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ABSTRACT

of the dissertation for the degree of Philosophy

**THE SIGNIFICANCE OF OCCULT HEPATITIS B VIRUS
INFECTION IN THE PROGRESSION OF CHRONIC LIVER
DISEASES**

Specialty: 3205.01- Internal diseases

Field of science: Medicine

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BAKU-2024

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GENERAL DESCRIPTION OF THE WORK

Relevance of the topic. Hepatitis B virus (HBV) is a major medical and social health problem. It is believed that more than half of the world's population has been infected with HBV virus, while approximately 350 million have chronic hepatitis B (CHB) infection¹. Every year, more than 4 million cases of acute hepatitis B (AHB) and about 1 million deaths as a result of HBV are registered worldwide, associated liver pathology². About 50% of chronically infected individuals die from complications such as liver failure, liver cirrhosis and hepatocellular carcinoma³. This problem also exists in Azerbaijan. The number of patients with CHB is approximately 280,000 people.

The high level of morbidity, frequent progression to chronic infection, loss of ability for patients to work, as well as the possibility of death entail enormous demographic and economic damage for the countries⁴. Until recently, it was believed that HBV infection can occur in one of three forms: acute hepatitis B, characterized by symptoms of acute liver damage and intoxication (with and without jaundice); chronic hepatitis B, characterized by long-term inflammatory damage to the liver; long-term persistence of HBV surface antigen (HBsAg) in serum in the absence of clinically evident hepatitis⁵.

¹ WHO. Global progress report on HIV, viral hepatitis, and sexually transmitted infections. July 15, - 2021.

² Raimondo, G. Occult HBV infection / G.Raimondo, G.Caccamo, R. Filomia Seminars in Immunopathology, - 2013. Vol. 35, No. 1, - p. 39–52.

³ European Association for the Study of the Liver. EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection // J. Hepatol., - 2017. 67, - p. 370–398.

⁴ Weng, C. Occult hepatitis B virus infection and the risk of hepatocellular carcinoma: a systematic review and meta-analysis / C. Weng, R. Kumar, R. Sultana [et al.] // Dig Med Res., - 2021 4, - p. 46–46.

⁵ Torbenson, M., Thomas, D.L. Occult hepatitis B // Lancet Infectious Diseases, - 2002. Vol.2, No. 8, - p. 479–486.

Hepatitis B virus is diagnosed by determining the circulation of the HBsAg antigen in the blood serum for at least 6 months after contamination. However, it has now been proven that seroconversion of HBsAg in acute hepatitis is not an absolute sign of elimination of the virus from the body and its complete recovery⁶.

Back in 1978, a case was reported of the development of acute hepatitis B in a recipient after a blood transfusion containing antibodies to the HBV capsid protein (anti-HBc) in the absence of HBsAg and antibodies to it (anti-HBs). Subsequently, it was shown that viral DNA can be detected in the blood serum and liver tissue of patients in whom serum HBsAg is not detected by available methods. This phenomenon is called “occult” or “latent” HBV (LHB) infection⁷.

Molecular evidence of occult infection consists of the presence of covalent closed circular DNA in the nuclei of hepatocytes following infection. Immunocompetent individuals have a lower risk of complications than immunocompromised patients⁸. However, under certain circumstances, acute hepatitis B may develop with clinical manifestations that include transmission of infection through blood or organ transplantation, leading to the progression of chronic liver disease, the emergence of hepatocellular carcinoma, and also viral reactivation that develops during immunosuppressive conditions. CHB is a disease that changes dynamically over time due to interactions between the virus and the patient's immune system.

⁶ Br´echot, C. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely occult? / V. Thiers, D. Kremsdorf “Hepatology, - 2001. Vol. 34, No. 1, - p. 194–203.

⁷ Lledó, J.L. Management of occult hepatitis B virus infection: an update for the clinician / J.L.Lledó, C.Fernández, M.L.Gutiérrez World Journal of Gastroenterology, - 2011. Vol. 17, No. 12, - p. 1563–1568.

⁸ Yip, T.C., Wong, G.L. Current knowledge of occult hepatitis B infection and clinical implications // Semin. Liver Dis., - 2019. 39, - p. 249–260.

The natural history of chronic hepatitis B can be divided into 4 phases: the immunotolerant phase, the immune activity phase, the immune control phase, and immune clearance. There are frequent cases of reverse phase shift and reactivation of the disease compared to the phase of immune clearance. It should be noted that in many countries of the world, including Azerbaijan, HBsAg is still the main, and sometimes the only serum marker of HBV determined in blood donors. Because of this, LHB infection is a serious problem for blood transfusion centers in those countries where, in addition to washed blood cells, plasma or even whole blood is used for transfusions to recipients since blood transfusions from people with occult HBV infection can lead to the development of recipient of acutely manifesting acute hepatitis or to the development of its chronic form⁹. In 2008, at a conference organized by the European Association for the Study of the Liver, the concept of “occult” HBV infection was defined as the presence of HBV DNA in the liver (regardless of its presence in the blood serum) in patients in whom HBsAg is not detected in the blood by available methods¹⁰. Currently, the criteria for latent infection are the absence of HBsAg, the presence of HBcAg, and determination of HBV DNA. The prevalence of latent HBV infection, according to various publications, varies in different study groups from 0 to 2.4% among HBsAg-negative, anti-HBc-positive (but anti-HBs +/-) blood donors in Western countries to 6% in similar groups of donors living in endemic areas¹¹. One of the reasons for interest in the study of latent hepatitis B infection is its possibility of reactivation.

⁹ Ji, D.Z.; Pang, X. Y.; Shen, D.T.; Liu, S.N.; Goyal, H.; Xu, H. G. Global prevalence of occult hepatitis B: A systematic review and meta-analysis. *J. Viral. Hepat.* 2022, 29, 317–329.

¹⁰ Raimondo, G. Statements from the Taormina expert meeting on occult hepatitis B virus infection / G.Raimondo, J.P.Allain, M.R.Brunetto [et al.] // *J Hepatol.*, - 2008. 49, No. 4, - p.652 –657.

¹¹ Makvandi, M. Update on occult hepatitis B virus infection // *World J. Gastroenterol.*, - 2016. 22(39), - p. 8720–8734.

Another important factor dictating the need for in-depth investigation of latent HBV infection is the possibility of its presence at co-infections with other viruses¹². According to the literature, a significant proportion of patients (30-45%) infected with the hepatitis C virus have a latent HBV infection. It is known that co-infection with HBV in patients with chronic hepatitis C (CHC) can significantly affect the course of the disease, the level of viral load, and the effectiveness of treatment¹³. The lack of information about the characteristics of the course of CHC during co-infection with HBV, which occurs in a latent form, does not allow us to develop recommendations for the diagnosis and management of such patients. The presence of co-infection with other, even non-hepatotropic viruses, may affect not only the clinical manifestations of the disease, but also the pathogenetic mechanisms of pathology development¹⁴. Literature data are largely contradictory, which is primarily due to the lack of dynamic studies and the small number of observations. In Azerbaijan, there is no information on the incidence of occult HBV infection, and the role and place of it in the progress of chronic liver diseases of various etiologies have not been studied.

Object and subject of research:

The study included 1014 individuals, among whom 512 were blood donors and 492 patients with various pathologies with concomitant chronic liver diseases or liver lesions with the absence of the surface antigen HBsAg in the blood serum. The group of patients also included transfusion-dependent patients with beta

¹² Zoulim, F. New insight on hepatitis B virus persistence from the study of intrahepatic viral cccDNA // *Journal of Hepatology*, - 2005. Vol. 42, no. 3, - p. 302–308.

¹³ Schüttler, C.G. Suppression of hepatitis B virus enhancer 1 and 2 by hepatitis C virus core protein / C.G.Schüttler, N.Fiedler, K.Schmidt [et al.] // *J Hepatol.*, - 2002. 37, - p.855-862.

¹⁴ Malagnino, V. Occult Hepatitis B Virus Infection: An Old Entity With Novel Clinical Involvements / V. Malagnino, D. B. Fofana, K. Lacombe [et al.] // *Open Forum Infect Dis.*, - 2018. 5, - p. 227.

thalassemia major and intermediate forms, patients with HIV, preoperatively screened patients and patients with cancer, as well as patients infected with viral hepatitis C.

Purpose of the study:

To determine the detectability and characteristics of the clinical course of occult HBV infection among various groups of patients and blood donors.

The tasks of the research:

1. To study the frequency of detection of latent HBV infection in blood donors and in a group of patients with various pathologies who have chronic diseases or liver damage in the absence of the surface antigen HBsAg.
2. To identify groups of people with an increased risk of acquiring latent HBV infection, as well as to determine factors influencing the development of HBV.
3. To compare clinical and morphological manifestations, as well as serological markers of HBV in a group of patients with chronic hepatitis C with and without latent HBV infection.
4. To assess the risk of reactivation of combined HBsAg-positive and latent HBV infections when treating hepatitis C with direct-acting antivirals, and to study the impact of the presence of both latent and overt infections on the sustained virological response.
5. To determine factors contributing to the timely detection of reactivation of latent HBV infection.

Research methods:

All examined groups of donors and patients were tested in the relevant institutions according to the guidelines approved by the Ministry of Health of Azerbaijan Republic using enzyme-linked immunosorbent assay (ELISA) for the presence of HBsAg and anti-HCV. To detect latent infection in the serum of donors and patients, anti-HBs, anti-HBc IgM, anti-HbcIgG were determined using commercially available ELISA kits. In the study groups, no surface

antigen HBsAg was detected in any patient. In individuals with a positive anti-HBcIgG indicator, which is a surrogate marker of latent infection, the presence of HBV DNA was additionally determined using the PCR method (Abbot real-time; sensitivity 12 Bv/ml).

To determine the presence of occult infection, serum samples were subjected to commercial enzyme-linked immunosorbent assays (ELISA) for anti-HBs, anti-HBc IgM, anti-HBc IgG using commercially available enzyme immunoassay kits. In children with a positive anti-HBc IgG, which is a surrogate marker of occult infection, the presence of HBV DNA was additionally determined.

Provisions of the dissertation submitted for defense:

1. Despite the absence of the surface antigen HBsAg in the blood serum, the incidence of latent HBV infection varies in different groups of subjects.
2. The highest prevalence of LHB is observed in patients with chronic hepatitis C and thalassemia.
3. Blood transfusions are the most important factor associated with LHB. Age, as well as previous surgical procedures, influence the prevalence of latent hepatitis B to a lesser extent.
4. When using chemotherapy drugs, as well as direct-acting antiviral drugs, there is a risk of clinical reactivation of LHB.
5. The presence of latent HBV infection in patients with chronic hepatitis C negatively affects the course of the disease.

Scientific novelty of the work:

- In the conditions of Azerbaijan, for the first time, a study is being conducted of the prevalence of latent HBV infection, its significance in the development of chronic liver diseases, as well as the risk of reactivation in certain groups of patients.
- Theoretical value of the research results: Based on the results obtained, it is possible to revise the diagnostic value of anti-HBc as a marker of latent hepatitis B, since in its absence in the body the virus can not only be present, but also actively

replicate, and also carry the risk of reactivation in immunocompetent conditions.

Practical significance:

The results of the study provided information on the prevalence of latent HBV infection among individuals previously considered not to have been in contact with the virus due to the absence of the HBsAg antigen. Determination of anti-HBc IgG and HBV DNA in serum made it possible to identify the presence of low concentrations of the virus and, accordingly, to identify the risk of transmission of the virus from these individuals during blood transfusion and organ transplantation, as well as to determine the possibility of reactivation of HBV in immunosuppressive conditions. An important role in the work is to identify the difference in the incidence of latent HBV infection among healthy individuals and various groups of patients, the assumption of risk factors for infection, as well as the influence of the presence of HBV on the development and course of liver diseases.

Approval of research work:

The initial discussion of the work was held at the interdepartmental conference of the departments: “III Internal Medicine” was held on 16th of February 2016. The work was tested at the scientific Seminar of the Dissertation Council on 21st of September 2023.

Discussion of the dissertation work: “The prevalence of occult hepatitis B infection in Azerbaijani population” at the International Hepato-Gastroenterological Congress (Antalya, Turkey, 4th of September 2017); “Occult hepatitis B infection in patients with chronic hepatitis C and the risk of reactivation during DAA treatment” – at the 27th Annual Conference of the Asian and Pacific Association for the Study of Liver (Bali, Indonesia 28th of March 2018), “Reactivation of overt and occult hepatitis B in patients with chronic hepatitis C undergoing treatment with DAAs” at the 27th Annual Conference of the Asian and Pacific Association for the

Study of the Liver (Bali, Indonesia 28th of March, 2018), at the Azerbaijan-Turkey Scientific and Practical Conference on the problem of hepatology (Baku, 9th of October 2022); at the Monothematic Conference of the Asian and Pacific Association for the Study of the Liver (Baku, 27th of June, 2019), at the First International Medical Forum (Nakhichevan, 18 – 21st of June 2022).

Application of the research.

The results of the study are used in educational and therapeutic practice at the III Department of Internal Medicine of the Azerbaijan Medical University.

The organization in which the dissertation work was carried out. The dissertation work was carried out at the III Department of Internal Medicine of the Azerbaijan Medical University, the private clinic Baku Medical Plaza, the Oncology Clinic of the Azerbaijan Medical University, the Thalassemia Center (National Center for Hematology and Blood Transfusion), the Central Blood Bank, as well as the Republican AIDS Center under the Ministry Healthcare of the Republic of Azerbaijan.

Published scientific works. 13 scientific papers have been published on the topic of the dissertation, including 6 articles and 7 theses. Published 2 scientific articles as one author. Published 4 articles with my supervisor.

Scope and structure of the dissertation work. The dissertation consists of 163 pages of computer text (185 391 characters), introduction (12,939 characters), literature review (51,122 characters), chapter “Materials and Methods” (20474 characters), chapter of personal research (33883 characters), chapter of discussion of the findings results (60 040), conclusions (1 999 characters), practical recommendations (698 characters) and a bibliography, including 140 sources (of which 2 domestic, 138 foreign). The work is illustrated with 19 tables, 3 diagrams and 11 drawings.

MATERIALS AND METHODS OF THE RESEARCH

The work was carried out at the Department of Internal Medicine, Azerbaijan Medical University and at the central branch of the private clinic Baku Medical Plaza. Blood samples were collected at the Department of Internal Medicine of the Azerbaijan Medical University, at the central branch of the private clinic Baku Medical Plaza, at the Central Blood Bank, at the Thalassemia Center, at the Oncology Center of the Azerbaijan Medical University, at the AIDS Center of the Ministry of Health of Azerbaijan Republic and were also provided by the Medera Clinic. When studying the incidence of latent HBV infection among people with chronic liver diseases, the main category was a group of patients with chronic hepatitis C. In addition to the assessment, they were monitored before and during treatment with direct-acting antivirals and the risk of viral reactivation was carefully monitored.

In accordance with the objectives, to compare the incidence of LHB among the general population, the study also included a group of blood donors, preoperative patients, a group of patients with HIV, patients with thalassemia, as well as patients with oncological diseases of the gastrointestinal tract receiving chemotherapy in combination with or without other treatment methods. The presence of occult HBV infection was assessed both in patients with and without concomitant chronic hepatitis C. The main criterion for selecting subjects was the absence of the surface antigen HBsAg.

For the purpose of qualitative detection of anti-HBc, in most cases the ARCHITECT Anti-HBc II assay kit (Abbott GmbH & Co. KG, Wiesbaden, Germany) was used, which was operated in the same system as described above, according to the manufacturer's instructions. This kit identifies IgM and IgG simultaneously. Also, tests were carried out using devices Acon, San Diego, California, Bioelisa anti-HBs, Biokit, Barcelona, Catalonia, Spain); HBeAg and anti-HBe were determined using Cobas e411, Roche Diagnostics,

Manheim, Germany) according to each manufacturer's instructions. Serums were sent to the laboratories of the Azerbaijan Medical University, the private clinic Baku Medical Plaza, Referans, Saglam Aile and Sunlab laboratories.

Liver biochemical enzymes such as alanine aminotransferase (ALAT), aspartate aminotransferase (AST), alkaline phosphatase, total, direct and indirect bilirubin, and gamma-glutamyltransferase (GGT) using a commercial kit (LabMax 560, LabTest, Lagoa Santa, Brazil).

An ultrasound examination in patients with hepatitis C was carried out to determine the state of liver disease before treatment at the Baku Medical Plaza clinic, and an examination to determine the extent of damage - fibroscan - was carried out using a device for non-invasive determination of the degree of liver fibrosis FibroScan® 502 TOUCH with an ultrasound sensor (M+), Echosens (France) - at the Avrasiya clinic. During the liver fibroscan examination, digital values of stiffness in kilopascals (kPa) were obtained, from which the degree of fibrosis can be determined: 2.0-5.8 kPa corresponds to the F0 stage of fibrosis (normal), 5.9-7.2 kPa - F1st, 7,3-9.2 kPa - F2st, 9.3-12.9 kPa - F3st, more than 13.0 kPa - F4st (cirrhosis).

HBV DNA testing was carried out using Abbott equipment (Abbott GmbH & Co. KG, Wiesbaden, Germany) in the Referans MMC laboratory and in the SUNLAB laboratory (specimens were sent to Turkish laboratories). Hepatitis B viral load, hepatitis C viral load, and hepatitis C virus genotype were assessed using Abbott real-time PCR (sensitivity 12 IU/ml). The study of a group of donors was conducted on the basis of the Central Blood Bank of the Scientific Research Institute of Hematology and Transfusiology of Azerbaijan and included 512 people who applied in December 2018 to donate blood (133 women (26.7%, average age 41.2 ± 3.65). 144 were also studied patients with planned surgical procedures, placed at the Department of Internal Medicine of the Faculty of Pediatrics of the Azerbaijan Medical University, the inpatient department of the

central branch of the private clinic Baku Medical Plaza, as well as the inpatient department of the Medera private clinic, were examined one day or on the day of the operation and depending on depending on the type of operation (61 women (42.4%, average age 52.7 ± 14.73). At the AIDS Center of the Ministry of Health of the Republic of Azerbaijan, 75 HIV- infected HBsAg-negative patients were examined. Of these, 9 had a positive anti-HCV test. The average age of the patients was 37.2 ± 6.8 ; 42 of them are women, 33 are men. The purpose of the study was to take blood samples from 68 patients diagnosed with thalassemia registered at the Azerbaijan Thalassemia Center (women 29.4%, mean age 30.4 ± 3.6). The study included 51 patients who were diagnosed with various gastrointestinal malignancies in the endoscopy department at the Oncology Center of the Azerbaijan Medical University and Baku Medical Plaza Clinic from January to July 2018 (women N=22, age 65.34 ± 4.73 ; 29 men, age 68.59 ± 3.85). Since anti-HBc testing is not included in routine screening for viral hepatitis in cancer patients in Azerbaijan, the purpose of our study is to estimate the prevalence of occult hepatitis B infection in HBsAg-negative patients with gastroenterological malignancies receiving chemotherapy that is accompanied by previously normal liver function tests. but increasing while taking anticancer drugs.

The main group of subjects consisted of 164 patients with viral hepatitis C, divided into 2 subgroups: Group I included 72 (43%) patients with positive anti-HBc-IgG (anti-HBs \pm) (49 men and 23 women, mean age \pm SD 47.5 ± 9.8 years). Group II included 92 (56%) anti-HBc-IgG negative patients (57 men and 35 women, mean \pm SD age 43.6 ± 12.5 years). In this group, the risk of reactivation of LHB was assessed during treatment with direct-acting antiviral drugs. To monitor virological/clinical reactivation of LHB, if present, HCV RNA, HBV DNA, ALAT and ASAT were tested before treatment and rechecked at 2, 4, 12 weeks during treatment, and at 4, 12 and 24 weeks after treatment. Clinical reactivation of LHB was

defined as a greater than or equal to 2-fold increase in serum alanine aminotransferase. In addition to the risk of reactivation, the impact of LHB on sustained virological response (SVR) during and after treatment with direct-acting antivirals (DAAs) and its correlation with baseline liver tissue was also assessed.

RESULTS OF THE RESEARCH

In the blood donors we examined, in whose blood serum there was no surface antigen HBsAg, the detection of HBV DNA was 1.5% among donors with negative hepatitis C virus and in 7.4% of donors with positive HCV. Characteristics of virological parameters of donors are presented in Table 1.

Table 1.

Virological indicators of the donor group

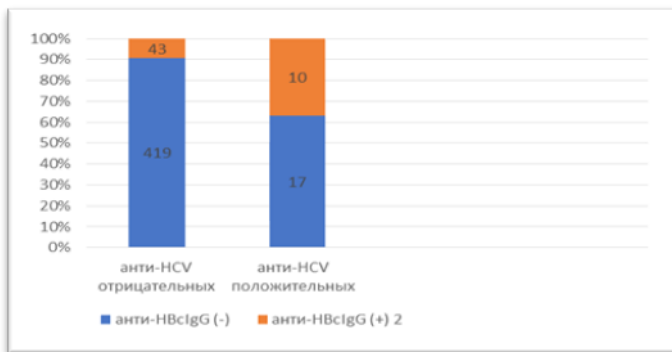
Variable	Patients N (%)
Total N (%)	512
HBsAg (+)	14 (2.7%)
Anti-HCV (+)	17 (3.3%)
HBsAg/anti-HCV co-infection	0/512 (0%)
anti-HBcIgG (+) in anti-HCV (-)	43/481 (8.9%)
anti-HBcIgG (+) in anti-HCV (+)	10/17 (37%)
anti-HBs (+) in anti-HCV (-)	26/498 (5.2%)
anti-HBs (+) in anti-HCV (+)	8/17 (18.6%)

When studying HBV DNA in individuals with a positive anti-HBcIgG indicator, the viral load was determined in 7 out of 43 (16.3%) anti-HCV negative and 2 out of 10 (20%) anti-HCV positive donors, as shown in Figure 1.

In a study of preoperative patients, 11.6% had positive anti-HBc IgG, 18.75% of them had a minimal viral load. The mean HBV

DNA value was 254 IU/ml. None of them have a positive anti-HBs status.

Among 6 patients with HCV, 50% had anti-HBcIgG; 33.3% had an HBV DNA count that was 114 IU/mL. Our study showed that among patients screened preoperatively, 2% had low levels of HBV DNA.



Pic. 1. The prevalence of OBH in patients with HCV and without it

Among patients diagnosed with HCV, 16.6% had HCV DNA when tested by PCR. Anti-HBcIgG was detected more often in the group of people aged 50 years and older than in the group of people aged 40 years and younger ($p < 0.05$).

Those with previous surgery also had a higher prevalence of positive anti-HBcIgG than those who had never had surgery (17.2% vs 6.4%; $p > 0.01$), but the prevalence of HBV DNA detection was similar in both groups ($p = 0.17$).

Among patients with HIV, anti-HBcIgG was detected in 18 of 42 women (42.8%) and 23 of 33 men (69.6%). Patient characteristics and virological profile are shown in Table 2.

Table 2.

Characteristics of patients and detection of viral markers among HIV-infected patients

	Females	Males
Total (N=75)	42 (56%)	33 (44%)
Mean age	32.1±3.59	39.6±4.64
Anti-HBcIgG	18 (42.8%)	23 (69.9%)
HBV DNA	3 (42.8%)	4 (57.1%)

Of the 41 patients with anti-HBcIgG (+) antibodies, 7 (17%) had HBV DNA detected. The HBV DNA value averaged 304 IU/ml; Anti-HBs were not detected in any of the patients with positive HBV DNA.

We aimed to identify latent hepatitis B in patients with β -thalassemia, in whose blood serum there was no surface antigen HBsAg. The studies were conducted in 68 transfusion-dependent patients with β -thalassemia. Of these, 36 (52.9%) patients had β -thalassemia major, and 32 (47.1%) had β -thalassemia intermedia. The age of the patients ranged from 18 to 53 years, and on average was 32.1±6.0 years.

Using the PCR method (Abbot real time; sensitivity 12 Bv/ml), the HBV DNA load was examined in all HBsAg-negative/anti-HBc-positive patients with β -thalassemia major and intermediate forms. A positive test result for anti-HBcIgG was obtained in 29 (63%) of 46 patients with β -thalassemia who did not have detectable HBsAg and did not have hepatitis C virus (anti-HCV). Of these, 8 (27.5%) patients with β -thalassemia had HBV DNA at low but detectable levels. HBV DNA ranged from 12 IU/ml to 303 IU/ml with a mean value of 259±18.6 IU/ml. Anti-HBs were not detected in any of the β -thalassemia patients who had a positive viral load.

Of the 22 β -thalassemia patients with concomitant HCV infection, 9 (40.9%) were positive for anti-HBcIgG, and 4 (26.6%) were positive for HBV DNA. The mean HBV DNA value was 204 ± 11.4 IU/ml. Among patients with positive HBcAg and negative HBV DNA, 17 had a positive Anti-HBs indicator. One patient with an HBV DNA viral load of 13 IU/mL tested positive for anti-HBs, although at a low level (19 IU/mL). In total, among patients with β -thalassemia without HCV, HBV DNA was detected in 8 (17.3%), and among patients with β -thalassemia with concomitant HCV - 4 (18.1%);. In the group with HCV, in one patient with β -thalassemia major, anti-HBcIgG, HBV DNA with low replication activity, and anti-HBs were simultaneously determined.

When examining cancer patients with increased aminotransferases, out of 51 patients, five (9.8%) patients were positive for anti-HBcIgG; detectable HBV DNA was observed in 2 (40%) samples ($p < 0.5$). HBV DNA levels were 69 IU/ml and 312 IU/ml, respectively. The overall mean pretreatment AST and ALT levels were 30.2 ± 43.5 and 28.9 ± 25.1 , respectively.

Fluorodeoxyuridine (5FUdR) for the treatment of colorectal liver metastases was associated with higher elevations in liver enzymes, possibly due to involvement of the liver tissue itself ($p = 0.01$). The greatest increase was noted after the fourth cycle of chemotherapy. In terms of the T-test, the difference was significant ($P = 0.035$). There was no significant difference between combining chemotherapy with other combination treatments such as surgery or radiation therapy and the risk of LHB reactivation ($p = 0.18$). During the 12-month follow-up period, HBsAg remained negative in all patients, as did anti-HBcIgM.

Patients with hepatitis C constitute the main group of subjects, as they reflect the influence of the presence of HBV on the course of the disease, the severity of the degree of damage at initial detection, and also represent a group of risks for clinical and virological reactivation during the treatment of HCV and the elimination of the

HBV C virus, which suppresses replication. Examination of patients with hepatitis C includes 2 separate groups that were observed at different intervals. In the first group, the incidence of LHB and the influence of its presence on liver parameters, as well as the degree of damage to liver tissue, were determined. The second study tracked patients with HCV treated with direct-acting antivirals and determined the risk of LHB reactivation. In our study, detection of HBcIgG was observed in 43% of patients with chronic hepatitis C. Detectable HBV DNA was observed in 25% of HBcIgG-positive and 10.9% of all patients with hepatitis C. HBV DNA was not detected in any of the HBcIgG-negative patients.

Table 3.

The description and characteristics of the patients

Variable	Number of patients N (%)
Gender	
Males	106 (64.6)
Females	58 (35.4)
Total	164(100)
HbcIgG:	
-	92(56.1)
+	72(43.9)
Treatment:	
Naive	137(83.5)
PEG + IFN	27(16.5)
Genotype:	
1	120(73)
2	9(5.5)
3	36(22)
Anti-HBS:	
Neg	123(75)
Pos	41(25)
SVR at week 24	
-	2(1.2)
+	162(98.8)

Of this group, 4 patients had a pre-treatment HBV DNA viral load of <2000 copies/mL and were considered inactive carriers. The overall range of pre-treatment HBV DNA was 147–12,580 IU/mL. In 7 patients (29.2%), HBV DNA levels increased by 1–2 log during treatment ($p>0.05$). A parallel increase in ALT levels was noted (median 105 ± 12.3 IU/l, $p>0.05$). All 7 patients were anti-HBs negative, and the baseline HBV DNA load was >2000 IU/mL.

Table 4.

Relationship between the degree of fibrosis, ALT, and the presence of HBcIgG

Variable	HBcIgG	N	Mean (m)	P
ALT	-	92	70.8±3.2	0.0001
	+	72	93.05±5.6	
Fibrosis	-	92	7.9±0.43	0.0001
	+	72	11.6±0.82	

DAA treatment did not cause reactivation of latent HBV. However, in the treatment of 24 patients with HCV and concomitant chronic HBV, in 7 (29.2%) HBV DNA levels increased by 1–2 log during treatment ($p>0.05$), and a parallel increase in ALT levels was noted (median 105 ± 12.3 IU/l, $p>0.05$). Overall SVR was not associated with the presence of overt or latent hepatitis B. Two patients (11.1%) had relapse, but none had evidence of HBV reactivation, such as increased ALT or increased DNA.

Among the study groups, the highest mean age was observed in cancer patients (66.9 ± 3.13), the lowest age among patients with thalassemia (32.1 ± 12.8), the highest number of women in the HIV group (56%), and the highest detection rates HCV among the study groups was observed in the group of patients with β -thalassemia (26.4%). Among patients with HCV, thalassemia and HIV, the

highest number of LHB was identified, which is explained by possible routes of infection with the virus.

Thanks to advances in molecular biology, latent viral infection is attracting increasing attention from clinicians and virologists. The clinical and biological significance and mechanisms of formation of latent HBV infection, as well as the difficulties of its diagnosis, are currently being studied quite intensively, are periodically updated with new data and are the subject of debate.

Because classic LHB is by definition a low-replicative form of infection, clinical forms of infection are unlikely. The patient most often does not know that he has an infection, and the diagnosis is made by chance, during a laboratory test. Currently, LHB is primarily a problem of transfusiology and transplantology.

During HBV infection, the spectrum and severity of clinical manifestations depend on the relationship between the virus and the host's immune system, ranging from asymptomatic carriage of the virus to severe damage to various organs and systems. For all forms of chronic HBV infection, its obligatory condition was the presence of HBsAg in the blood serum.

The disappearance of HBsAg and the appearance of antibodies to it were considered as a sign of the body being freed from the virus, i.e., the cessation of infection. However, clinical and morphological studies of earlier years indicated the identity of the activity and stage of the liver process in patients with chronic liver diseases with the presence of "isolated" anti-HBc and in patients with HBsAg.

The mechanisms of development of low-level HBV replication remain unexplored. It is known that alcohol can interfere with the mechanisms of viral replication, and in alcohol abusers, anti-HBc is often the only marker of chronic HBV infection. Also, superinfection with hepatitis D and/or C virus can have an inhibitory effect on HBV replication, which leads to a decrease in the level of viremia and clearance of HBeAg, and in the case of HCV, not only HBeAg, but also HBsAg disappears.

A similar effect is observed with the human immunodeficiency virus. In the absence of other factors, mutations in various parts of the virus genome, primarily in the crossover region of the C and X genes, which are responsible for virus replication, play an important role in the formation of low-level HBV replication.

Certain virus-host factors are associated with the risk of HBV reactivation in LHB patients. Host factors include, for example, the intensity of immunosuppression, especially when rituximab is used in combination with steroids and hematopoietic stem cell transplantation. Viral factors include absence of anti-HBs before chemotherapy, decline in anti-HBs during chemotherapy, detectable serum HBV DNA, viral genotype, and mutations in the Pre-cor and Cor promoters.

The degree of immunosuppression determines the frequency and severity of LHB reactivation, such as aggressive forms of chemotherapy or immunosuppression. Several studies have highlighted the increasing incidence of reverse seroconversion in anti-HBs-positive patients following courses of anti-CD20-containing chemotherapy (rituximab) and/or autologous hematopoietic stem cell transplantation for hematologic malignancies.

Also, subclinical reactivation of LHB is possible in HBsAg-negative patients after solid organ transplantation without clinical hepatitis. There is a small risk of occult transmission of HBV from HBsAg-negative and anti-HBc-positive donors after kidney, heart, or bone marrow transplantation, but this risk is increased (17–90%) with orthotopic liver transplantation, especially if the recipient is negative for all serum markers of HBV.

Most often, such hepatitis has a benign course, and it is less severe compared to hepatitis B, which develops as a result of reactivation of HBV in an anti-HBc-positive recipient.

Since the persistence of cccDNA is an indicator of contact with the virus, the question arises about the role of latent HBV infection in

the development of chronic diffuse liver damage. However, clinical analysis of cccDNA is limited due to the invasiveness of liver biopsy.

CONCLUSIONS

1. The presence of LHB infection was established in the examined individuals whose blood serum did not contain HBsAg antigen. LHB infection was detected in 1.5% of donors with negative viral markers, in 2% of patients screened before surgery, in 10.9% of patients with chronic hepatitis C, in 27.5% of transfusion-dependent patients with beta thalassemia, 9.3% of patients with HIV and in 3.9% of cancer patients with an increase in aminotransferases during chemotherapy (2,3,4,5,6,8,9,10,11,12,13,14).
2. In groups where donors and patients with and without HCV were examined, the presence of HCV was characterized by a higher frequency of detection of latent hepatitis B viral infection, which is explained by the common route of infection. The prevalence of LHB is associated with blood transfusions, previous operations, and is also observed more often in the older age group. (1.5, 10,11,12,13,14)
3. When comparing a group of patients with HCV with concomitant LHB and without latent LHB, it was found that the presence of LHB was accompanied by higher rates of ALT and liver fibrosis (ALT – 70.8 ± 3.2 Me/l versus 93.0 ± 5.6 Me/l, $p = 0.001$; Fibroscan – 7.9 ± 0.43 kPa versus 11.6 ± 0.82 kPa, $p = 0.001$), which indicates a negative effect of the presence of the virus on the course of chronic liver disease (2,4,7,10).
4. When treating a group of patients with LHB with direct-acting antiviral drugs, no clinical or virological reactivation was observed, while when treating combined HBV/HCV infection, in 29.2% of patients, the level of HBV DNA increased by 1–2

log during treatment ($p < 0.05$), and there was also a parallel increase in ALT levels (median 105 ± 12.3 IU/l, $p < 0.05$). The overall SVR rate of HCV treatment was not associated with the presence of overt and latent hepatitis B and was 98.7%, which is consistent with international data on treatment with direct-acting antivirals (2,4,7,8,9,13).

5. As a result of the work, it was found that, given the risk of clinical and virological reactivation of HBV when using DAAs (sofosbuvir, daclatasvir, ledipasvir, etc.), as well as in the treatment of cancer patients, regular scheduled monitoring of liver functional parameters plays an important role and assessment of changes in viral load, as well as adequate correction if they increase (2,4,7,9).

PRACTICAL RECOMMENDATIONS

1. According to our data, the diagnostic value of HBsAg as the main marker of hepatitis B is ambiguous, since according to the information we received, even in the absence of the surface antigen HBsAg in the blood serum of patients, the virus can be latent and even actively replicate.
2. When patients undergo chemotherapy and immunotherapy, before starting treatment, an additional examination is necessary for the presence of anti-HBs and anti-HBc antibodies in the serum. In case of positive anti-HBc and negative anti-HBs, periodic monitoring of ALT increases is recommended for timely detection of LHB reactivation with further testing by highly sensitive methods, such as the use of PCR to detect viral DNA.

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LIST OF ABBREVIATIONS USED

VHB	- viral hepatitis B
AHB	- acute hepatitis B
CHB	- chronic hepatitis B
LHB	- latent (occult) hepatitis B
HCV	- viral hepatitis C
HDV	- viral hepatitis D
HIV	- human immunodeficiency virus
NAFLD	- non-alcoholic fatty liver disease
CKD	- chronic liver disease
HCC	- hepatocellular carcinoma
ALT	- alanine transferase
AST	- aspartame transferase
GGT	- gamma-glutamic acid
ALP	- alkaline phosphatase
TB	- total bilirubin
PCR	- polymerase chain reaction
DNA	- Deoxyribonucleic acid
cccDNA	- covalently closed circular DNA
RNA	- Ribonucleic acid
mRNA	- micro-ribonucleic acid
US	- ultrasound examination
NAT	- Nucleic Acid Testing
IF	- interferon
TNFα	- tumor necrosis factor α
SVR	- sustained virological response.
RVR	- rapid virological response
DAAs	- direct acting antivirals
5FU (5-FU)	- 5-fluorodeoxyuridine
AASLD	- American Association for the Study of Liver Diseases
EASL	- European Association for the Study of Liver Diseases
APASL	- Asian Pacific Association for the Study of Liver

The defense will be held on the 28th of March 2024 at 14:00 at the meeting of the Dissertation council ED 2.27 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at Azerbaijan Medical University.

Address: Bakikhanov str 23, Baku Azerbaijan (conference room)

Dissertation is accessible at the Library of the Azerbaijan Medical University.

Electronic version of the abstract is available on the official website of the Azerbaijan Medical University.

Abstract was sent to the required addresses on 21 February 2024.

Signed for print: _____

Paper format: 60x841/16

Volume: 28734

Number of hard copies: 100