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RELATIONSHIP OF CLINICAL COURSE, BIOCHEMICAL AND IMMUNOLOGICAL CHANGES IN PATIENTS WITH CHRONIC HEPATITIS B+C

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Abstract

Recent studies have shown that parenteral viral hepatitis is one of the main causes of chronic diffuse liver diseases. The number of patients not only with chronic hepatitis C and B, but also patients with hepatitis of mixed etiology is increasing annually. At the same time, there is a potentiating effect of the association of HBV and HCV with a high risk of developing progressive forms of chronic viral hepatitis or hepatocellular carcinoma. Correlation of the clinical course, biochemical indices in patients with viral mixed infection of the liver have not been studied enough. In the studies of a number of authors, it has been shown that with mixed infection, erased and latent forms of the disease are rarely observed. **The aim** of the study is to study the immune status and biochemical parameters in patients with chronic hepatitis B+C for a more accurate assessment of the severity of the pathological process. **Materials and methods.** 62 patients with chronic hepatitis B+C were examined. All the patients under examination were monitored in the hepatological center of the Odessa Municipal Infectious Hospital (Ukraine). The assessment of the severity of the patients' condition was carried out using biochemical indicators. Confirmation of the diagnosis based

on determination (qualitatively and quantitatively) HCV RNA and HBV DNA by polymerase chain reaction. Subpopulations of B- and T-lymphocytes (CD19+, CD16+, CD8+, CD4+, CD3+) were determined by the immunofluorescence method using a set of polyclonal and monoclonal antibodies to establish differential antigens of human lymphocytes using the Eurostar immunofluorescence microscope. **Results and discussions.** When analyzing the main clinical syndromes in dynamics, it was found that in patients with chronic hepatitis B+C with initial of fibrosis (F0-F1), less pronounced changes in the general condition were observed than in patients with moderate (F2) and advanced fibrosis (F3). The presence of a correlation between the degree of fibrosis and immunological indices in patients with chronic hepatitis B+C allows us to use the information obtained as one of the additional criteria for the severity of the pathological process. The expressiveness of changes of immune status indicators can serve as an additional criterion for the degree of morphological abnormalities in liver tissue.

Key words: chronic hepatitis B+C; morphological abnormalities; liver tissue.

Introduction

Recent studies have shown that parenteral viral hepatitis is one of the main causes of chronic diffuse liver diseases. The number of patients not only with chronic hepatitis C and B, but also patients with hepatitis of mixed etiology is increasing annually. At the same time, there is a potentiating effect of the association of HBV and HCV with a high risk of developing progressive forms of chronic viral hepatitis or hepatocellular carcinoma [1].

Chronic viral hepatitis are the result of superinfection, less often - coinfection with sequential or simultaneous activity of several hepatotropic viruses. At the same time, HCV replication is observed in 64% of cases, and HBV replication - in 58% of cases [2].

Simultaneous replication of more than one type of hepatitis virus contributes to the progression of a chronic process with transformation into liver cirrhosis [3].

In most cases of co-infection (HBV+HCV), only HCV replication is detected. At the initial stage of the disease, replication of both viruses probably takes place, however, later on, replication of one of the viruses, more often HBV, is suppressed. At the same time, HCV infection can induce HBeAg seroconversion [4].

There are reports in the literature that when infected with several viruses, the genome of one or two viruses is suppressed (viral interference). However, simultaneous reproduction with a cumulative effect is also possible, which leads to a progressive course of the pathological process [5].

Correlation of the clinical course, biochemical indices in patients with viral mixed infection of the liver have not been studied enough. In the studies of a number of authors, it has been shown that with mixed infection, erased and latent forms of the disease are rarely observed. Hepatitis B+C is characterized by high biochemical activity and more pronounced changes in the histological structure of the liver, a pronounced tendency to the formation of portal hypertension syndrome already at the early stages of the disease [6].

In many viral diseases, immunosuppression is developing not only in chronic hepatitis B+C. There are a few studies of the immune status, which indicate that with mixed infection there is a high level of CD16+, a significant decrease in CD4+, a tendency to a decrease in CD8+, as well as defects in the macrophage link [7]. Information in the works of various authors are contradictory and need further study.

It has been established that in patients with co-infection HBV+HCV the level of many cytokines increases: TNF α , IL-1 β , IL-6, IL-8, TGF β . However, the obtained data on the study of the cytokine profile are often contradictory, which is explained by the different age and duration of the disease in the examined patients [8].

Thus, the problem of combined viral hepatitis requires further research in order to develop effective methods of diagnosis and treatment.

The aim of the study is to study the immune status and biochemical parameters in patients with chronic hepatitis B+C for a more accurate assessment of the severity of the pathological process.

Materials and methods

62 patients with chronic hepatitis B+C were examined. All examined patients were under monitoring in the hepatological center of the Odesa Infectious Hospital. There are 42 men and there only 20 women in group of studied patients. The control group consisted of 30 practically healthy persons of a similar age. There were 15 women and 15 men.

All patients who were included in the study have given free and informed consent. The methodology of this investigation is in accordance with the requirements of the Committee on Bioethics of the Odessa National Medical University (protocol 179 of 19.11.2010).

At the moment of admission such clinical syndromes as general intoxication (fatigue, general weakness), dyspeptic (lack or decreased appetite, nausea, vomiting, feeling of heaviness in the right hypochondrium), jaundice, hepato- and splenomegaly were marked.

The assessment of the severity of the patients' condition was carried out using such biochemical indicators as the concentration of total bilirubin and its fractions, the activity of ALT and AST, the thymol test, the prothrombin index, the concentration of total protein,

albumin and globulin.

Confirmation of the diagnosis based on determination (qualitatively and quantitatively) HCV RNA and HBV DNA by polymerase chain reaction. Hepatitis markers were also detected by enzyme immunoassay: HBsAg, HBeAg, aHBcor-IgM, aHCV-IgM.

Subpopulations of B- and T-lymphocytes (CD19+, CD16+, CD8+, CD4+, CD3+) were determined by the immunofluorescence method using a set of polyclonal and monoclonal antibodies to establish differential antigens of human lymphocytes using the Eurostar immunofluorescence microscope.

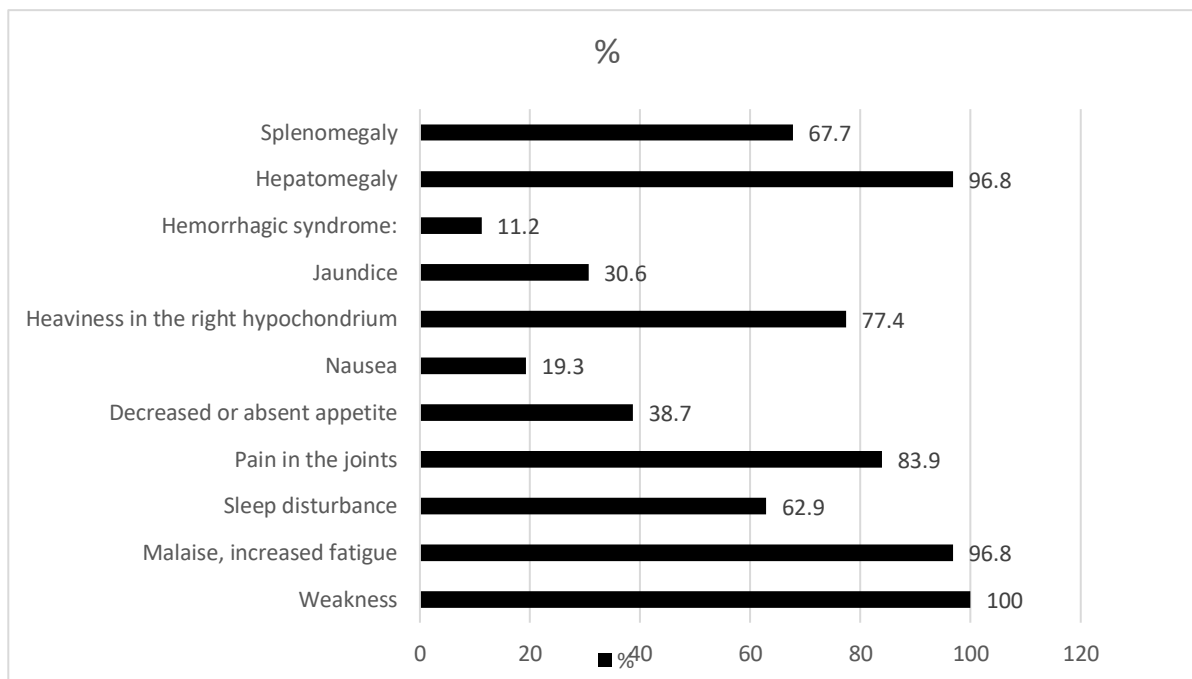
The degree of liver fibrosis was established by FibroScan. FibroScan is a noninvasive method for assessing the degree of liver fibrosis, which is implemented using a special apparatus. The FibroScan liver examination is based on the measurement of liver elasticity. The ultrasonic sensor of the instrument generates medium amplitude and low frequency oscillations. These vibrations pass through the skin, subcutaneous tissues and create in the liver.

The obtained data were processed using the PSPP program and LibreOffice open source software. Comparison of immunological and biochemical parameters in patients with hepatitis B+C and practically healthy individuals was carried out using the Mann-Whitney test. The relationship of fibrotic changes in hepatic tissue, parametric and non-parametric parameters was evaluated using the Spearman rank correlation coefficient.

Results and discussions

When analyzing the main clinical syndromes in dynamics, it was found that in patients with chronic hepatitis B+C with initial of fibrosis (F0-F1), less pronounced changes in the general condition were observed than in patients with moderate (F2) and advanced fibrosis (F3).

Asthenovegetative syndrome was determined in all patients with chronic hepatitis B+C, dyspeptic syndrome - in 96.8% (60 patients), arthralgia - in 40.3% (25 patients). Jaundice was registered in 30.6% (19 patients). The hepatomegaly 96.8% (60 patients) and splenomegaly 67.7% (42 patients) were revealed (Figure 1).



The clinical picture of chronic hepatitis was characterized by periods of exacerbation and remission of the studied signs, but all patients before starting antiviral therapy noted significant changes in the general condition that reduced the quality of life.

A comparison of biochemical parameters of patients with chronic hepatitis B+C and healthy persons is presented in table 1.

Table 1

Biochemical indexes in patients with chronic hepatitis B+C(M±m)

	Patients with chronic hepatitis B+C, n=62	Healthy persons, n=30
Total bilirubin, μmol/l	19,3±1,33	10,4±1,11
ALT, mmol/l/h	3,29 ± 0,91	0,58 ± 0,04
AST, mmol/l/h	2,27 ± 0,74	0,35 ± 0,02
Thymol test	8,9 ± 0,07	3,5 ± 0,04
Total protein, g/l	64,09 ± 0,68	84,09 ± 0,71
Albumin, g/l	35,45 ± 0,55	53,45 ± 0,45
Globulins γ, g/l	7,29 ± 0,17	12,11 ± 0,06
Prothrombin index, %	94,04 ± 0,39	94,04 ± 0,39

In patients with chronic hepatitis B+C changes of different biochemical indices were determined: in comparison with the indicators of healthy persons, the level of ALT increased

by 4.8 times, AST - by 6.5 times. The level of total protein in patients with chronic hepatitis B+C was 1.3 times lower than in healthy persons, albumin by 1.5 times, and γ -globulins by 1.7 times.

In the group of patients with hepatitis B+C, the distribution of patients according to the degree of fibrosis according to the results of FibroScan was as follows: the minimal degree of fibrosis (F0-F1) of the liver was detected in 40.3% (25 patients), moderate degree (F2) - in 17.8% (11 patients) and advanced fibrosis (F3) – in 41.9% (26 patients).

The investigation of lymphocyte`s subpopulations of in the peripheral blood of patients with chronic hepatitis B+C established a low expression of CD3+ and CD4+, as well as an increase in the number of cells expressing CD8+ and CD19+ antigens compared to the indicators of healthy persons (Table. 2).

Table. 2

Immunological indexes in patients with chronic hepatitis B+C(M \pm m)

Index	Patients with chronic hepatitis B+C,n=62	Healthy persons,n=30
Leukocytes, 10 ⁹ /l	5,40 \pm 0,42*	6,80 \pm 0,26
Lymphocytes, %	25,7 \pm 1,23*	31,2 \pm 1,04
Lymphocytes, abs.	1,56 \pm 0,04*	1,86 \pm 0,05
CD3+, abs.	1,10 \pm 0,02*	1,52 \pm 0,01
CD3+, %	49,12 \pm 2,60*	71,8 \pm 1,92
CD4+, abs.	0,39 \pm 0,04*	0,78 \pm 0,06
CD4+, %.	35,83 \pm 2,81*	41,2 \pm 1,5
CD8+, abs.	0,56 \pm 0,04*	0,48 \pm 0,02
CD8+, %.	29,71 \pm 1,32*	20,5 \pm 1,3
CD16+, abs.	0,12 \pm 1,8*	0,22 \pm 0,02
CD16+, %	7,18 \pm 1,07*	14,1 \pm 1,6
CD19+, abs.	0,32 \pm 0,17 *	0,24 \pm 0,19
CD19+, %.	16,14 \pm 1,24*	10,8 \pm 1,2

The relationship between fibrotic changes in the liver tissue, biochemical indices and immune status indicators was evaluated using the Spearman rank correlation coefficient (Table 3).

Table. 3

Correlation between biochemical and immunological indices of patients with chronic hepatitis B+C

	Degree of fibrosis	CD3+, %	CD4+, %	CD8+, %	CD16+, %	CD19+, %
Degree of fibrosis	1	-0,778**	-0,821**	0,171	-0,759**	0,673**
CD3+, %	-0,778**	1	0,904**	-0,406*	0,912**	-0,625**
CD4+, %	-0,821**	0,904**	1	-0,440*	0,932**	-0,659**
CD8+, %	0,171	-0,406*	-0,440*	1	-0,445*	0,320
CD16+, %	-0,759**	0,912**	0,932**	-0,445*	1	-0,752**
CD19+, %	0,673**	-0,625**	-0,659**	0,320	-0,752**	1

** - $p < 0,01$

The relationship between the following indicators was revealed:

- inverse average correlation between the degree of fibrosis and the content of CD3+, $p < 0.01$ (in patients with chronic hepatitis B+C with initial degree of fibrosis, a greater number of CD3+ lymphocytes is noted);
- a strong inverse correlation between the degree of fibrosis and the CD4+ content, $p < 0.01$ (in patients with chronic hepatitis B+C with initial degree of fibrosis, a greater number of CD4+ lymphocytes is noted);
- inverse average correlation between the degree of fibrosis and the content of CD16+, $p < 0.01$ (in patients with chronic hepatitis B+C with initial degree of fibrosis, a greater number of CD16+ lymphocytes is noted);
- direct average correlation between the degree of fibrosis and the content of CD19+, $p < 0.01$ (in patients with chronic hepatitis B+C with initial degree of fibrosis, a smaller amount of CD19+ lymphocytes is noted);

The presence of a correlation between the degree of fibrosis and immunological indices in patients with chronic hepatitis B+C allows us to use the information obtained as one of the additional criteria for the severity of the pathological process. The expressiveness of changes of immune status indicators can serve as an additional criterion for the degree of morphological abnormalities in liver tissue.

Conclusions

1. The obtained data on the relationship between the degree of liver fibrosis and immunological parameters in patients with chronic hepatitis B+C can be used in clinical practice to assess the features of the course and outcome of the disease to create an individual patient management plan.

2. More pronounced changes in biochemical and immunological indicators are noted in patients with chronic hepatitis B+C with the degree of fibrosis F2-F3.

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The article presented is a result of the author's research work

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki. The methodology of this investigation is in accordance with the requirements of the Committee on Bioethics of the Odessa National Medical University (protocol 179 of 19.11.2010).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The author declares no conflict of interest.