

Relationships between HBeAg status of patients with chronic hepatitis B and changes in serum TNF- α , viral load and severity of morphological changes in the liver according to non-invasive tests

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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Aim – to investigate the relationship between HBeAg status of patients with chronic hepatitis B and the content of TNF- α in the serum, the level of viral load and the severity of morphological changes in the liver according to non-invasive tests.

Material and methods. 70 patients with CHB were examined. Noninvasive methods were used to determine the severity of morphological changes in the liver. The content of HBV-DNA in the blood was determined by PCR, HBeAg, anti-HBe, TNF- α by ELISA. Statistical processing was performed in Statistica 13 for Windows (StatSoft Inc., No. JPZ804I382130ARCN10-J).

Results. HBeAg-negative patients (78.6 %) with anti-HBe (89.1 %) predominate among patients with CHB. Lower frequency of seroconversion in patients with stages F 2–4, compared with patients with stages F 0–1 (85.7 % vs. 100 %, $P < 0.05$).

The highest level of HBV-DNA in the blood was in HBeAg-positive patients, compared with HBeAg-negative with stages F 0–1 ($P < 0.05$), of whom 83.3 % of patients had HBV-DNA >20000 IU/ml (83.3 % vs. 17.7 %). Viral load in HBeAg-positive patients with activity A 0–1 was the highest ($P < 0.05$), namely 4 times more often HBV-DNA was >20000 IU/ml, compared with HBeAg-negative ($P < 0.05$) A 0–1.

The content of TNF- α in the serum of patients with CHB was higher than in healthy individuals ($P < 0.05$). The highest content of TNF- α in the blood in HBeAg-positive patients with F 2–4, compared with HBeAg-negative with F 2–4 ($P < 0.05$). The severity of liver fibrosis correlated with the level of TNF- α ($r = 0.31$, $P < 0.05$).

Conclusions. HBeAg-negative (78.6 %) predominate among patients with CHB. In the presence of HBeAg-positive patients F 0–1 viral load is highest ($P < 0.05$). HBeAg-negative patients are 2.7 times more likely ($P < 0.05$) to have a viral load of HBV-DNA >20000 IU/ml in the presence of A 2–3 than in A 0–1. The highest content of TNF- α is in the serum of HBeAg-positive patients with F 2–4, compared with HBeAg-negative patients and the corresponding liver fibrosis ($P < 0.05$).

Ключові слова:

хронічний гепатит В, HBeAg-статус, цитокіни, фіброз печінки.

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Взаємозв'язки HBeAg-статусу хворих на хронічний гепатит В зі змінами вмісту TNF- α в сироватці крові, рівнем вірусного навантаження та ступенем виразності морфологічних змін у печінці за даними неінвазивних тестів

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Мета роботи – дослідити взаємозв'язки HBeAg-статусу хворих на хронічний гепатит В (ХГВ) із вмістом TNF- α в сироватці крові, рівнем вірусного навантаження та ступенем виразності морфологічних змін у печінці за даними неінвазивних тестів.

Матеріали та методи. Обстежили 70 хворих на ХГВ. Під час визначення ступеня виразності морфологічних змін у печінці застосовували неінвазивні методи. Методом ПЛР визначили вміст HBV-DNA в крові, методом ІФА – HBeAg, anti-HBe, TNF- α . Статистичне опрацювання результатів виконали в програмі Statistica 13 for Windows (StatSoft Inc., No. JPZ804I382130ARCN10-J).

Результати. Серед хворих на ХГВ переважають HBeAg-негативні пацієнти (78,6 %) з наявністю anti-HBe (89,1 %). Частота сероконверсії нижча у хворих зі стадіями F 2–4 порівняно з пацієнтами зі стадіями F 0–1 (85,7 % проти 100,0 %, $p < 0,05$).

Найвищий рівень HBV-DNA в крові встановлено у HBeAg-позитивних пацієнтів порівняно з HBeAg-негативними зі стадіями F 0–1 ($p < 0,05$), серед яких 83,3 % пацієнтів мали HBV-DNA >20000 IU/ml (83,3 % проти 17,7 %). Вірусне навантаження у HBeAg-позитивних хворих з активністю А 0–1 найбільше ($p < 0,05$): в 4 рази частіше HBV-DNA було >20000 IU/ml порівняно з HBeAg-негативними ($p < 0,05$) А 0–1.

Вміст TNF- α в сироватці крові хворих на ХГВ вищий, ніж у здорових ($p < 0,05$). Найвищий вміст TNF- α в крові – у HBeAg-позитивних хворих із F 2–4 порівняно з HBeAg-негативними з F 2–4 ($p < 0,05$). Ступінь виразності фіброзу печінки корелював із рівнем TNF- α ($r = 0,31$, $p < 0,05$).

Висновки. Серед хворих на ХГВ переважають HBeAg-негативні (78,6 %). Якщо у HBeAg-позитивних хворих наявне F 0–1, вірусне навантаження найвище ($p < 0,05$). У HBeAg-негативних пацієнтів у 2,7 рази частіше ($p < 0,05$) вірусне навантаження HBV-DNA >20000 IU/ml у разі А 2–3, ніж при А 0–1. Найвищий вміст TNF- α в сироватці крові – у HBeAg-позитивних хворих із F 2–4 порівняно з HBeAg-негативними пацієнтами та відповідним фіброзом печінки ($p < 0,05$).

Взаимосвязи HBeAg-статуса больных хроническим гепатитом В с изменениями содержания TNF- α в сыворотке крови, уровнем вирусной нагрузки и степенью выраженности морфологических изменений в печени по данным неинвазивных тестов

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Цель работы – исследовать взаимосвязи HBeAg-статуса больных хроническим гепатитом В (ХГВ) с содержанием TNF- α в сыворотке крови, уровнем вирусной нагрузки и степенью выраженности морфологических изменений в печени по данным неинвазивных тестов.

Материалы и методы. Обследовали 70 больных ХГВ. При определении степени выраженности морфологических изменений в печени применяли неинвазивные методы. Методом ПЦР определено содержание HBV-DNA в крови, методом ИФА – HBeAg, anti-HBe, TNF- α . Статистическая обработка проведена в программе Statistica 13 for Windows (StatSoft Inc., No. JPZ804I382130ARCN10-J).

Результаты. Среди больных ХГВ преобладают HBeAg-негативные пациенты (78,6 %) с наличием anti-HBe (89,1 %). Частота сероконверсии ниже у больных со стадиями F 2–4 по сравнению с пациентами со стадиями F 0–1 (85,7 % против 100,0 %, $p < 0,05$).

Самый высокий уровень HBV-DNA в крови определен у HBeAg-позитивных пациентов по сравнению с HBeAg-негативными со стадиями F 0–1 ($p < 0,05$), среди которых 83,3 % пациентов имели HBV-DNA >20000 IU/ml (83,3 % против 17,7 %). Вирусная нагрузка у HBeAg-позитивных больных с активностью A 0–1 высокая ($p < 0,05$): в 4 раза чаще HBV-DNA было >20000 IU/ml по сравнению с HBeAg-отрицательными ($p < 0,05$) A 0–1.

Содержание TNF- α в сыворотке крови больных ХГВ выше, чем у здоровых ($p < 0,05$). Самое высокое содержание TNF- α в крови – у HBeAg-позитивных больных с F 2–4 по сравнению с HBeAg-отрицательными с F 2–4 ($p < 0,05$). Степень выраженности фиброза печени коррелировала с уровнем TNF- α ($r = 0,31$, $p < 0,05$).

Выводы. Среди больных ХГВ преобладают HBeAg-негативные (78,6 %). При наличии у HBeAg-позитивных больных F 0–1 вирусная нагрузка самая высокая ($p < 0,05$). У HBeAg-негативных пациентов в 2,7 раза чаще ($p < 0,05$) вирусная нагрузка HBV-DNA >20000 IU/ml при A 2–3, чем при A 0–1. Самое высокое содержание TNF- α в сыворотке крови – у HBeAg-позитивных больных с F 2–4 по сравнению с HBeAg-негативными пациентами и соответствующим фиброзом печени ($p < 0,05$).

Ключевые слова:
хронический гепатит В, HBeAg-статус, цитокины, фиброз печени.

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Chronic hepatitis B (CHB) has a worldwide prevalence of 3.9 % and is a global medical and social problem, causing about 2 million deaths due to the development and progression of HBV-associated liver cirrhosis and hepatocellular carcinoma [1]. It is known that in the natural course of CHB, the formation of liver cirrhosis in a 5-year cumulative incidence is estimated at 8–20 %. The annual risk of liver decompensation in the presence of cirrhosis and the development of hepatocellular carcinoma varies between 1–5 % [1–4].

The natural course of CHB has significant variability, which makes it difficult to monitor patients [5]. The rate of progression of fibrotic changes in the liver largely depends on the ability of the immune system to control viral load [6,7]. In the process of replication, hepatitis B virus (HBV) antigens can affect the immune response. Of particular note is HBeAg, which plays a significant role in modulating the immune response by reducing the efficiency of the T cell [8]. Existing international recommendations have some differences in the criteria that need to be determined when monitoring patients with CHB to address treatment tactics [4]. Only the recommendations of the American Association for the Study of Liver Diseases (AASLD) are not only more complex, but also necessarily take into account the HBeAg status of the patient in relation to morphological changes in the liver and viral load [9].

Cytokines play a leading role in the immunopathogenesis of CHB progression, with considerable attention paid to tumor necrosis factor alpha (TNF- α). TNF- α is produced during inflammation by macrophages/monocytes and is responsible for a wide range of signaling events

in cells. It is not only a mediator of hepatotoxicity, but is also considered an inducer of hepatocyte proliferation and liver regeneration. However, in the long course of the disease is involved in the formation and progression of fibrotic changes in the liver [10].

The study of the immunopathogenesis of the progression of morphological changes in the liver in CHB continues, taking into account the relationship between changes in cytokine regulation and viral replication parameters. Liver biopsy is the «gold standard» for assessing the degree of liver fibrosis. However, this study is invasive and is accompanied by pain in 74 % of patients and the development of complications in 5.6 % of patients [11]. Therefore, in modern conditions, non-invasive methods for assessing morphological changes in the liver have emerged, which are based either on biochemical blood tests followed by mathematical modeling (fibrotest, actitest) or imaging methods (elastometry) [12–14]. The choice of non-invasive diagnostic method requires the doctor to take into account a number of factors that may have a negative impact on the informativeness of the data obtained. The use of elastography is limited in patients with abdominal obesity and severe hepatic steatosis, and the use of fibrotest is inappropriate in patients with hyperbilirubinemia and severe intrahepatic cholestasis due to high blood activity of gammaglutamyltranspeptidase [15]. However, the advantage of these methods is their availability, low cost, no side effects for patients during their implementation and the possibility of repeated use to assess the dynamics of change, including to assess the antifibrotic effect of treatment [12,16].

Aim

To investigate the relationship between HBeAg status of patients with chronic hepatitis B with the content of TNF- α in the serum, the level of viral load and the severity of morphological changes in the liver according to non-invasive tests.

Materials and methods

The study included 70 patients with CHB who were under dispensary observation at the non-profit municipal enterprise "Regional Infectious Diseases Clinical Hospital" of the Zaporizhzhia Regional Council. The age of patients ranged from 20 to 78 years. The median age was 44.5 [35.0; 58.0] years. There were 24 women (34.3 %) and 46 men (65.7 %).

All patients were included in the study on a random basis and with informed consent. Non-invasive methods were used to determine the severity of fibrotic and necro-inflammatory changes in the liver. The degree of fibrosis in 52 patients was determined by elastometry, in 18 fibrotest was performed. Necrosis-inflammatory activity in 18 was assessed on the basis of actitect and in 52 patients by the level of elevated serum alanineaminotransferase according to the classification of chronic hepatitis (Los Angeles, 1994).

In all patients, a study of the quantitative content of HBV-DNA in the blood was conducted by polymerase chain reaction. The presence of HBeAg and anti-HBe in the serum, the quantitative content of tumor necrosis factor alpha (TNF- α) (Elabscience, USA) was determined by enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assays were performed on the basis of the Scientific medical-laboratory center of Zaporizhzhia State Medical University (Head – Prof. A. Abramov). A control group of 30 healthy individuals was formed to assess changes in serum TNF- α levels.

Statistical data processing was performed in Statistica 13 for Windows (StatSoft Inc., No. JP-Z8041382130ARCN10-J). The Mann-Whitney test was used to assess the significant difference between quantitative traits in independent groups, and the χ^2 method was used between qualitative traits. Correlation analysis was performed by the Spearman method.

Results

Analysis of the frequency of HBeAg detection in the serum of patients with CHB showed a predominance of HBeAg-negative patients, part of whom was 78.6 % (55 of 70). HBeAg/anti-HBe seroconversion analysis showed the presence in the serum of the majority of HBeAg-negative anti-HBe patients, namely in 89.1 % (49 of 55). Comparison of the frequency of HBeAg/anti-HBe seroconversion in HBeAg-negative patients with varying degrees of morphological changes in the liver (according to non-invasive tests) found a lower rate of seroconversion in patients with F 2–4 liver fibrosis stages, compared with patients with early liver fibrosis F 0–1 (85.7 % vs. 100.0 %, $\chi^2 = 5.14$, $P < 0.05$). The frequency of anti-HBe detection did not depend on

the severity of necrotic-inflammatory changes in the liver ($P > 0.05$) (Table 1).

According to the results of the studies, it was found that HBeAg-negative patients predominated among patients with CHB, regardless of the liver fibrosis severity ($P > 0.05$). Thus, in the presence of initial liver fibrosis manifestations, the share of HBeAg-negative patients was 85.0 % (34 of 40), and in the presence of F 2–4 fibrosis – 70.0 % (21 of 30).

Analysis of viral load depending on HBeAg status and the severity of fibrotic changes in the liver showed that the highest level of HBV-DNA in the blood was found in HBeAg-positive patients with early liver fibrosis stages, which probably exceeded the same in HBeAg-negative patients with similar liver fibrosis stages ($P < 0.05$). This pattern was confirmed by a greater number of patients with HBV-DNA levels in the blood above 20.000 IU/ml (83.3 % vs. 17.7 %), compared with HBeAg-negative patients with F 0–1 liver fibrosis stages. There were no statistically significant ($P > 0.05$) relationships between the dependence of viral load on HBeAg status in patients with CHB with F 2–4 liver fibrosis stages (Table 2).

Analysis of the frequency of patients detection with CHB with different HBeAg status showed no statistically significant relationship with the severity of necro-inflammatory changes in the liver ($P > 0.05$). In the presence of A 0–1 degree of necro-inflammatory activity in the liver, the share of HBeAg-negative patients was 81.4 % (48 of 59), and in the presence of A 2–3 degree – 63.6 % (7 of 11).

The level of viral load in HBeAg-positive patients with A 0–1 severity of necrosis-inflammatory process was higher ($P < 0.05$), compared not only with HBeAg-negative patients with a similar level of necrosis-inflammatory process, but also compared with HBeAg-positive patients with A 2–3 degree of necro-inflammatory activity ($P < 0.05$). The established pattern is confirmed by almost 4 times more frequent detection of HBV-DNA >20000 IU/ml in HBeAg-positive patients compared with HBeAg-negative (81.8 % vs. 20.8 %, $\chi^2 = 15.24$, $P < 0.05$) in the A 0–1 presence. In HBeAg-negative patients, a significant relationship was found between the frequency of HBV-DNA viral load detection (>20000 IU/ml) with the severity of necro-inflammatory activity, which confirms 2.7 times more frequent viral load detection of this level (57.1 % vs. 20.8 %, $\chi^2 = 4.24$, $P < 0.05$) in the presence of A 2–3 necro-inflammatory activity (Table 3).

According to the results of the analysis of TNF- α content in the serum of patients with CHB, it was found that the content of this cytokine was higher than in healthy individuals ($P < 0.05$), regardless of HBeAg status and the severity of morphological changes in the liver according to noninvasive tests. However, the highest content of TNF- α in blood serum was registered in HBeAg-positive patients with F 2–4 liver fibrosis degrees, compared with HBeAg-negative patients and the corresponding severity of liver fibrosis ($P < 0.05$) (Table 4). The established pattern was confirmed by the detection of a direct correlation by Spearman's method between the severity of liver fibrosis and the level of TNF- α in serum ($r = 0.31$, $P < 0.05$).

Table 1. Frequency of HBeAg/anti-HBe seroconversion in HBeAg-negative patients with varying degrees of morphological changes in the liver according to the results of non-invasive tests, abs (%)

Index	HBeAg-negative patients with CHB (n = 55)			
	Liver fibrosis		Necrotic-inflammatory changes	
	F 0–1 (n = 34)	F 2–4 (n = 21)	A 0–1 (n = 48)	A 2–3 (n = 7)
anti-HBe-positive	34 (100 %)	18 (85.7 %)*	46 (95.8 %)	6 (85.7 %)

*: the difference is significant compared with HBeAg-negative patients with F 0–1 liver fibrosis stages (P < 0.05).

Table 2. Viral load in patients with CHB depending on HBeAg status and the liver fibrosis severity, Me [Q₂₅; Q₇₅] or abs (%)

Index, units	Patients with CHB (n = 70)			
	F 0–1 (n = 40)		F 2–4 (n = 30)	
	HBeAg positive (n = 6)	HBeAg negative (n = 34)	HBeAg positive (n = 9)	HBeAg negative (n = 21)
HBV-DNA, IU/ml	1.5 × 10 ⁶ [6.2 × 10 ⁴ ; 2.0 × 10 ⁷]	1.1 × 10 ³ [1.5 × 10 ² ; 1.0 × 10 ⁴]*	5.6 × 10 ⁴ [5.0 × 10 ¹ ; 5.3 × 10 ⁶]	1.9 × 10 ³ [2.5 × 10 ² ; 7.2 × 10 ⁴]
HBV-DNA <2000 IU/ml, abs (%)	1 (16.6 %)	19 (55.9 %)	3 (33.3 %)	11 (52.4 %)
HBV-DNA 2000–20000 IU/ml, abs (%)	0	9 (26.5 %)	1 (11.1 %)	2 (9.5 %)
HBV-DNA >20000 IU/ml, abs (%)	5 (83.3 %)	6 (17.7 %)	5 (55.6 %)	8 (38.1 %)

*: the difference is significant compared to HBeAg-positive patients with F 0–1 liver fibrosis stages (P < 0.05).

Table 3. Viral load in patients with CHB depending on HBeAg status and the severity of necro-inflammatory changes in the liver, Me [Q₂₅; Q₇₅] or abs (%)

Index, units	Patients with CHB (n = 70)			
	A 0–1 (n = 59)		A 2–3 (n = 11)	
	HBeAg positive (n = 11)	HBeAg negative (n = 48)	HBeAg positive (n = 4)	HBeAg negative (n = 7)
HBV-DNA, IU/ml	2.9 × 10 ⁶ [5.6 × 10 ⁴ ; 2.0 × 10 ⁷]	1.1 × 10 ³ [1.6 × 10 ² ; 1.2 × 10 ⁴]*	6.3 × 10 ³ [5.0 × 10 ¹ ; 4.3 × 10 ⁴]*	4.9 × 10 ⁴ [5.2 × 10 ² ; 9.8 × 10 ⁴]
HBV-DNA <2000 IU/ml, abs (%)	2 (18.2 %)	27 (56.3 %)	2 (50.0 %)	3 (42.9 %)
HBV-DNA 2000–20000 IU/ml, abs (%)	0	11 (22.9 %)	1 (25.0 %)	0
HBV-DNA >20000 IU/ml, abs (%)	9 (81.8 %)	10 (20.8 %)*	1 (25.0 %)	4 (57.1 %)**

*: the difference is significant compared to HBeAg-positive patients with necro-inflammatory activity A 0–1 in the liver (P < 0.05); **: compared with HBeAg-negative patients with necro-inflammatory activity A 0–1 in the liver (P < 0.05).

Table 4. The content of TNF-α in the serum of patients with CHB depending on HBeAg status and the degree of morphological changes in the liver according to non-invasive tests, Me [Q₂₅; Q₇₅]

Index	Healthy people (n = 30)	Patients with CHB (n = 70)			
The severity of liver fibrosis					
TNF-α, pg/ml	2.6 [0.04; 3.02]	F 0–1 (n = 40)		F 2–4 (n = 30)	
		HBeAg-positive (n = 6)	HBeAg-negative (n = 34)	HBeAg-positive (n = 9)	HBeAg-negative (n = 21)
		4.01 [3.50; 4.46]*	4.42 [3.02; 5.40]*	14.27 [13.35; 18.23]*	4.93 [3.50; 6.37]**
The severity of necro-inflammatory changes in the liver					
TNF-α, pg/ml	2.6 [0.04; 3.02]	A 0–1 (n = 59)		A 2–3 (n = 11)	
		HBeAg-positive (n = 11)	HBeAg-negative (n = 48)	HBeAg-positive (n = 4)	HBeAg-negative (n = 7)
		4.04 [3.50; 14.00]*	4.39 [3.02; 5.89]*	16.25 [13.81; 19.86]*	5.80 [4.43; 14.98]*

*: the difference is significant compared to healthy (p < 0.05); **: the difference is significant compared with HBeAg-positive patients with F 2–4 liver fibrosis stages (P < 0.05).

Discussion

According to modern literature, HBeAg is considered as an antigen of the virus, which when circulating in the blood of patients with CHB, is able to change the immune response. This leads to decrease in the effectiveness of the cellular immune response [17,18]. However, most researchers today note that HBeAg-negative patients predominate among patients with CHB [19,20]. According to the results of our study, it was also recorded that HBeAg-negative patients predominate among the examined patients with CHB, the share of whom is 78.6 % (55 of 70). Such changes in the structure of patients with CHB currently have two main explanations. On the one hand, this is due to the decrease in the number of young people

due to compulsory vaccination. On the other hand, we observe an increase in the proportion of patients in older age groups in whom seroconversion occurred with the appearance of anti-HBe in the long-term natural course of CHB [17,18]. The occurrence of mutations in the pre-core/core region with subsequent loss of HBeAg expression while maintaining high HBV replicative activity is not excluded [17,18,20]. HBeAg-negative CHB may have an unstable course with persistent necro-inflammatory changes in the liver and viral load levels above 2000 IU/ml [21]. The data obtained in our study also to some extent demonstrate the instability of HBeAg-negative CHB, namely in HBeAg-negative patients 2.7 times more often (P < 0.05) was detected viral load above 20000 IU/ml in

the presence of necro-inflammatory activity A 2–3 than in the presence of A 0–1.

Immunopathogenetic mechanisms of morphological changes progression in the liver of patients with CHB today continue to be studied. It is known that in CHB, the immune response to virus antigens is ineffective. This leads to almost continuous necro-inflammatory activity, the appearance of a stable regenerative reaction in the liver. These processes are accompanied by the transformation of stellate liver cells into activated myofibroblasts with excessive production and accumulation of extracellular matrix components, which leads to the progression of fibrotic changes in the liver [22–24]. Cytokines play a leading role in the progression of fibrotic changes in the liver. Particular attention is drawn to cytokines that have pro-inflammatory and profibrogenic properties, in particular TNF- α [24,25]. According to researchers [24,25], the level of increased TNF- α in the serum correlates with severe liver fibrosis and more pronounced inflammation. According to the results of our study, a relationship was found between the level of this cytokine elevation from HBeAg status and the degree of liver fibrosis in patients with CHB. The highest content of TNF- α in blood serum was recorded in HBeAg-positive patients with F 2–4 liver fibrosis stages, compared with HBeAg-negative patients and the corresponding liver fibrosis degree ($P < 0.05$). The established pattern can be explained on the one hand by the negative effect of HBeAg on the cellular immune response [17,18], on the other hand by the increase in activity of stellate cells, which show very high sensitivity to anti-inflammatory cytokines, and the ability of TNF- α to prevent stellate cell apoptosis [26–28].

Conclusions

1. HBeAg-negative patients predominate among patients with CHB in 78.6 % with seroconversion of HBeAg/anti-HBe in 89.1 %. In the presence of HBeAg-negative patients with liver fibrosis F 2–4 degree, the frequency of seroconversion is lower compared with patients with initial stages of liver fibrosis F 0–1 (85.7 % vs. 100.0 %, $\chi^2 = 5.14$, $P < 0.05$).

2. HBeAg-negative patients predominate regardless of the severity of liver fibrosis (85.0 % at F 0–1 and 70.0 % at F 2–4). In the presence of HBeAg-positive patients with CHB in the initial stages of liver fibrosis, the viral load is the highest ($P < 0.05$) due to the higher number of patients with HBV-DNA levels above 20000 IU/ml (83.3 % vs. 17.7 %), compared with HBeAg-negative patients with stages of liver fibrosis F 0–1.

3. HBeAg-negative patients predominate among patients with CHB, regardless of the severity of necro-inflammatory changes in the liver (81.4 % at A 0–1 and 63.6 % at A 2–3). In HBeAg-positive patients with the severity of the necro-inflammatory process A 0–1 viral load is higher ($P < 0.05$), compared with both HBeAg-negative patients with A 0–1 and HBeAg-positive patients with A 2–3 ($P < 0.05$). HBeAg-positive patients have 4 times more often ($P < 0.05$) HBV-DNA level >20000 IU/ml, compared with HBeAg-negative in the presence of A 0–1. HBeAg-negative patients are 2.7 times more likely ($P < 0.05$) to have a viral load of HBV-DNA >20000 IU/

ml in the presence of necro-inflammatory activity A 2–3 than in the presence of A 0–1.

4. In patients with CHB, the content of TNF- α in the serum, regardless of HBeAg status and the severity of morphological changes in the liver according to non-invasive tests is higher than in healthy people ($P < 0.05$). The highest content of TNF- α in blood serum is registered in HBeAg-positive patients with degrees of liver fibrosis F 2–4, compared with HBeAg-negative patients and the corresponding severity of liver fibrosis ($P < 0.05$).

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