Characteristics of fibrous formation and its relationship with the level of pro- and anti-inflammatory cytokines in patients with CHC

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Abstract. To solve the tasks set, 120 patients with chronic viral hepatitis were examined, including 52 patients with C with extrahepatic manifestations and 68 patients with CHC without extrahepatic manifestations under the age of 70 years. The results of the study showed that in CHC patients with cryoglobulinemia, with an increase in the stage of fibrosis, the content of IL-10 tended to gradually increase and the content of IL-18 gradually began to significantly decrease.

1 Introduction

Viral hepatitis is still one of the most urgent problems of world health, taking the 7th place among the causes of mortality from all diseases. According to the latest WHO estimates, approximately 58 million people worldwide suffer from chronic hepatitis C, with about 1.5 million new infections occurring each year. An estimated 3.2 million children and adolescents suffer from chronic hepatitis C [1, 10, 13]. According to EASL, CHC, accounting for 70–90% of primary liver cancers, is the 5th leading cause of cancer in Europe, with 1–13 new cases and 1–10 deaths per 100,000 inhabitants per year. CHC develops at a rate of up to 8% per year after the occurrence of cirrhosis associated with infection with viral hepatitis C (HCV) (average 1–4%) [2, 9, 15]. In addition, CHC can occur in the early stages of fibrosis or even without it [3, 11, 16].

Among chronic liver diseases, viral hepatitis ranks first, accounting for 40-60% of the total number of patients with chronic hepatitis. Currently, there are more than 175 million people in the world infected with the hepatitis C virus. The significance of this pathology is due to the high incidence rate, an increase in the number of virus carriers, a change in the age structure of those infected with a predominance of young people and an increase in the percentage of extrahepatic manifestations [4, 5, 12].

In CHC patients with extrahepatic manifestations, liver elastometry has a diagnostic value, which can be used as a non-invasive diagnosis of liver fibrosis. Liver elastometry allows comparing their diagnostic accuracy with the results of a morphological study of liver tissue, which is a very important criterion at all stages of development. Evaluation of hepatitis activity limits the therapeutic possibility of treatment, and the use of

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elastometry only as an independent method for monitoring the development of liver fibrosis [6, 7, 8].

The short duration and non-invasive nature of the study carried out during traditional ultrasound examination of the liver makes it possible to use elastometry as a guideline for screening the study of liver fibrosis and assessing its dynamics in patients with extrahepatic viral hepatitis C in wide clinical practice [9, 10, 11].

A special place in the literature is given to the immunological aspects of this pathology, where materials are given on the assessment of the immunocytokine status of CHC patients with extrahepatic manifestations. Information is provided on the role of IL-10 and IL-18 in the development of hepatitis C. However, the issues of humoral immunity and assessment of the features of changes in the concentration of cytokines depending on extrahepatic manifestations in patients with CHC, as well as the relationship between the stages of liver fibrosis and the content of cytokines, remain little studied. In this regard, studies devoted to this problem have not lost their relevance and relevance in theoretical and practical medicine.

The purpose of the study: to study the characteristics of fibrosis and its relationship with the level of pro- and anti-inflammatory cytokines in patients with CHC.

2 Materials and methods

120 patients with chronic viral hepatitis were examined, including 52 patients with C with extrahepatic manifestations (main group) and 68 patients with chronic viral hepatitis C without extrahepatic manifestations (comparison group) under the age of 70 years; in addition, 25 healthy individuals were examined (control group).

The diagnosis of CHC in patients was established on the basis of epidemiological anamnesis, clinical data, and laboratory and instrumental diagnostics.

Hematological parameters were studied using an automatic hematological analyzer BC-20S Mindray (China). Biochemical parameters of the blood test: total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, glucose (GLU), urea, creatinine were determined using an automatic biochemical analyzer MINDRAY BC-30 (China).

For our scientific work, a qualitative, quantitative analysis for the hepatitis C virus (viral RNA) and genotyping of the virus by PCR diagnostics was determined using DTlite 4 (RF).

The concentration of pro-inflammatory IL-18, anti-inflammatory IL-10 in blood serum was determined by solid-phase ELISA using the test systems of Vector-Best JSC (Novosibirsk, Russia), in accordance with the manufacturer's recommendations.

Liver elastography was performed using the French device Fibroscan ECHOSENS 430 (France). Elastometry is a modern method for assessing and diagnosing liver fibrosis in chronic hepatitis C. It was also found that, in contrast to the later stages of fibrosis (F-3, F-4 for METAVIR), the diagnostic significance of the method decreases at low stages of fibrosis (F- 1, F-2 for METAVIR).

Transient elastography results are expressed in kilopascals (kPa) and represent an average of ten measurements ranging from 2.5 to 75 kPa. Normal values are more than 5.5 kPa.

3 Result and discussion

According to the results of liver elastography of the examined patients, the distribution of fibrotic processes in patients with chronic hepatitis C shows that in the first group of patients (main group, n=52) fibrotic processes amounted to:

F0 - 9.62±4.09% (n=5), F1 - 30.77±6.40% (n=16), F2 - 32.69±6.50% (n=17), F3 - 19.23±5.47% (n=10), F4 - 7.69±3.69% (n=4) of cases (Table 1).

Table 1. Distribution of fibrous processes in patients with CHC in comparative terms.

Stages of fibrosis	with CG, n=52		without CG, n=68	
	abs	%	abs	%
F0	5	9,62±4,09	13	19,12±4,77* ↑
F1	16	30,77±6,40	23	33,82±5,74 ↔
F2	17	32,69±6,50	23	33,82±5,74 ↔
F3	10	19,23±5,47	8	11,76±3,91 ↔
F4	4	7,69±3,69	1	1,47±1,46* ↓

Note: * - sign of reliability between parameters with and without cryoglobulinemia; ↑, ↓ - direction of changes; ↔ - no reliability; CG - cryoglobulinemia.

In patients of the second group (comparison group, n=68), fibrotic processes were: F0 - 19.12±4.77% (n=13), F1 - 33.82±5.74% (n=23), F2 - 33.82±5.74% (n=23), F3 - 11.76±3.91% (n=8), F4 - 1.47±1.46% (n=1) cases.

Comparison of the given parameters in table. 4.2 showed that the F0 values were significantly higher in patients with chronic hepatitis C without cryoglobulinemia (comparison group) - respectively 9.62±4.09% versus 19.12±4.77% ($P<0.05$), in addition, F4 parameters were significantly lower in the comparison group - respectively 7.69±3.69% versus 1.47±1.46% ($P<0.05$).

If in the first case the difference between the compared groups in fibrous lesions of the F0-F1 stage was equal, 1.31 times in favor of the comparison group ($P<0.05$). In the second case, fibrous lesions of the F2-F3 stage, this difference reached 1.13 times, but the benefit was already in the main group.

These facts indicate that the fibroscopic picture in patients of the first group is significantly worse than in patients of the second group. If we consider that there is only one sign between the compared groups (the presence of cryoglobulinemia), then it becomes clear that the presence of cryoglobulins in the blood of patients with CHC is a complicating course of this pathology in patients, and also accelerates the process of fibrosis in the liver.

Thus, it was found that among patients of the first group, extrahepatic manifestations associated with cryoglobulinemia are more common, in addition, these patients more often have higher stages of liver fibrosis, which suggests that the presence of cryoglobulins in the blood of patients is an aggravating factor in the course and outcome of CHC in examined patients.

Next, we presented comparative parameters of the results of the study on the detection of fibrotic processes and elastometric parameters detected by liver elastometry with the level of cytokines in the serum of CHC patients with extrahepatic manifestations.

To do this, all the results on the detection of fibrotic processes were compared with the indices of blood serum cytokines in the dynamics of the formation of the fibrous process. The results obtained were given separately for IL-10 and IL-18, since the purpose of these cytokines was different.

Comparative parameters of IL-10 and the presence of a fibrous process are given in table. 2 in a comparative aspect in CHC patients with and without cryoglobulinemia.

Table 2. Distribution of fibrous processes in patients with CHC in comparative terms.

Stages of fibrosis	with CG, n=52		without CG, n=68	
	Fibrous process abs / %	IL-10 (normal 18,61±1,02 pg/мl)	Fibrous process abs / %	IL-10 (normal 18,61±1,02 pg/мl)
F0	5/9,62	9,42±0,99	13/19,12	6,29±0,84
F1	16/30,77	10,04±0,83	23/33,82	6,19±0,96
F2	17/32,69	10,76±0,91	23/33,82	6,85±0,73
F3	10/19,23	12,11±0,78	8/11,76	7,32±0,91
F4	4/7,69	15,24±0,87	1/1,47	7,53±0,90

Note: * - sign of reliability between parameters with and without cryoglobulemia; ↑, ↓ - direction of changes; ↔ - no reliability; CG - cryoglobulinemia.

As can be seen from Table. 2 at the stage of fibrosis F0 (healthy liver), the content of IL-10 (9.42±0.99 pg/ml) remained below the control values (18.61±1.02 pg/ml), such a significantly reduced state is typical for this anti-inflammatory cytokine, as there were no signs of overt inflammation. Such a reduced state was also determined in patients without cryoglobulinemia (6.29±0.84 pg/ml). There is a significant difference between the compared groups ($P<0.05$).

With an increase in the stage of fibrosis, the content of IL-10 in patients with cryoglobulinemia gradually began to increase. In patients with fibrosis stage F1 and F2, the content of IL-10 in the blood serum increased to 10.04±0.83 pg/ml and 10.76±0.91 pg/ml, respectively, although the results did not differ significantly from those with the stage fibrosis F0 ($P>0.05$), but an increase trend is visible. This fact indicates the relationship between the content of cytokines in the blood serum and the stage of fibrosis in patients. Further, with an increase in the stage of fibrosis, the tendency to increase the anti-inflammatory cytokine IL-10 remained, so at the stage of fibrosis F3 and F4, the content of this cytokine was 12.11±0.78 pg/ml and 15.24±0.87 pg/ml, respectively, which is reliable 1.29 and 1.62 times more than the initially obtained figures at the stage of fibrosis F0 ($P<0.05$).

We obtained a somewhat different picture in a comparative study of the obtained results on the dynamics of changes in the content of cytokines depending on the stage of fibrosis in patients without cryoglobulinemia. If at the stage of fibrosis F0 of IL-10 was 6.29 ± 0.84 pg/ml, then with an increase in the stage of fibrosis, the content of IL-10 changed slightly ($P> 0.05$), even at the stage of fibrosis F4, the increase in the content of this cytokine was insignificant (7.53±0.90 pg/ml), not significantly different from the previous indicators ($P>0.05$).

Thus, a comparative study of the content of IL-10 depending on the stage of fibrosis in patients with CHC with and without cryoglobulinemia showed that in CHC patients with cryoglobulinemia, with an increase in the stage of fibrosis, the content of IL-10 tended to gradually increase, but at F1 and F2, the content of this cytokine did not differ from these individuals with F0 fibrosis stage ($P>0.05$). Further, with an increase in the stage of fibrosis, the trend of increasing IL-10 remained, at the stage of fibrosis F3 and F4, the content of this cytokine was 1.29 and 1.62 times significantly higher than the initially obtained figures at the stage of fibrosis F0.

Identical studies were carried out with the pro-inflammatory cytokine IL-18, where the relationship between an increase in the stage of fibrosis and the content of IL-18 in the blood serum of CHC patients with and without cryoglobulinemia was also comparatively studied. The results are shown in table. 3.

It was established that the initial content of IL-18 was higher than the normative values (68.02±2.36 pg/ml) in both compared groups - respectively 204.46±16.64 pg/ml in patients with and 164.68±12, 20 pg/ml without cryoglobulinemia ($P<0.001$). But with an

increase in the stage of fibrosis in patients with cryoglobulinemia, the content of IL-18 gradually began to decrease - from 204.46±16.64 pg/ml at the stage of fibrosis F0 to 134.23±14.76 pg/ml at the stage of fibrosis F4 (P< 0.001).

Table 3. Distribution of fibrous processes in patients with CHC in comparative terms.

Stages of fibrosis	with CG, n=52		without CG, n=68	
	Fibrous process	IL-18 (normal 68,02±2,36 pg/мl)	Fibrous process	IL-18 (normal 68,02±2,36 pg/мl)
	abs/ %		abs / %	
F0	5/9,62	204,46±16,64	13/19,12	164,68±12,20
F1	16/30,77	186,71±12,33	23/33,82	158,17±10,38
F2	17/32,69	169,94±13,52	23/33,82	151,32±10,07
F3	10/19,23	161,59±15,04	8/11,76	153,94±11,84
F4	4/7,69	134,23±14,76	1/1,47	149,87±12,12

Note: * - sign of reliability between parameters with and without cryoglobulinemia; ↑, ↓ - direction of changes; ↔ - no reliability; CGE - cryoglobulinemia.

Other results were obtained in CHC patients without cryoglobulinemia, where the content of the cytokine IL-18 was reduced slightly and not significantly depending on the stage of fibrosis (P>0.05).

4 Conclusions

Upon completion of the study, we came to the following conclusions: in CHC patients with cryoglobulinemia, with an increase in the stage of fibrosis, the content of IL-10 tended to gradually increase; .29 and 1.62 times significantly more than the initially obtained figures at the stage of fibrosis F0;

With an increase in the stage of fibrosis in patients with cryoglobulinemia, the content of IL-18 gradually began to significantly decrease. Other results were obtained in CHC patients without cryoglobulinemia, where the content of the cytokine IL-18 was reduced slightly and not significantly depending on the stage of fibrosis.

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