3.12 ± 0.16 pg/ml vs. 10.11 ± 0.33 pg/ml, $p<0.001$) (Table 1) indicating the involvement of these cytokines in the activation of pathological changes in the body in case of chronic hepatitis C, especially if this group of patients suffered from the comorbidity when the target organ, namely liver, was pathogenetically affected. The content of anti-inflammatory cytokine IL-4 was significantly increased in patients with CHC and concomitant DM in comparison with the indices of healthy people (9.65 ± 0.20 pg/ml vs. 2.06 ± 0.19pg/ml, $p<0.001$) and patients with CHC without comorbidity ($p<0.001$) (Table 1).

The tendency to increase in IL-2 level in the patients of all studied groups was detected after 2 weeks of treatment compared to that before the treatment ($p<0.001$) with no statistically significant difference between the groups ($p>0.05$). The level of IL-4 did not decrease in all study groups after 2 weeks of treatment, although statistically significant difference between Groups I and IV was observed (7.89 ± 0.39 pg/ml vs. 9.69 ± 0.38 pg/ml, $p<0.01$).

IL-2 level in the patients of Group I increased on average by 2.1 times, but remained lower by 1.6 times in comparison with the indices of healthy individuals (7.70 ± 0.34 pg/ml vs. 10.11 ± 0.33 pg/ml, $p<0.001$) (Figure 1). IL-2 level in the patients of Group II increased compared to the data before the treatment (7.46 ± 0.11 pg/ml, 7.52 ± 0.17 pg/ml and 8.53 ± 0.14 pg/ml vs. 3.13 ± 0.28 pg/ml and 10.11 ± 0.33 pg/ml, $p<0.001$), but it remained still below the control indices ($p<0.001$) and indices of group IV ($p<0.01$, $p<0.05$). The level of IL-2 in the patients of Group III significantly increased during the treatment in comparison with its initial indices (from 3.18 ± 0.28 pg/ml to 6.28 ± 0.22 pg/ml, $p<0.001$), but it was significantly lower compared to the control group and Group II (p <0.001).

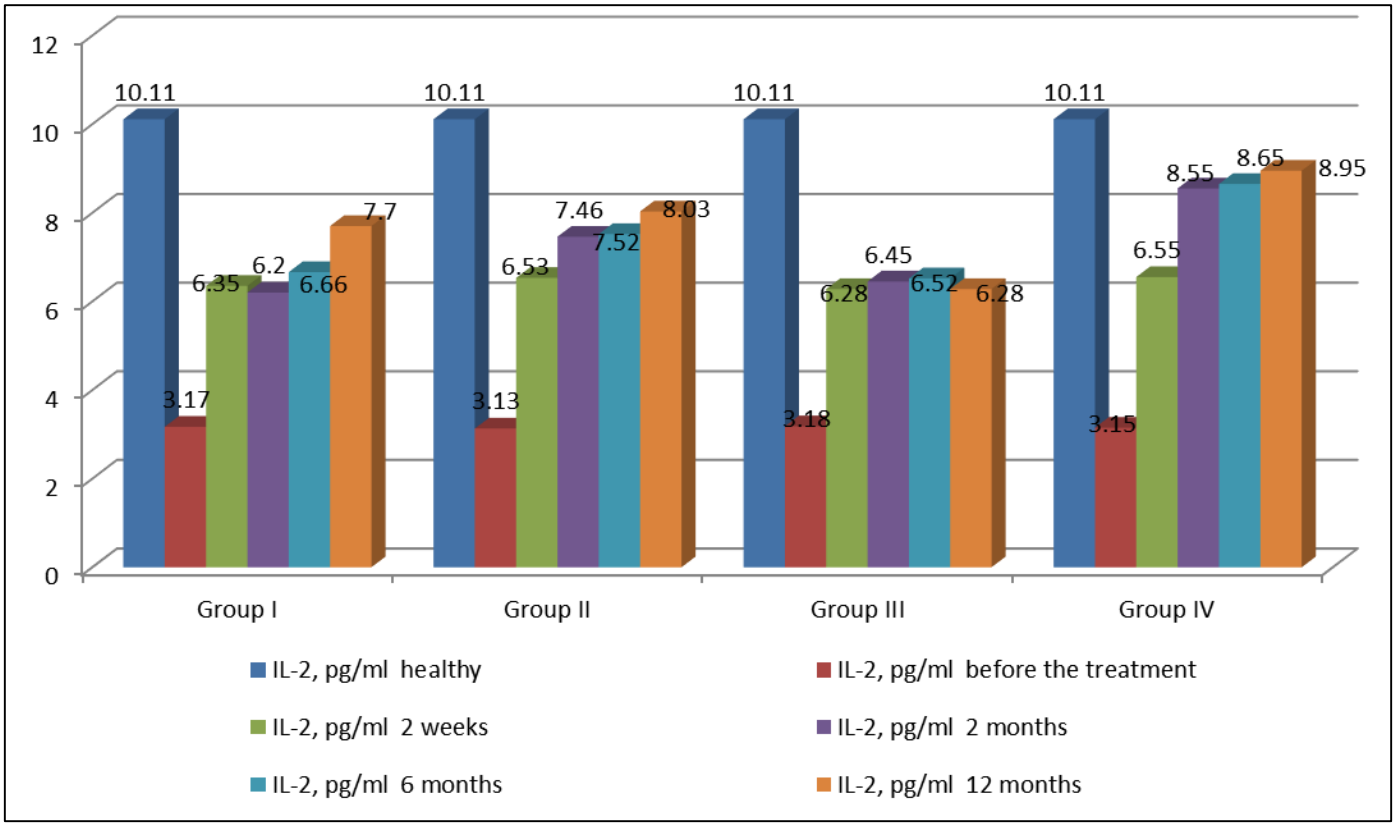

Fig 1: IL - 2 dynamics under the influence of treatment

IL-2 level in patients of Group IV increased by 2.6 times on average in 2 months in comparison with the initial data (8.60 ± 0.30 pg/ml vs. 3.15 ± 0.35 pg/ml, $p<0.001$), by 3 times in 6 months (9.15 ± 0.27 pg/ml vs. 3.15 ± 0.35 pg/ml, $p<0.001$) and by 2.6 times at the end of the therapy (8.15 ± 0.39 pg/ml vs. 3.15 ± 0.35 pg/ml, $p<0.001$) not being different from the indices of the control group ($p>0.05$) and being significantly higher in comparison with other study groups ($p<0.01$, $p<0.05$, $p<0.001$).

IL-4 level in patients of Group I decreased during the treatment ($p<0.001$) but did not reach the normal level ($p<0.001$) and was higher compared to Group IV ($p<0.001$) throughout the treatment (see Figure 2).

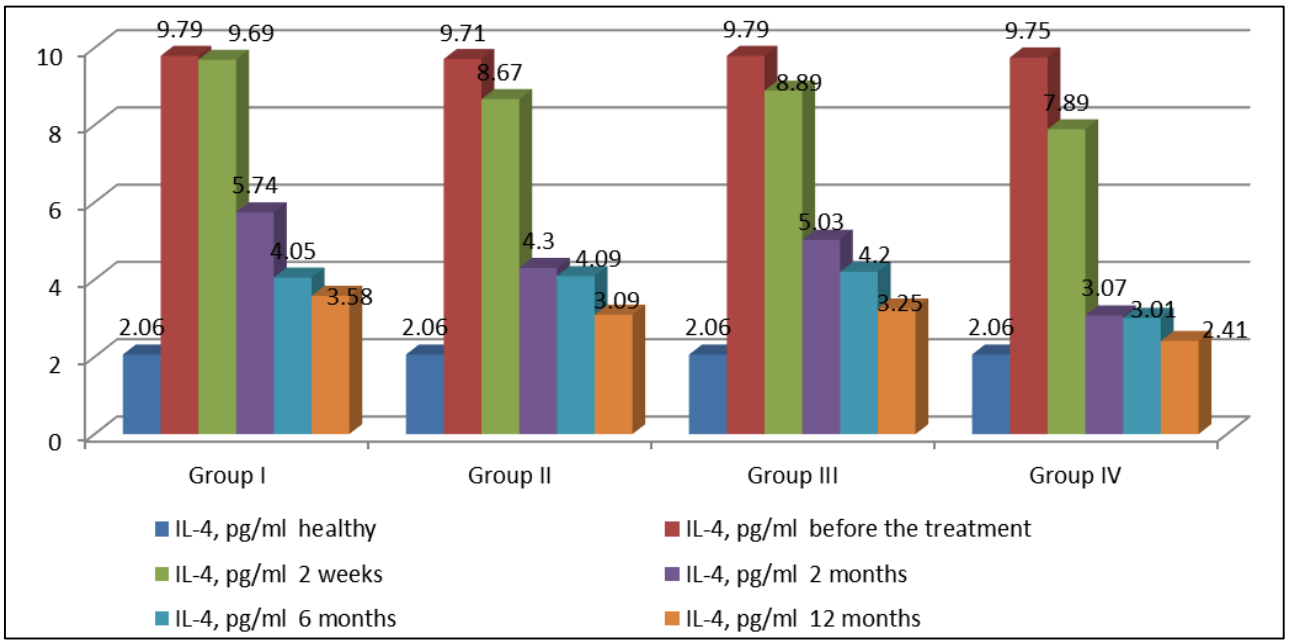

Fig 2: IL - 4 content dynamics under the influence of treatment

The level of IL-4 in the patients of Group III significantly decreased during the treatment as well ($p<0.001$), however it did not approximate the normal level ($p<0.001$) and was higher compared to Group IV in 2 months of the treatment and at the end of the treatment ($p<0.001$, $p<0$ 01). With ALA use in the treatment we observed the decrease in this index (from 9.71 ± 0.27 pg/ml to 3.09 ± 0.53 pg/ml, $p<0.001$), however it remained above the normal level ($p<0.001$) and was not significantly different from the indices in Group IV ($p>0.05$). Stabilization of this index occurred in the patients of Group IV. The levels of IL-4 in this group decreased to normal during the treatment remaining at the same level at the end of the treatment as well (3.07 ± 0.64 pg/ml, 3.01 ± 0.43 pg/ml and 2.41 ± 0.09 pg/ml vs. 2.06 ± 0.19 pg/ml, $p<0.001$) (Figure 2).

Thus, the data obtained during the research indicate that the improvement of CHC treatment in patients with concomitant diabetes mellitus type II using alpha-lipoic acid and lactulose on the background of AVT has a significant positive effect on cytokines level rebalancing. It may be safely suggested that this proposed treatment regimen will intensify T-cell immune responses providing more efficient AVT and faster elimination of hepatitis C virus.

Conclusions

1. Significant decrease in IL-2 level in the patients with concomitant DM type II by 3.2 times on average ($p<0.001$) in comparison with the control group and by 1.3 times compared to the group of patients without comorbidity ($p<0.001$) was detected during the research.
2. The level of IL-4 in the main group was 4.8 times higher than in the control group and 1.3 times higher compared to the group of patients without comorbidity ($p<0.001$).
3. IL-2 level increased by 2.6 times ($p<0.001$) on average after the treatment compared to the data before the treatment, but was 1.3 times below normal ($p<0.001$) in case of alpha lipoic acid use along with AVT in patients with CHC and concomitant DM type II. IL-4 level in blood serum was 3.2 times lower after the treatment in comparison with the indices before the treatment ($p<0.001$) and remained increased compared to the indices of the control group ($p<0.001$).
4. IL-2 level was significantly lower than in healthy individuals (6.28 ± 0.22 pg/ml vs. 10.11 ± 0.33 pg/ml, $p<0.001$) and increased by 1.6 times on average in comparison with its level before the treatment ($p<0.001$) in patients with chronic hepatitis C with concomitant diabetes mellitus type II after the use of lactulose in the treatment. IL-4 content in blood serum decreased by 3 times compared to the indices before the treatment ($p<0.001$) and remained significantly higher than in the control group (3.25 ± 0.25 pg/ml vs. 2.06 ± 0.19 pg/ml, $p<0.001$).
5. Treatment improvement with alpha-lipoic acid and lactulose promote cytokines rebalancing (IL-2 content constituted 8.95 ± 0.45 pg/ml and IL-4 content was 2.41 ± 0.09 pg/ml) with a significant difference in comparison with other study groups.

References

1. Hural AD, Mariyevskiy VF, Serheeva TA, Hepat C. in Ukraine: Epidemiological aspects. Suchasni infektsii. 2008; 1:53-60.
2. Zaitsev IA, Kobzeva VV, Shevliakova NA. Yestestvennoe techenie virusnogo hepatita C. News of medicine and pharmacy. 2009; 281:49-52.
3. Buyeverov A. Hepatitis C and concomitant hepatic pathology. Vrach. 2009; 5:22-26.
4. Tanashchuk EL, Serov VV. Patogenes NVV I NSU-infektsii pri alkogolnoi bolezni pecheni. Klinicheskaya meditsina. 2002; 1:5-9.
5. Vorozhbyt OB. The problem of extrahepatic lesions of chronic hepatitis C. Medical practice. 2002; 3(IX):97-101.
6. Vlasenko AV. Funktsionalnyi stan pechinky u khvorykh na tsukrovyi diabet, zhyrovu dystrofiiu pechinky ta pry iikh poiednanni. Vrachebnaya praktika. 2007; 3(57):105-109.
7. Gubergrits NB. Pancreas lesion at viral hepatitis. Contemporary Gastroenterology. 2008; 2(40):87-92.
8. Kuliesh OV. Course of chronic hepatitis c with insulin resistance syndrome depending on presence of metabolic disorders. Hepatology. 2013; 4(22):43-48.
9. Moucari R, Asselah T, Voitot H. Insulin resistance in

chronic hepatitis C: association with genotype I and 4, serum HCV RNA levels, and liver fibrosis. Gastroenterology. 2008; 134:416-423.
10. Neiko YeM, Virstiuk NH. Vplyv tsytokiniv na perebih khronichnoho hepatytu C. Zhurnal AMN Ukrainy. 2001; 7(3):576-583.
11. Malyi VP, Hololobova OV. Stan tsytokinovoi rehuliatsii u khvoryks na KhHS. Suchasni infektsii. 2007; 2:13-17.
12. Ivashkin VT, Mammaev SN, Lukina EA. Osobennosti imunnogo otveta u bolnykh khronicheskim virusnym hepatitom C. The Russian Journal of Gastroenterology, Hepatology, Coloproctology. 2001; 3:24-28.
13. Hempel G, Galle PR, Lohr HF. Characterzation of HCV-specific cellular and humoral immune responses referred of virus elimination. J Hepatology. 2000; 32(2):172.
14. Sybirna NO, Barska ML, Lapovets LYe. Deiaki pokaznyky klitynnoho i humoralnoho imunitetu u khvorykh na tsukrovyi diabet I typu. Laboratorna diahnostyka. 2003; 4:47-49.
15. Kotliarenko NM, Chebotariov VF, Zamotaieva HA. Interleikin-1 ta interleikin-2 v patohenezi insulinzalezhnoho tsukrovoho diabetu. Zhurnal AMN Ukrainy. 1999; 5(2):274-289.