Am J Transl Res 2024;16(6):2301-2309
www.ajtr.org /ISSN:1943-8141/AJTR0156652

Original Article

Quantitative PCR-based high-sensitivity detection of HBV-DNA levels reflects liver function deterioration in patients with hepatitis B virus-related cirrhosis

Hao Zhang[1], Jiayun Ding[2], Yingzhen Zhou[1]

[1]Laboratory Department, Suzhou Hospital of Integrated Traditional Chinese and Western Medicine, Suzhou 215000, Jiangsu, China; [2]Laboratory Department, Suzhou Guangci Cancer Hospital, Suzhou 215000, Jiangsu, China

Received March 18, 2024; Accepted May 20, 2024; Epub June 15, 2024; Published June 30, 2024

Abstract: Objectives: To investigate the clinical implication of quantitative polymerase chain reaction (PCR)-based high-sensitivity detection of hepatitis B virus (HBV)-DNA levels in patients with HBV-related liver cirrhosis (LC). Methods: From January 2020 to December 2022, 100 fasting serum samples were collected and retrospectively analyzed from patients with treated HBV-related LC attending the Suzhou Hospital of Integrated Traditional Chinese and Western Medicine and Suzhou Guangci Cancer Hospital. Patients were divided into a negative group (HBV-DNA < 20 IU/mL) and a positive group (HBV-DNA ≥ 20 IU/mL) according to their high-sensitivity HBV-DNA test results. The clinical characteristics and serological indicators of the two groups were compared, mainly including gender, age, liver function [total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL), and indirect bilirubin (IBIL)], lipids [total cholesterol (TC) and triglycerides (TG)], platelets (PLT), five serum liver fibrosis markers [cholyglycine (CG), hyaluronic acid (HA), laminin (LN), precollagen type III (PCIII), and type IV collagen (IV-C)], serum gastrointestinal tumor markers [α-fetoprotein (AFP) and carcinoembryonic antigen (CEA)], and hepatitis B surface antigen (HBsAg). The differences between the two groups in terms of liver function Child-Pugh grades and the incidence of hepatocellular carcinoma (HCC) were also compared. Results: There were 39 patients in the positive group, including 29 males and 10 females, and 61 patients in the negative group, including 38 males and 23 females, with no statistically significant differences in gender and age distribution between the two groups ($P > 0.05$). The levels of serological indicators (TP, ALB, AST, GGT, ALP, TBIL, DBIL, IBIL, TC, TG, PLT, CG, HA, LN, PCIII, IV-C, AFP, CEA, and HBsAg) in both groups showed no significant differences ($P > 0.05$), but the ALT level in the positive group was higher than that in the negative group ($P < 0.0001$). The positive group had worse Child-Pugh grades and higher HCC incidence compared to the negative group ($P < 0.0001$, $P = 0.028$). Conclusions: Patients with HBV-related LC and HBV-DNA ≥ 20 IU/mL have higher serum ALT levels, worse liver function Child-Pugh grades, and higher HCC incidence than those with HBV-DNA < 20 IU/mL. High-sensitivity HBV-DNA quantification can reflect the deterioration of liver function in patients with HBV-related LC to some extent.

Keywords: Quantitative PCR assay, hepatitis B virus-related liver cirrhosis, high-sensitivity HBV-DNA quantification

Introduction

Hepatitis B virus (HBV) infection is a worldwide disease, with approximately 257 million patients with chronic HBV infectionand 887,000 deaths from HBV infection-related liver diseases each year [1]. Related studies have shown an intrinsic link between hepatitis B, liver fibrosis, liver cirrhosis (LC), and hepatocellular carcinoma (HCC) in a progressive manner [2]. In patients with chronic hepatitis B (CHB), progression to HBV-related LC followed by further HCC is the natural course of CHB, and in a few cases of CHB, HCC may occur directly. CHB has been shown to increase the risk of HCC by approximately 10-30 times compared to non-HBV [3]. Chronic viral hepatitis creates a microenvironment in the liver with a cycle of hepatocyte death and regeneration that favors the growth of hepatocytes with a proliferative advantage, ultimately leading to cancer [4]. In China, the number of patients with LC and HCC due to HBV infection accounts for 60% and 80%, respectively [5], and preven-

https://doi.org/10.62347/BDLO2786

tion of end-stage liver diseases such as HBV-related LC and HCC is an important therapeutic goal for chronic HBV infection.

Studies showed that the HBV-DNA level is an independent risk factor for the development of LC and HCC [6, 7]. The higher the HBV-DNA load, the higher the risk of LC and HCC [8, 9]. The most common mutation in HBV-related HCC is HBV-DNA integration into chromosomes [10]. Approximately 90% of HBV-related HCC cells contain integrated HBV DNAs, usually at multiple chromosomal loci [11, 12]. However, HBV-DNA integration is an incidental event during HBV infection and can be found in only 0.1%-1% of infected hepatocytes, but it becomes the most common mutation in the majority of HBV-related HCC cases [13]. Over the past few years, the widespread use of nucleoside anti-HBV drugs has resulted in significant suppression of HBV replication in treated patients. However, in clinical practice, some patients still exhibit low-level viremia (LLV) after long-term treatment with potent nucleoside analogues. Although the definition of LLV has not been standardized, it usually refers to a condition with serum HBV-DNA < 2000 IU/mL, and a poor prognosis is strongly associated with LLV, especially in patients with LC [14]. The REVEAL study showed an increased risk of cirrhosis and HCC with increasing HBV-DNA level [15]. Moreover, conventional reagent tests are prone to false negatives, leading to missed diagnosis or misdiagnosis. The platform for discovering HBV integration sites in the chromosomes of HCC cells has rapidly evolved from the classical Southern blot to reverse transcription-polymerase chain reaction (PCR), which allows rapid estimation of the copy number of HBV integrations in HCC or pre-cancerous lesions but is of limited value for identifying the details of HBV integrations at the level of nucleotide sequences [16, 17]. Thus, the use of highly sensitive and specific assays is essential to guide the clinical management and treatment of patients with HBV-related LC and low viral load. This study focuses on the clinical implication of quantitative PCR-based high-sensitivity detection of HBV-DNA levels in patients with HBV-related LC.

Materials and methods

General information

From January 2020 to December 2022, 100 fasting serum samples were collected and retrospectively analyzed from patients with treated HBV-related LC attending the Suzhou Hospital of Integrated Traditional Chinese and Western Medicine and Suzhou Guangci Cancer Hospital. Patients were divided into a negative group (HBV-DNA < 20 IU/mL) and a positive group (HBV-DNA ≥ 20 IU/mL) according to their high-sensitivity HBV-DNA test results. This study was approved by the Ethics Committee of Suzhou Hospital of Integrated Traditional Chinese and Western Medicine.

Diagnostic criteria for HBV-related LC [18, 19]

(1) History of and blood testing with current HBV infection, i.e. positive hepatitis B surface antigen (HBsAg), or a clear history of chronic HBV infection (previous HBsAg positive > 6 months, current HBsAg positive, and hepatitis B core antibody positive), with exclusion of other etiologies. (2) Histopathologic findings of liver biopsy consistent with LC presentation. (3) Those who met 2 or more of the following: a) imaging studies showed signs of LC and/or portal hypertension; b) endoscopy indicated esophagogastric fundic varices; c) liver elastography showed liver stiffness consistent with LC; d) blood biochemistry revealed reduced albumin (< 35 g/L) and/or prolonged prothrombin time (more than 3 s longer than control); e) hematology tests showed platelets (PLT) < 100*10^9/L.

Staging criteria for LC

(1) Compensated stage of LC: Pathologic or clinical diagnosis of LC, never without serious complications such as ruptured esophagogastric varices bleeding, ascites or hepatic encephalopathy. (2) Decompensated stage of LC: LC patients who presented with the above-mentioned serious complications.

Inclusion criteria

(1) Patients had been on antiviral therapies for at least 2 years. (2) Patients were tested for HBV-DNA levels < 500 IU/mL using conventional reagents. (3) Patients were aged 18-80 years. (4) Patients had a qualitative positive or negative serological test for HBsAg but positive HBV-DNA test. (5) Liver ultrasound with homeopathic elastography suggested LC, or abdominal imaging suggested LC/HCC, or liver puncture biopsy suggested LC/HCC. (6) Patients had complete clinical information and signed the informed consent form.

Exclusion criteria

(1) Liver diseases were caused by infection with hepatitis C virus, hepatitis D virus, hepatitis E virus or other hepatophilic viruses. (2) Abnormal liver function or LC was caused by drug-related liver damage. (3) LC was caused by parasitic worms such as schistosome infection. (4) Patients were diagnosed with alcoholic liver disease, non-alcoholic fatty liver, autoimmune liver disease, hepatomegaly, or other metabolic liver diseases. (5) Patients had a history of chronic heavy alcohol consumption (equivalent ethanol intake > 140 g/week for males and > 70 g/week for females). (6) Patients had concomitant malignancy or serious diseases of other systems.

High-sensitivity HBV-DNA assay and reagents

For the HBV quantitative test, the Natch S Automated Nucleic Acid Extraction System and diagnostic reagents were supplied by Sansure Biotech Inc., China, including nucleic acid extraction and amplification reagent kit (magnetic bead method). HBV DNAs were extracted from serum samples using the magnetic bead method. A pair of specific primers, a specific fluorescent probe (TaqMan probe labeled with FAM luciferin at the 5' end), and a PCR reaction solution designed for the conserved region of HBV nucleic acid were used. On a fluorescence quantitative PCR instrument (Bio-Rad, Hercules, CA), real-time fluorescence quantitative PCR detection technology was applied to achieve quantitative detection of HBV DNAs through changes in fluorescence signals. The kit contained a positive internal control (HBV internal standard), and the 5' end of the HBV internal standard TaqMan probe was labeled with HEX fluorescein to monitor whether the sample to be tested had PCR inhibitors by detecting whether the internal standard was normal or not, to avoid PCR false negatives. This reagent kit was also equipped with UNG enzyme+dUTP pollution prevention measures. Before PCR amplification, pre-treatment was carried out at 50°C, and UNG enzyme was used to fully degrade potential PCR product contamination to eliminate possible false positives. In addition, internal reference fluorescence ROX was installed to correct errors caused by sample addition and differences between different tubes, making it easier for the instrument to automatically analyze and report the ratio of fluorescence to internal reference fluorescence ROX, and making quantification more accurate. The sensitivity of the reagent kit was 20 copies/mL.

Two mL of morning fasting venous blood samples were collected from patients using a sterile syringe, transferred to a sterile collection tube, and centrifuged at 1600 rpm for 5 min at room temperature. The serum was separated and transferred to a 1.5-mL sterile centrifuge tube by a pipette for immediate detection or stored at -20±5°C for later use. Before conducting the test, the operator should be familiar with and master the operation of various instruments to be used and the precautions to be taken for quality control of each test. Laboratory management should be strictly in accordance with the laboratory management practice for PCR gene amplification, and laboratory personnel must undergo professional and uniform training. The test process should be strictly partitioned, and the consumables used should be sterilized and disposable. Specialized instruments and equipment should be used for each stage of test operations, and the cross-use of supplies for each stage in each area was not allowed. It was also important that all reagents should be fully thawed and mixed at room temperature before use. For serum samples with negative test results, it should be determined whether the amplification signal of HBV internal standard was normal to ensure the normal use of test operations and detection reagents as well as the appearance of suppressed samples to avoid false negative results. For positive test samples, the amplification signal of HBV internal standard could be disregarded. The above tests were performed by one laboratory staff each from the Department of Laboratory Medicine of Suzhou Hospital of Integrated Traditional Chinese and Western Medicine and Suzhou Guangci Cancer Hospital.

Clinical data collection

Clinical and test data of all patients were collected. The positive rate of HBV-DNA detection by ultrasensitive reagents in all patients was analyzed. The differences in clinical characteristics and serological indicators between the positive and negative groups were compared,

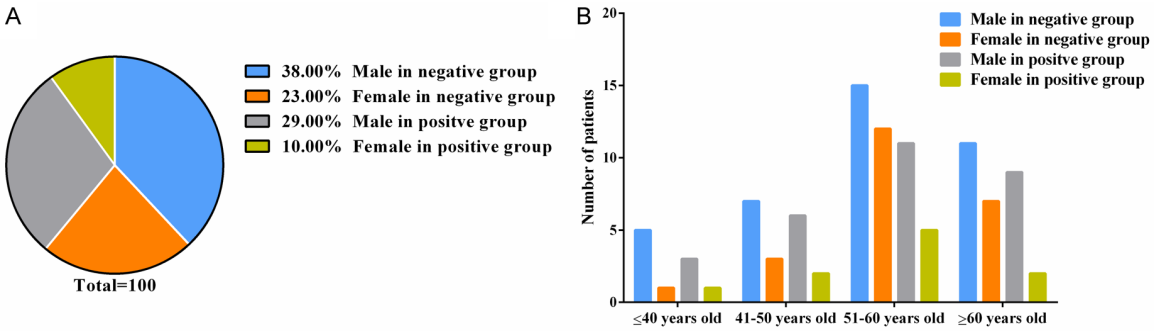

Figure 1. Distribution of gender and age. A. Gender distribution of 100 patients; B. Age distribution of 100 patients.

mainly including gender, age, liver function [total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL), and indirect bilirubin (IBIL)], lipids [total cholesterol (TC) and triglycerides (TG)], PLT, five serum liver fibrosis markers [cholyglycine (CG), hyaluronic acid (HA), laminin (LN), precollagen type III (PCIII), and type IV collagen (IV-C)], serum gastrointestinal tumor markers [α-fetoprotein (AFP) and carcinoembryonic antigen (CEA)], and HBsAg. The differences between the two groups in terms of liver function Child-Pugh grades and the incidence of HCC were also compared.

Data processing

SPSS 20.0 software was used. The measured data were expressed as mean ± standard deviation. The counted data were expressed as percentage. The differences in the distribution of liver function, blood lipids, PLT, five serum liver fibrosis markers, and serum gastrointestinal tumor markers between the two groups were determined by T-test; the differences in the distribution of gender, age, HBsAg level, Child-Pugh grade, and HCC incidence were determined by chi-square test. $P < 0.05$ was considered statistically significant.

Results

Gender and age distribution of the positive and negative groups

After the high-sensitivity HBV-DNA test for 100 patients with HBV-related LC who met the inclusion criteria, there were 39 patients in the positive group, including 29 males and 10 females, and 61 patients in the negative group, including 38 males and 23 females. The differences in gender and age distribution between the two groups were not significant ($P > 0.05$) (**Figure 1**).

Liver function test results in the positive and negative groups

The differences in TP, ALB, AST, GGT, ALP, TBIL, DBIL, and IBIL levels between the two groups were not significant ($P > 0.05$), but the ALT level in the positive group was higher than that of the negative group ($P < 0.05$) (**Figure 2**).

Lipid and PLT test results in the positive and negative groups

The differences in TC, TG, and PLT levels between the two groups were not significant ($P > 0.05$) (**Figure 3**).

Results of five serum liver fibrosis markers in the positive and negative groups

The differences in CG, HA, LN, PCIII, and IV-C levels between the two groups were not significant ($P > 0.05$) (**Figure 4**).

Results of serum gastrointestinal tumor markers in the positive and negative groups

The differences in AFP and CEA levels between the two groups were not significant ($P > 0.05$) (**Figure 5**).

Differences in the distribution of HBsAg levels between the positive and negative groups

The difference in HBsAg distribution between the two groups was not significant ($P > 0.05$) (**Figure 6**).

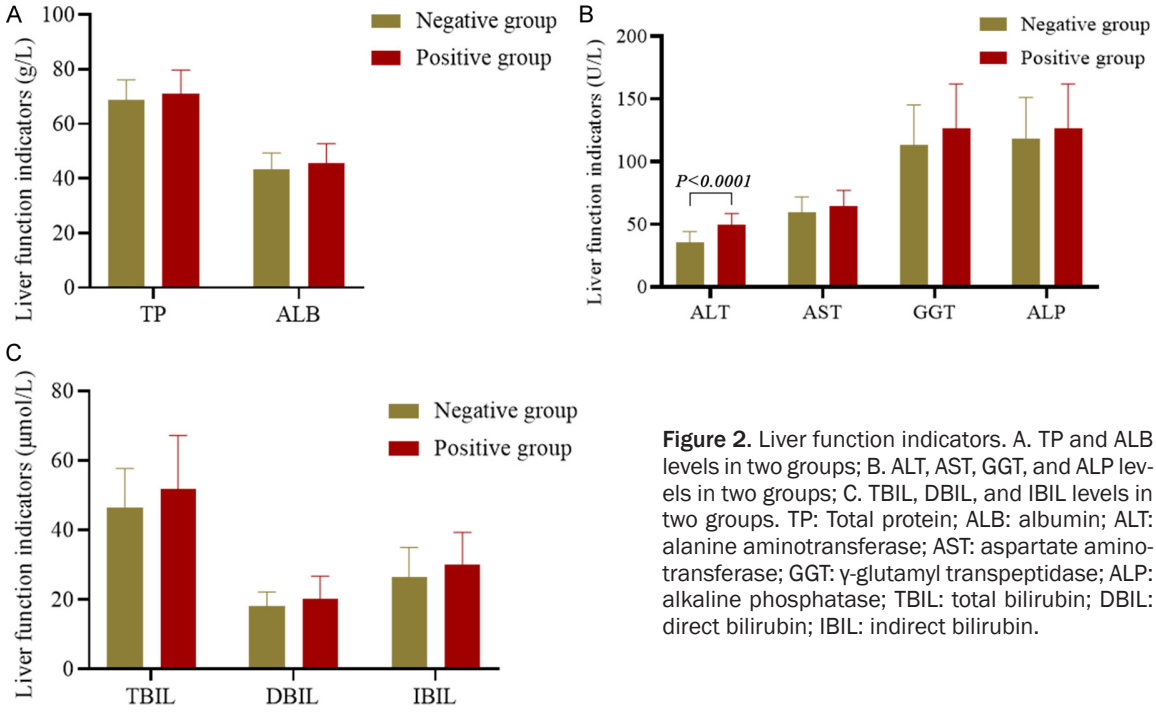

Figure 2. Liver function indicators. A. TP and ALB levels in two groups; B. ALT, AST, GGT, and ALP levels in two groups; C. TBIL, DBIL, and IBIL levels in two groups. TP: Total protein; ALB: albumin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: γ-glutamyl transpeptidase; ALP: alkaline phosphatase; TBIL: total bilirubin; DBIL: direct bilirubin; IBIL: indirect bilirubin.

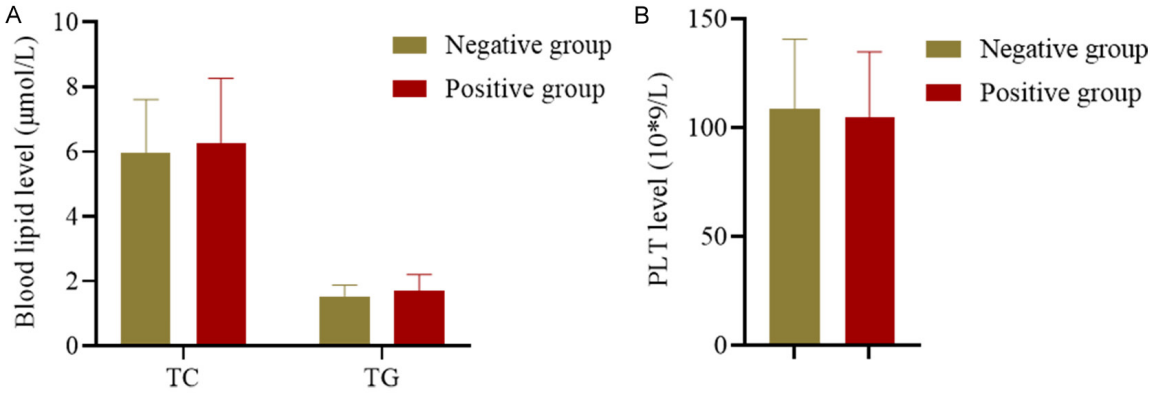

Figure 3. Blood lipid and platelet (PLT) levels. A. Blood lipid level (μmol/L); B. PLT level (10*9/L). TC: Total cholesterol; TG: triglycerides; PLT: platelets.

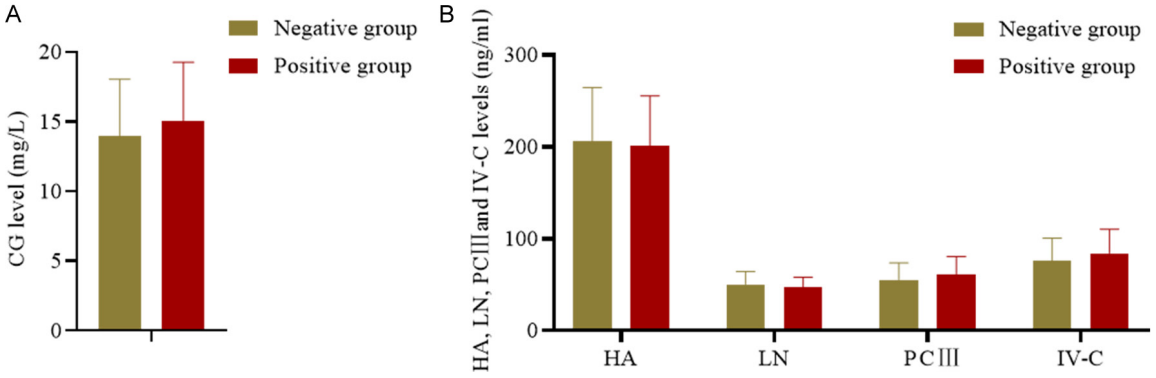

Figure 4. Five serum liver fibrosis markers. A. CG level of the patients; B. HA, LN, PCIII, and IV-C levels of the patients. CG: Cholyglycine; HA: hyaluronic acid; LN: laminin; PCIII: precollagen type III; IV-C: type IV collagen.

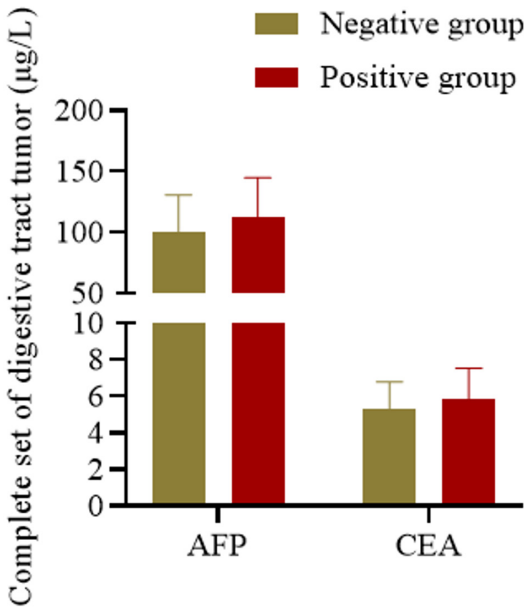

Figure 5. Serum gastrointestinal tumor markers (μg/L). AFP: α-fetoprotein; CEA: carcinoembryonic antigen.

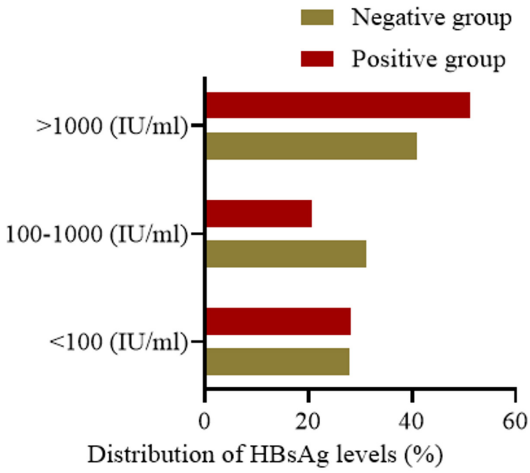

Figure 6. Distribution of HBsAg levels (%). HBsAg: Hepatitis B surface antigen.

Differences in the distribution of Child-Pugh grades between the positive and negative groups

The Child-Pugh grade of the positive group was worse than that of the negative group, ($P < 0.05$) (**Figure 7**).

Comparison of the incidence of HCC in the positive and negative groups

Among 100 patients with HBV-related LC, 3 patients in the positive group were complicated by HCC and 0 patient in the negative group, ($P < 0.05$) (**Figure 8**).

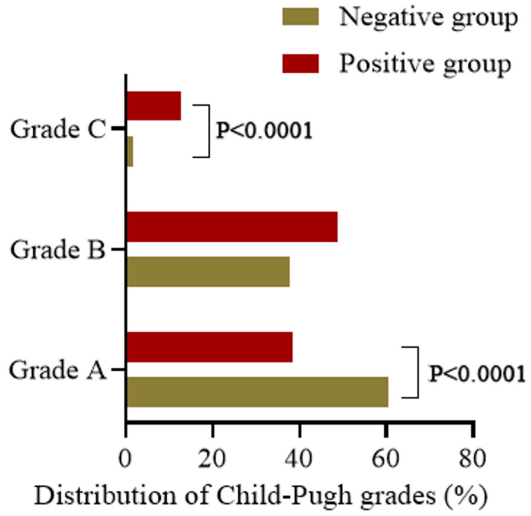

Figure 7. Distribution of Child-Pugh grades (%).

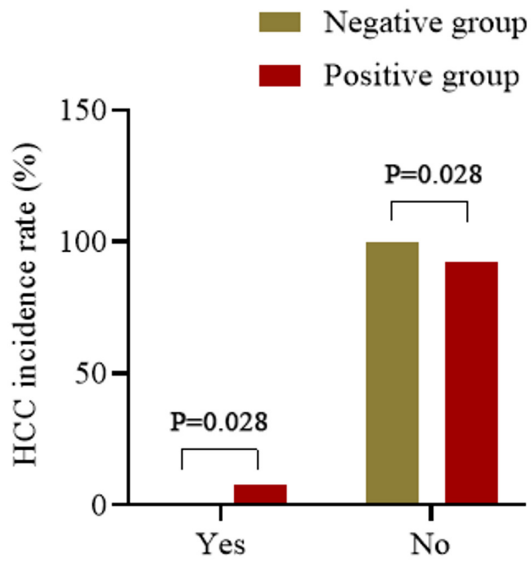

Figure 8. Incidence rate of HCC (%). HCC: Hepatocellular carcinoma.

Discussion

Previous studies have hinted that baseline serum HBV-DNA levels may be associated with HBsAg serological conversion after treatment [20]. Numerous studies have pointed out that the risk of liver fibrosis, LC decompensation, and HCC is significantly reduced in patients with low viral load who develop HBsAg serological conversion spontaneously or after antiviral therapy [21-23]. To date, however, there are no

clear indications for antiviral therapy in this population, and whether this population can benefit from antiviral therapy is controversial and needs to be confirmed by further studies.

Viral replication is more common in patients with HBV-related LC, and is active in approximately 30% to 70% of patients with compensated HBV-related LC. For most of these patients, the clinical ordinary fluorescent probe method PCR is used to detect HBV-DNA levels (lower limit of detection < 1000 IU/mL), which is prone to false negative results for patients with low viral load due to its low sensitivity, thus misleading treatment decision. The high-sensitivity HBV-DNA assay (lower limit of detection < 10 IU/mL) has high sensitivity and specificity and helps to better determine HBV-DNA replication in patients with CHB and low viral load. Jiang et al. [24] studied 72 CHB patients treated with nucleoside analogues who had achieved virological response and met discontinuation criteria to assess the relationship between the extent of HBV-DNA replication and virological rebound after drug discontinuation, with a follow-up period of ≥ 6 months. HBV-DNA values at the time point of drug discontinuation were measured using high-sensitivity PCR reagents, and 41.7% of patients were found to have positive high-sensitivity test results. In this study, the positive detection rate (HBV-DNA ≥ 20 IU/mL) was 39% and the negative detection rate (HBV-DNA < 20 IU/mL) was 61% in 100 patients with HBV-related LC who were tested with high-sensitivity HBV-DNA reagents. After grouping based on the test results, it was found that there were no significant differences in gender, age distribution, or levels of serological indicators (TP, ALB, AST, GGT, ALP, TBIL, DBIL, IBIL, TC, TG, PLT, CG, HA, LN, PC III, IV-C, AFP, CEA, and HBsAg) between the HBV-DNA positive and negative groups. The above indicated that conventional reagent testing had some false-negative results. The clinic should use high-sensitivity reagents for patients with HBV-related LC who are difficult to identify by conventional biochemical indicators and clinical features to improve the detection rate of HBV-DNA and reduce viral recurrence.

Child-Pugh grading is a commonly used clinical grading scale for quantitative assessment of liver reserve function in patients with LC, which includes serum albumin, serum bilirubin, hepatic encephalopathy, ascites, and prothrombin time [25]. Patients with Child-Pugh grade A are generally in the compensated stage of LC and those with Child-Pugh grade B/C in the decompensated stage of LC [26]. In patients with HBV-related LC in the decompensated stage, patients with low HBV-DNA levels have a higher incidence of complications such as spontaneous peritonitis, gastrointestinal bleeding, and HCC compared with those with high HBV-DNA levels [27]. In this study, the differences in the distribution of ALT and Child-Pugh grades of liver function between patients in the positive and negative groups were statistically significant, with higher ALT levels and worse Child-Pugh grade in the positive group than in the negative group. The above suggested that in patients with HBV-related LC and low serum HBV-DNA levels, patients with HBV-DNA ≥ 20 IU/mL were more likely to develop serious complications such as LC decompensation compared with those with HBV-DNA < 20 IU/mL. Therefore, clinical workers should closely follow up and observe this population for a long time in order to find more sensitive indicators for early prediction of disease progression and to guide the clinical management of this population.

Serum HBV-DNA quantification is the most direct indicator of active HBV replication [28]. Molecular biology studies have confirmed that HBV DNAs can integrate into hepatocyte chromosomal DNA, leading to hepatocellular carcinogenesis [29]. Some studies have shown that some patients are chronically seronegative for HBV DNAs before developing HCC [30]. In this study, 100 patients with HBV-DNA levels < 500 IU/mL (determined by conventional reagents) were tested with high-sensitivity reagents; three of them were complicated by HCC, and all these three HCC patients were in the positive group. The above showed that patients with HBV-related LC had the possibility of progressing to HCC despite the low level of viral replication, and the HCC incidence was higher in the positive group than in the negative group. Therefore, clinical attention and treatment should be given to this group of patients to prevent decompensation.

Several studies have shown that although some patients have undetectable serum HBV DNAs during antiviral therapy, infection persists in a subset of patients [31-33]. Accurate and sensitive detection reagents can more effectively guide patients to initiate or adjust

antiviral treatment regimens in a timely manner, resulting in better treatment outcomes, and they are key factors in initiating initial antiviral therapy, determining optimal treatment regimens, judging treatment efficacy and safe treatment endpoints, early detecting drug resistance, and detecting virologic relapse in CHB patients. Future clinical work should place emphasis on long-term effective antiviral therapy to suppress serum HBV-DNA levels until which are undetectable by highly sensitive methods, which is a basic requirement for complete clearance of HBV DNAs. In addition, in view of our findings, it may be possible to propose the hypothesis that further grouping to determine whether antiviral treatment improves each abnormal indicator in the positive group may provide more valuable basic data for clinical work. The limitation of this study is that, due to the limited observation time and observation samples, it was not possible to determine the requirement of this group of patients for further antiviral therapy and the treatment effect, and there is still a need to establish a large follow-up cohort for further in-depth study.

In summary, patients with HBV-related LC and HBV-DNA ≥ 20 IU/mL have higher serum ALT levels, worse liver function Child-Pugh grades, and higher HCC incidence than those with HBV-DNA < 20 IU/mL. High-sensitivity HBV-DNA quantification can reflect the deterioration of liver function in patients with HBV-related LC to some extent.

Disclosure of conflict of interest

None.

Address correspondence to: Yingzhen Zhou, Laboratory Department, Suzhou Hospital of Integrated Traditional Chinese and Western Medicine, No. 39, Xia Sha Tang, Mudu, Wuzhong District, Suzhou 215000, Jiangsu, China. Tel: +86-15995772860; E-mail: 15995772860@163.com

References

[1] Zhao F, Xie X, Tan X, Yu H, Tian M, Lv H, Qin C, Qi J and Zhu Q. The functions of hepatitis B virus encoding proteins: viral persistence and liver pathogenesis. Front Immunol 2021; 12: 691766.

[2] Rizzo GEM, Cabibbo G and Craxì A. Hepatitis B virus-associated hepatocellular carcinoma. Viruses 2022; 14: 986.

[3] Xue X, Liao W and Xing Y. Comparison of clinical features and outcomes between HBV-related and non-B non-C hepatocellular carcinoma. Infect Agent Cancer 2020; 15: 11.

[4] Yeh SH, Li CL, Lin YY, Ho MC, Wang YC, Tseng ST and Chen PJ. Hepatitis B virus DNA integration drives carcinogenesis and provides a new biomarker for HBV-related HCC. Cell Mol Gastroenterol Hepatol 2023; 15: 921-929.

[5] Tian T, Song C, Jiang L, Dai J, Lin Y, Xu X, Yu C, Ge Z, Ding Y, Wen Y, Liu B, Shao Y, Shi P, Zhu C, Liu Y, Jing S, Wang Z, Hu Z and Li J. Hepatitis B virus infection and the risk of cancer among the Chinese population. Int J Cancer 2020; 147: 3075-3084.

[6] Harputluoglu M and Carr BI. Hepatitis B before and after hepatocellular carcinoma. J Gastrointest Cancer 2021; 52: 1206-1210.

[7] Sato K, Inoue J, Akahane T, Kobayashi T, Takai S, Nakamura T, Sato T, Kimura O, Ninomiya M, Iwata T, Sano A, Tsuruoka M, Onuki M, Sawahashi S, Niitsuma H and Masamune A. Switching to tenofovir alafenamide fumarate in chronic hepatitis B patients who had detectable HBV DNA during treatment with entecavir. Tohoku J Exp Med 2022; 258: 277-285.

[8] Sun Y, Wu X, Zhou J, Meng T, Wang B, Chen S, Liu H, Wang T, Zhao X, Wu S, Kong Y, Ou X, Wee A, Theise ND, Qiu C, Zhang W, Lu F, Jia J and You H. Persistent low level of hepatitis B virus promotes fibrosis progression during therapy. Clin Gastroenterol Hepatol 2020; 18: 2582-2591, e2586.

[9] Afifi AM, Elgenidy A, Hashim M, Awad AK and Jalal PK. Hepatitis B virus core-related antigen (HBcrAg) as a prognostic marker for the development of hepatocellular carcinoma: a mini systematic review of the literature. Rev Med Virol 2022; 32: e2353.

[10] Bousali M, Papatheodoridis G, Paraskevis D and Karamitros T. Hepatitis B virus DNA integration, chronic infections and hepatocellular carcinoma. Microorganisms 2021; 9: 1787.

[11] Li CL, Li CY, Lin YY, Ho MC, Chen DS, Chen PJ and Yeh SH. Androgen receptor enhances hepatic telomerase reverse transcriptase gene transcription after hepatitis B virus integration or point mutation in promoter region. Hepatology 2019; 69: 498-512.

[12] Péneau C, Imbeaud S, La Bella T, Hirsch TZ, Caruso S, Calderaro J, Paradis V, Blanc JF, Letouzé E, Nault JC, Amaddeo G and Zucman-Rossi J. Hepatitis B virus integrations promote local and distant oncogenic driver alterations in hepatocellular carcinoma. Gut 2022; 71: 616-626.

[13] Tu T, Budzinska MA, Vondran FWR, Shackel NA and Urban S. Hepatitis B virus DNA integration occurs early in the viral life cycle in an in vitro infection model via sodium taurocholate co-

transporting polypeptide-dependent uptake of enveloped virus particles. J Virol 2018; 92: e02007-17.
[14] Li T, Kong Y, Liu YY, Liu TF, Ma AD, Li LQ, Pei ZY and Zhang LY. Demographic characteristics and associated influencing factors in treated patients with chronic hepatitis B with hypoviremia: a single-center retrospective cross-sectional study. Zhonghua Gan Zang Bing Za Zhi 2023; 31: 42-48.
[15] Iloeje UH, Yang HI, Su J, Jen CL, You SL and Chen CJ; Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-In HBV (the REVEAL-HBV) Study Group. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006; 130: 678-686.
[16] Pollicino T and Caminiti G. HBV-integration studies in the clinic: role in the natural history of infection. Viruses 2021; 13: 368.
[17] Zhang D, Zhang K, Protzer U and Zeng C. HBV integration induces complex interactions between host and viral genomic functions at the insertion site. J Clin Transl Hepatol 2021; 9: 399-408.
[18] European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu; 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: 370-398.
[19] Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS Jr, Bzowej NH and Wong JB. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology 2018; 67: 1560-1599.
[20] Yuen MF, Heo J, Jang JW, Yoon JH, Kweon YO, Park SJ, Tami Y, You S, Yates P, Tao Y, Cremer J, Campbell F, Elston R, Theodore D, Paff M, Bennett CF and Kwoh TJ. Safety, tolerability and antiviral activity of the antisense oligonucleotide bepirovirsen in patients with chronic hepatitis B: a phase 2 randomized controlled trial. Nat Med 2021; 27: 1725-1734.
[21] Kim SK, Fujii T, Kim SR, Nakai A, Lim YS, Hagiwara S and Kudo M. Hepatitis B virus treatment and hepatocellular carcinoma: controversies and approaches to consensus. Liver Cancer 2022; 11: 497-510.
[22] Lok AS, McMahon BJ, Brown RS Jr, Wong JB, Ahmed AT, Farah W, Almasri J, Alahdab F, Benkhadra K, Mouchli MA, Singh S, Mohamed EA, Abu Dabrh AM, Prokop LJ, Wang Z, Murad MH and Mohammed K. Antiviral therapy for chronic hepatitis B viral infection in adults: a systematic review and meta-analysis. Hepatology 2016; 63: 284-306.
[23] Zhang Q, Cai DC, Hu P and Ren H. Low-level viremia in nucleoside analog-treated chronic hepatitis B patients. Chin Med J (Engl) 2021; 134: 2810-2817.
[24] Jiang JN, Huang ZL, He LX, Huang YH, Su MH, Xie R, Liang YX, Fu WD, Huang XH, Guo WW, Zhong SH, Liu ZH, Li SH, Zhu TF and Gao ZL. Residual amount of HBV DNA in serum is related to relapse in chronic hepatitis B patients after cessation of nucleos(t)ide analogs. J Clin Gastroenterol 2015; 49: 323-328.
[25] Rimini M, Rovesti G and Casadei-Gardini A. Child Pugh and ALBI grade: past, present or future? Ann Transl Med 2020; 8: 1044.
[26] Zhao Y, Ren M, Lu G, Lu X, Yin Y, Zhang D, Wang X, Ma W, Li Y, Cai G, Lin Y and He S. The prognosis analysis of liver cirrhosis with acute variceal bleeding and validation of current prognostic models: a large scale retrospective cohort study. Biomed Res Int 2020; 2020: 7372868.
[27] Xu QH, Jie YS, Lin SZ, Shu X, Chen N, Xie QF and Li G. The relationship between the levels of HBV DNA loads and both the clinical characteristics and 48-week prognosis in patients with decompensated cirrhosis due to hepatitis B. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi 2009; 23: 282-284.
[28] Hayashi S, Nagaoka K and Tanaka Y. Blood-based biomarkers in hepatitis B virus-related hepatocellular carcinoma, including the viral genome and glycosylated proteins. Int J Mol Sci 2021; 22: 11051.
[29] D'Souza S, Lau KC, Coffin CS and Patel TR. Molecular mechanisms of viral hepatitis induced hepatocellular carcinoma. World J Gastroenterol 2020; 26: 5759-5783.
[30] Chang JI, Sinn DH, Cho H, Kim S, Kang W, Gwak GY, Paik YH, Choi MS, Lee JH, Koh KC and Paik SW. Clinical outcomes of hepatitis B virus-related hepatocellular carcinoma patients with undetectable serum HBV DNA levels. Dig Dis Sci 2022; 67: 4565-4573.
[31] Mak LY, Wong DK, Pollicino T, Raimondo G, Hollinger FB and Yuen MF. Occult hepatitis B infection and hepatocellular carcinoma: epidemiology, virology, hepatocarcinogenesis and clinical significance. J Hepatol 2020; 73: 952-964.
[32] Inoue T and Tanaka Y. Novel biomarkers for the management of chronic hepatitis B. Clin Mol Hepatol 2020; 26: 261-279.
[33] Mak LY, Huang Q, Wong DK, Stamm L, Cheung KS, Ko KL, Yan R, Ouyang L, Fung J, Seto WK and Yuen MF. Residual HBV DNA and pgRNA viraemia is associated with hepatocellular carcinoma in chronic hepatitis B patients on antiviral therapy. J Gastroenterol 2021; 56: 479-488.