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CHANGES IN IMMUNOLOGICAL PARAMETERS IN CIRRHOSIS OF THE LIVER IN THE OUTCOME OF CHRONIC HEPATITIS C VIRUS INFECTION

Tashpolotova A.Sh.[1], Alisherova A.Sh.[2], Suranbaeva G.S.[1,2], Nurmatov K.K.[1]
[1]*Scientific and Production Centre for Preventive Medicine of the Ministry of Health of the Kyrgyz Republic, Bishkek, e-mail: npopm@mail.ru*
[2] *I.K. Akhunbaev Kyrgyz State Medical Academy, Bishkek*

The paper presents an assessment of the state of the immune status in patients with liver cirrhosis in the outcome of chronic hepatitis C virus (HCV) infection in relation to the severity of liver cirrhosis (according to Child-Pugh). When assessing T cell immunity, it was determined that the level of T lymphocytes (35.2±1.5) was significantly lower for the pronounced degree of disease activity of class C according to Child-Pugh. In this group of patients, T lymphocytes (T-helper cells) were also low at 26.3±2.0%. The relative content of T lymphocytes is observed to decrease with the lengthening of the infectious process. The smallest number of Tlymphocytes was found in patients with a disease lasting 11 and more than 15 years (P<0.005). In patients with liver cirrhosis in the outcome of chronic HCV infection, besides changes in immunological parameters, the leading clinical syndrome was the astheno-vegetative one, which manifested itself mainly in general weakness and was observed in the vast majority of patients (89.0%). Dyspeptic manifestations were reflected in complaints of poor appetite (78.5%), nausea (58.7%), vomiting (19.5%), aching pain in the right hypochondrium (70.7%). Arthralgia and skin itching were observed in 56.0% and 55.0% of patients, respectively. Hemorrhagic syndrome, in the form of nosebleeds and bleeding gums, was observed in 72 (63.3%) patients. Esophageal varices of varying degrees was diagnosed in more than half of the patients (57.1%). Portal hypertension was a very severe, life-threatening manifestation of the bleeding esophageal varices (29%). The results obtained by us in the immunological study can be additionally used as prognostic criteria for the course of liver cirrhosis in the outcome of chronic hepatitis C.

Keywords: chronic hepatitis C virus infection, liver cirrhosis, clinical features, immune status, outcome

The problem of chronic viral hepatitis C infection has been acquiring particular importance throughout the world for many years because of the growth of the number of infected people becoming a medico-social problem despite the achieved progress [1, 2, 3]. According to the recent years' data of the World Health Organization the number of patients with hepatitis C virus (HCV) infection in the world is estimated at about 3% of the world's population or 170 million people of whom 129 million are chronic HCV cases. Annually 500 thousand people die from the consequences of hepatitis C infection, and a double increase in these numbers is forecast over the next 10 years [4]. Currently, hepatitis C infection has come to occupy the 1st place in the etiologic structure of chronic liver diseases. With unfavorable course chronic HCV infection presents a high risk of liver cirrhosis (LC) and hepatocellular carcinoma (HCC) with population prevalence rates from 3% (range: 0.3% to 14.5%) to 5% [5].

In patients with chronic viral hepatitis C, antiviral immune response plays a significant role in the development of fibrosing processes. There is a considerable role of natural killers (NK cells) and T-lymphocytes which are presented in large quantities in the liver [6]. High cytotoxicity of lymphocytes and natural killers is considered to be associated unfavorable prognosis of HCV-infection, since the excess nonspecific injury of hepatocytes stimulates the fibrosis of the hepatic parenchyma [7]. At present, there are few studies on the peculiarities of the immune status of patients with liver cirrhosis in the outcome of chronic HCV infection. Previous studies dealt mostly with the immunopathogenesis of chronic HCV infection and its role in the progression of liver fibrosis [8].

Cirrhosis of the liver in the Kyrgyz Republic also causes special concern, because its prevalence increased from 30.6 0/0000 in 2008 to 37.6 0/0000 in 2017 (1,3-fold) over the decade. The greatest changes in the rates were observed in the southern region of the Kyrgyz Republic. Thus, in the Jalal-Abad region, the rate increased 1.9 times during this period. This problem in our country acquires high medico-social importance due to the increasing related mortality and disability ratess among the working-age population [9, 10, 11].

Thus, all patients with an unfavorable course of chronic HCV infection develop liver fibrosis and hepatocellular carcinoma. In this regard, the search for significant criteria for the progression of chronic HCV infection, i.e. especially in transition to cirrhosis, remains an extremely urgent issue for clinician doctors. The timely solution of this problem will help determine the choice of adequate tactics in the management of patients with cirrhosis of the liver in the outcome of chronic HCV infection.

Purpose of the study

To study changes in immunological parameters in patients with cirrhosis of the liver in the outcome of chronic HCV infection.

Materials and methods

The paper presents the results of immunological biochemical surveys of 224 patients aged 16 to 60 years with the diagnosis of liver cirrhosis in the outcome of chronic HCV infection. The diagnosis of liver cirrhosis in the outcome of chronic HCV infection was established on the basis of comprehensive clinical and epidemiological data, anamnesis, physical examination, the results of biochemical studies, determination of HCV RNA amounts and HCV RNA genotyping by the method of polymerase chain reaction (PCR). The laboratory identification of the etiological factor was carried out in the Republican Reference Laboratory for the Diagnosis of Viral Infections (Scientific and Production Centre for Preventive Medicine) by enzyme immunoassay (EIA). Viral hepatitis markers for HVA (anti-HAV-IgM), HBV (HBsAg, a-HBC-IgM, IgG, HBeAg), HDV (a-HDV-IgG), C (a-HCVIg total) were determined. All patients underwent immune status examination. The set of immunological methods included first and second level tests. With the help of the rosette test, the absolute and relative content of T-lymphocytes ε-rosette forming cells, regulatory subpopulations of T-cells by the sensitivity of ε-receptors to theophylline (ε-theophylline resistant and ε-theophylline-sensitive cells), absolute and relative content of B-lymphocytes (M-rosette-forming cells) were determined. The phagocytic characteristic of neutrophilic granulocytes was determined with respect to latex particles with the counting of the phagocytic index and the phagocytic number. The quantitative analysis of serum immunoglobulins of G, M, A classes was performed using Mancini radial immunodiffusion assay in gel.

Statistical analysis of data was carried out using the software application packages EpInfo and Microsoft Excel-2000. Significance of difference was determined by Student's T-test.

Results of research and discussion

Chronic viral hepatitis with the outcome in the cirrhosis is characterized by a variety of clinical manifestations. The main ones were asthenovegetative, dyspeptic, hemorrhagic, edematic-ascitic, depressive and hepatoprive syndrome. The hepatoprive syndrome in patients manifested itself mainly in hepatic encephalopathy (emotional lability – 57.1%, sleep disorder – 49.0%, nervousness – 66.0% of cases).

The leading syndrome in patients was asthenovegetative, which manifested itself mainly in general weakness and was noted in the overwhelming majority of patients (89.0%). Dyspeptic syndrome expresses itself in complaints about poor appetite (78.5%), nausea (58.7%), vomiting (19.5%), and pain in the right hypochondrium (70.7%). Arthralgia and itching of the skin were observed in 56.0% and 55.0% of patients, respectively. Hemorrhagic syndrome, in the form of nasal bleeding and gum bleeding, was observed in 72 (63.3%) patients.

Esophageal varicose veins dilatation to varying degrees was diagnosed in more than half of patients (57.1%). A very severe, threatening manifestation of portal hypertension was bleeding from esophageal varices (29%). The overwhelming majority of patients had a compensated liver cirrhosis phase – 52.2%. (class A by Child-Pugh). In 30.5% of patients a Child-Pugh class B was recorded. And the rest of the patients had the decompensated stage of the disease (class C by Child-Pugh – 17.3%).

When assessing T-cell immunity, it was determined that the level of T lymphocytes was significantly lower for a pronounced degree of activity of the disease in patients with class C by Child-Pugh – 35.2 ± 1.5 (P_{1-2} <0.05; P_{1-2} <0.01). In this group of patients, T-lymphocytes (T-helpers) were also low at 26.3 ± 2.0%, which turned out to be statistically insignificant (P_{1-2}> 0.05; P_{1-3}> 0.05). Changes in the content of suppressors T-cells were of an ambiguous character for all degrees of liver cirrhosis.

The level of the phagocytic index in Child-Pugh class A and B patients remained within normal values unlike Child-Pugh class C patients. The content of B lymphocytes decreased insignificantly in Child-Pugh class C patients equaling 26.2 ± 3.4 (p> 0.05).

The apoptosis index was significantly higher among patients with pronounced disease activity (class C) compared to those with minimal disease activity of class A (p <0.05). Both the apoptosis level and the cytoproliferation index and their ratio are important for prediction of the prognosis of chronic hepatitis as well as for the timely diagnosis of hepatocellular carcinoma (Table 1).

The relative content of T lymphocytes decreases as the infectious process is getting longer. According to table 2, the least amount of T lymphocytes was found in patients with a duration of the disease of 11 to more than 15 years (p <0.005).

Table 1

Changes in biochemical and immunological parameters in different degrees of severity of liver cirrhosis (according to Child-Pugh)

Symptoms	Class A (1)	Class B (2)	Class C (3)	P
AST μkat	0,32±0,1	0,26±0,2	0,20±0,1	P_{1-2}>0,05 P_{1-3}<0,001
ALT μkat	0,28±0,1	0,32±0,2	0,24±0,1	P_{1-2}>0,05 P_{1-3}<0,001
Total bilirubin mmol/L	15,7±1,2	26,7±1,8	38,9±1,6	P_{1-2}<0,01 P_{1-3}<0,001
Total protein g/L	72,1±3,0	60,4±4,2	52,3±5,1	P_{1-2}>0,05 P_{1-3}>0,05
Prothrombin ratio (%)	70,1±3,2	58,2±2,4	54,2±2,3	P_{1-2}>0,05 P_{1-3}>0,05
Thymol test (units)	12,1±0,4	14,3±0,4	18,4±0,7	P_{1-2}<0,05 P_{1-3}<0,05
Albumin (g/L)	52,0±1,3	45,0±1,2	30,4±1,0	P_{1-2}<0,001 P_{1-3}<0,001
γ-globulin (%)	28,2±0,9	32,0±0,7	38,0±0,8	P_{1-2}<0,05 P_{1-3}<0,001
Leukocytes (thous/μL)	4,5±2,9	4,1±1,7	3,0±1,4	P_{1-2}>0,05 P_{1-3}>0,05
T lymphocytes (%)	46,1±2,5	38,0±1,2	35,2±1,5	P_{1-2}<0,05 P_{1-3}<0,01
T helpers (%)	33,5±2,4	28,4±2,7	26,3±2,0	P_{1-2}>0,05 P_{1-3}>0,05
T suppressors	31,0±4,0	32,3±4,0	33,3±4,1	P_{1-2}>0,05 P_{1-3}>0,05
B lymphocytes	32,7±3,9	28,7±1,3	26,2±3,4	P_{1-2}>0,05 P_{1-3}>0,05
Phagocytic index	67,7±5,3	50,2±5,2	40,5±3,2	P_{1-2}<0,05 P_{1-3}<0,001
CD 95 (apoptosis)	28,1±2,2	40,1±3,1	58,2±3,6	P_{1-2}>0,05 P_{1-3}<0,05

A similar trend was with T helper cells. For all clinical groups, the relative number of B lymphocytes remained at the control level, serving a compensatory function. In patients with a duration of the disease of more than 11 years, the IgG level progressively and significantly decreased ($p<0.005$), which is a result of the decreasing number of B cells experiencing helper impact by immunoregulatory subpopulations of T lymphocytes and enhancement of their suppressor action leading to the decrease of IgG concentration. The dynamic change in IgG levels is similar to that of B cells, T helper and other immunocompetent cells. The apoptosis number in patients with cirrhosis has a distinct tendency to increase with the increasing duration of the disease. On the whole, the apoptosis amount in patients with a 10-15 years' duration of the disease is higher than in controls.

Conclusions

The results of the study of clinico-biochemical, immunological parameters in patients with cirrhosis in the outcome of chronic HCV infection urge for their hospitalization in specialized facilities. Considering the heterogeneity of the clinical manifestations of liver cirrhosis in the outcome of chronic HCV infection, a promising approach is to make out the leading syndrome together with specified therapy tactics. The main syndromes were: asthenovegetative, dyspeptic, hemorrhagic, edematic-ascitic, depressive and hepatoprive.

Table 2

Parameters of immune status in cirrhosis of the liver by the duration of the infectious process (n=224)

Parameters of immune status	1 to 5 years	6 to 10 years	11 to ≥ 15 years	P
	M±m (1)	M±m (2)	M±m (3)	
T lymphocytes (50-70%)	47,0±1,3	39,2±1,8	35,0±1,6	P1-2 < 0.05 P1-3 < 0.05
T helper cells (theophylline resistant (33-46%)	37,0±1,4	27,0±6,8	26,2±7,1	P1-2 > 0.05 P1-3 > 0.05
T suppressor cells (theophylline sensitive) (17-30%)	28,6±1,3	35,0±4,2	53,0±5,6	P1-2 > 0.05 P1-3 > 0.05
Ea rosette forming cells (up to 5%)	7,4±0,6	12,0±5,6	13,3±5,3	P1-2 > 0.05 P1-3 > 0.05
Phagocytic index (60-90%)	56,0±2,0	54,2±6,1	37,0±7,0	P1-2 > 0.05 P1-3 < 0.05
Circulating immune complexes (110 Om-Eg)	88,7±4,3	105,2±23,6	145,1±27,4	P1-2 > 0.05 P1-3 < 0.05
Apoptosis (10-39%)	33,2±1,8	48,0±8,2	58,0±9,2	P1-2 > 0.05 P1-3 < 0.05
IgA (0,8-28 g/L)	1,9±0,9	1,4±0,1	2,1±0,1	P1-2 > 0.05 P1-3 > 0.05
IgG (5,6-16,4 g/L)	11,8±0,3	8,2±1,2	6,2±1,3	P1-2 < 0.05 P1-3 < 0.05
IgM (0,5-2,0 g/L)	1,5±0,5	1,4±0,1	2,3±0,1	P1-2 > 0.05 P1-3 > 0.05

When assessing T cellular immunity, it was found that the level of T lymphocytes (35.2±1.5) was significantly lower for the pronounced degree of disease activity – Class C by the Child-Pugh scoring. In this group of patients, T lymphocytes (T-helper cells) were also low at a level of 26.3±2.0%. The content of T suppressor cells varied in an ambiguous pattern for all degrees of liver cirrhosis. The insufficiency of T cellular immunity leads to the suppression of the microbicidal function of phagocytes, prolonged persistence of the pathogen, promoting disease progression and development of adverse complications. The changes found in the immunological study can be additionally used as prognostic predictors of the course of the liver cirrhosis in the outcome of chronic HCV infection. In the future, clinicians should use an individual approach and in-depth examination to determine the correct tactics for management of patients with liver cirrhosis.

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