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Original Article

Clinical significance of liver biopsy in the diagnosis of liver disease and the evaluation of the clinical efficacy of antiviral treatment for chronic hepatitis B

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Abstract: Objective: To explore the diagnostic value of liver biopsy in patients with acute/chronic liver diseases and to evaluate the application value of repeated liver biopsy in assessing the efficacy of antiviral therapy in patients with chronic hepatitis B. Methods: This retrospective study involved 146 patients with acute and chronic liver diseases who underwent liver biopsy at the Affiliated Hospital of Putian University from January 2018 to December 2023. Differential diagnoses were made for patients with liver diseases based on their pathological results from liver biopsy. Additionally, the effectiveness of antiviral treatment and changes in liver fibrosis in patients with hepatitis B infection before and after antiviral therapy were assessed using repeated liver biopsy. Results: The overall concordance rate between clinical and histopathological diagnoses was 79.45% (116/146). Specifically, the highest concordance rate was for chronic hepatitis B at 82.61% (76/92), followed by fatty liver disease at 77.78% (7/9), autoimmune liver disease at 75% (12/16), and drug-induced liver injury at 72.72% (16/22), and lastly, hepatitis B-related cirrhosis at 71.43% (5/7). After antiviral therapy, the number of cases with positive HBeAg and HBV-DNA significantly decreased compared to before treatment, while the number of cases with negative HBeAg increased, showing a statistically significant difference ($P<0.001$). The number of patients at fibrosis stages S3-S4 decreased after antiviral therapy compared to before treatment ($P=0.040$ and $P=0.028$), while the number of patients at stage S2 increased ($P=0.040$). Conclusion: Liver biopsy aids in the diagnosis of liver diseases and can effectively evaluate the degree of liver fibrosis before and after antiviral therapy for chronic hepatitis B.

Keywords: Hepatitis B, liver biopsy, liver fibrosis, pathological staging, entecavir

Introduction

Hepatitis B is a prevalent disease in hepatology, characterized by hepatic cell damage following infection with hepatitis B virus (HBV). These injuries can result in liver function abnormalities, progressing to liver fibrosis, cirrhosis, and even liver cancer [1, 2]. China, accounting for about one-third of the total HBV-infected population worldwide, remains one of the hardest hits of hepatitis B [3]. Although recent years have seen a decline in HBV infection rates due to enhanced healthcare focus and widespread vaccination, there is still a significant risk of HBV infection, particularly in remote and medically underserved regions [4, 5].

Antiviral therapy is commonly used in the clinical treatment of hepatitis B. Numerous studies have shown that entecavir can inhibit the activity of HBV polymerase, exerting an antiviral effect and is considered a first-line drug for the treatment of chronic hepatitis B [6]. While non-invasive clinical indicators are often used to evaluate the efficacy of antiviral therapy, and many clinical indicators can effectively assess the degree of liver fibrosis in chronic hepatitis B [7, 8], these indicators alone cannot completely replace ultrasound-guided liver biopsy in assessing liver disease progression [9]. Furthermore, liver biopsy plays a crucial role in the differential diagnosis of liver diseases [10]. For initially undiagnosed liver diseases, liver biopsy is a commonly used clinical detection method. However, there are few reports on repeated liver biopsy after antiviral therapy. This study evaluates the application value of repeated liver biopsy in assessing the efficacy of antiviral therapy for hepatitis B.

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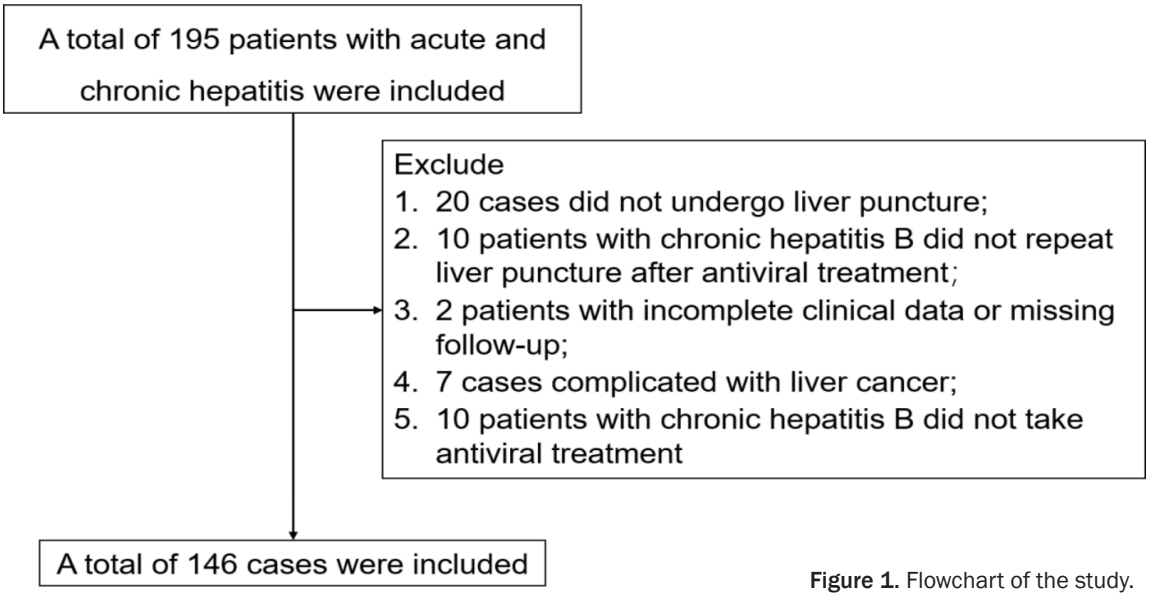

Figure 1. Flowchart of the study.

Methods and materials

Case enrollment

A retrospective study was conducted on 146 patients with acute/chronic liver diseases treated at The Affiliated Hospital of Putian University from January 2018 to December 2023. This study was approved by the Ethics Committee of the Affiliated Hospital of Putian University. Informed consent was obtained from all patients undergoing liver biopsy.

Inclusion and exclusion criteria

Inclusion criteria: ① Patients with clinical indicators or ultrasound suggesting acute or chronic liver disease, who underwent liver biopsy for confirmation; ② Patients who had not received antiviral treatment before liver biopsy diagnosis; ③ Chronic hepatitis B patients who received standardized antiviral treatment; ④ Chronic hepatitis B patients who underwent repeated liver biopsy after antiviral treatment; ⑤ Complete clinical and pathological data.

Exclusion criteria: ① Patients with severe cardiac, hepatic, pulmonary, or renal insufficiency; ② Patients with malignant liver tumors; ③ Patients unable to comply with antiviral treatment after liver biopsy.

The initial screen identified 195 patients with clinically diagnosed acute/chronic liver diseases. According to the exclusion criteria, 20 patients were excluded for not undergoing liver biopsy, 7 for concurrent liver cancer, 10 for not receiving antiviral treatment after hepatitis B diagnosis, 10 for not undergoing repeated liver biopsy after antiviral treatment, and 2 for having incomplete clinical data or were lost to follow-up. Finally, 146 patients were included in the study, of whom, 92 were diagnosed with chronic hepatitis B. The specific process is shown in **Figure 1**.

Data extraction

By retrieving relevant information of patients who underwent liver biopsy from the inpatient and outpatient systems of our hospital, the clinical data of the included patients were extracted. This data includes patient gender, age, BMI, liver biopsy pathology, liver biopsy results after antiviral treatment, hepatitis B serology before and after antiviral treatment, and patient follow-up status for statistical analysis.

Methods

Liver biopsy procedures: The liver biopsy was performed under the guidance of ultrasound (Resona7, Shenzhen Mindray Bio-Medical Electronics Co., Ltd.), with a probe frequency of 1.0-5.0 MHz to locate the biopsy site. Patients fasted before the procedure. The positions of major blood vessels and bile ducts within the liver were identified and marked preoperatively. The puncture area was routinely disinfected with povidone-iodine. Patients underwent local

infiltration anesthesia using lidocaine. The patient was positioned in a left lateral decubitus position, and the 9th-10th intercostal space along the right anterior axillary line was selected as the puncture point. Under real-time ultrasound guidance, the puncture needle was carefully advanced to 1 cm below the liver capsule, avoiding major blood vessels and bile ducts. Approximately 1-2 cm of liver tissue was sampled using a disposable automatic biopsy needle.

Pathological slice reading: After liver biopsy, the obtained liver tissue was fixed in a 10% formalin solution and sent to the Department of Pathology for sectioning and examination. The pathological results were determined by two physicians with at least associate professor titles.

Liver fibrosis staging: According to the 2015 *Classification Standards for Fibrosis Staging*, pathological diagnosis was based on the presence of pseudolobules and the extent of fibrosis spreading to the portal areas. The fibrosis stages were categorized from S0-S4, where S0 denotes no fibrosis, S1 mild liver fibrosis, S2-S3 moderate liver fibrosis, and S4 severe liver fibrosis [11].

Antiviral treatment regimen for chronic hepatitis B (CHB) patients: All patients diagnosed with CHB underwent standard antiviral regimen: oral administration of Entecavir dispersible tablets, 1 tablet each time, once daily, taken on an empty stomach in the morning. A follow-up liver biopsy was conducted 24 weeks after the commencement of the antiviral treatment for the observation of pathological changes in the liver.

Diagnostic criteria and efficacy evaluation

Pathological diagnosis analysis: The pathological results of 146 liver biopsy cases were analyzed, and the concordance rate between pathological diagnosis and clinical diagnosis was further examined.

(1) CHB [11]: Patients were diagnosed with CHB if there was a history of hepatitis B or Hepatitis B surface antigen (HBsAg) positivity for over 6 months, with current HBsAg and/or HBV DNA still positive. Clinical diagnosis is based on serological, virological, biochemical tests, and other clinical and auxiliary examination results of HBV infection.

(2) Hepatitis B-related cirrhosis (HBC) [11]: Patients met the diagnostic criteria for hepatitis B and cirrhosis as evidenced by ultrasonography. Clinical staging of cirrhosis is divided into five stages: Stages 1-2 are compensatory, with Stage 1 showing no varices or ascites, and Stage 2 showing varices without bleeding or ascites. Stages 3-5 are decompensatory, with Stage 3 showing ascites without bleeding, with or without varices; Stage 4 showing bleeding, with or without ascites; and Stage 5 indicating sepsis.

(3) Drug-induced liver injury (DILI) [12]: Patients were confirmed to have DILI if they had medication histories before the advent of abnormal liver function, and with biochemical diagnostic criteria including one of the following: ALT≥5 × ULN or ALP≥2 × ULN, especially with increased 5'-nucleotidase or γ-GGT and exclusion of bone disease-induced ALP elevation or ALT≥3 × ULN and TBil≥2 × ULN; exclusion of other causes of liver injury such as viruses, immune, alcohol, genetic metabolism, bile ducts, blood vessels; and availability and verification of drug information causing liver injury.

(4) Autoimmune liver disease (ALD) [13]: Patients were diagnosed with ALD based on the presence of abnormal liver functions and positive autoimmune antibodies after excluding virus, alcohol, hereditary and metabolic factors, bile or blood vessel issues.

(5) Non-alcoholic fatty liver disease (NAFLD) [14]: Patients were confirmed to have mild NAFLD as their ultrasonography showing normal liver morphology, enhanced anterior echo, no significant attenuation of posterior echo, clear intrahepatic tubular structures, normal or slightly elevated liver function and blood lipids; patients were diagnosed with moderate NAFLD if their ultrasonography showed mild or moderate enlargement in the liver, enhanced anterior echo and weakened posterior echo by half, unclear intrahepatic tubular structures, and increased TG level; patients were confirmed with severe NAFLD if their ultrasonography showing significantly enlarged liver, enhanced anterior echo, three-quarters attenuation of posterior echo, unclear intrahepatic tubular structures as well as significantly increased tri-

Table 1. The general data of included patients

Item	Patients with liver disease (n=146)
Gender (male:female)	94:52
Age (year)	53.5±9.6
Body mass index (kg/m^2)	23.69±2.69

glycerides (TG) and ALT levels, and significantly decreased high-density lipoprotein cholesterol (HDL-C) level.

(6) Alcoholic fatty liver disease (AFLD) [15]: Patients were diagnosed with AFLD if their ultrasonographic results showed mild or moderate liver enlargement, enhanced anterior echo, slightly coarser parenchymal echo, and slightly attenuated posterior echo, clear intrahepatic tubular structures, with no varicosity or ascites observed; patients were confirmed with alcoholic hepatitis if their ultrasonographic results demonstrated enlarged liver, significantly coarser parenchymal echo, visible tubular structures in the intrahepatic portal vein's liver segment or subhepatic segment branches, or the presence of multiple dilated hepatic artery branches; patients were diagnosed with liver cirrhosis as their ultrasonographic results demonstrated shrunken liver, thickened liver capsule, diffusely enhanced parenchymal echo, widened portal veins, accompanied by varying degrees of edema and ascites within the gallbladder wall.

Serum marker testing: Fasting blood samples were collected from patients in the morning, and changes in hepatitis B serology and HBV-DNA before and after antiviral treatment in chronic hepatitis B patients were analyzed using the hepatitis B virus test kit (PCR-fluorescent probe method) (Daan Gene Co., Ltd. of Sun Yat-sen University, approval number: National Medical Device Registration Certificate No. 2014-3400032).

Statistical analysis

Statistical analyses were conducted using SPSS 22.0 software. Continuous variables conforming to the normal distribution were expressed as mean ± standard deviation, and an independent samples t-test was applied for comparison. Continuous variables not conforming to the normal distribution were expressed as quartile, and compared using the Mann-Whitney U test. Count data were subjected to Pearson's chi-square test. A *P*-value of <0.05 was considered statistically significant.

Results

General data

The general data of included patients are detailed in **Table 1**, containing 94 males and 52 females, with an average age of (53.5±9.6) years and a BMI of (23.69±2.69) kg/m^2.

Clinical and pathological diagnoses for included patients

The liver biopsy of the included 146 patients revealed 92 patients (63.01%) had CHB, 22 (15.07%) with DILI, and 16 patients (10.96%) had ALD, with 7 cases (4.79%) having HBC, 6 cases (4.11%) having NAFLD, and only 3 cases (2.05%) having AFLD. The overall concordance rate between the clinical diagnosis and pathological examinations for live diseases was 79.45% (116/146), among which the highest concordance rate was 82.61% (76/92) for CHB, 77.78% (7/9) for NAFLD and AFLD, 75% (12/16) for ALD, 72.72% (16/22) for DILI, and 71.43% (5/7) for HBC, as shown in **Table 2**.

Changes in hepatitis B markers before and after antiviral treatment in the 92 CHB patients

Among the 92 patients with hepatitis B, the number of cases with HBeAg-negative, HBeAg-positive, and HBV-DNA positive before treatment were 27, 65, and 92, respectively. After treatment, the number of HBeAg-positive and HBV-DNA positive cases significantly decreased compared to before treatment, while the number of HBeAg-negative cases increased, showing a statistically significant difference (P<0.05). See **Table 3** for details.

Pathological types and clinical efficacy analysis before and after treatment in 92 CHB patients

After 24 weeks of antiviral treatment, the pathological results from the second liver biopsy showed that patients with fibrosis S2-S4 experienced an improvement in liver fibrosis, supported by the observation that the number of patients in S3-S4 decreased while that of patients in S2 increased (all P<0.05). However, there are individual patients whose degree of

Table 2. Pathological diagnosis results from liver biopsy

Item	CHB [n (%)]	NAFLD [n (%)]	AFLD [n (%)]	ALD [n (%)]	DILI [n (%)]	HBC [n (%)]
Clinical diagnosis	92 (63.01%)	6 (4.11%)	3 (2.05%)	16 (10.96%)	22 (15.07%)	7 (4.79%)
Concordance rate between clinical and pathological diagnosis	82.61% (76/92)	83.33% (5/6)	66.67% (2/3)	75% (12/16)	72.72% (16/22)	71.43% (5/7)

Note: CHB, chronic hepatitis B; NAFLD, non-alcoholic fatty liver disease; AFLD, alcoholic fatty liver disease; ALD, autoimmune liver disease; DILI, Drug-induced liver injury; HBC, hepatitis B-related cirrhosis.

Table 3. Changes in hepatitis B markers in the 92 CHB patients before and after treatment

	No. of cases	HBeAg- [n (%)]	HBeAg+ [n (%)]	HBV-DNA+ [n (%)]
Pre-treatment	92	27 (29.35)	65 (70.65)	92 (100)
Post-treatment	92	55 (59.78)	37 (40.22)	36 (39.13)
P	-	17.274		80.500
χ^2	-	<0.001		<0.001

Note: CHB, chronic hepatitis B.

Table 4. Comparison of the pathological results of the 92 CHB patients before and after treatment

Staging	Before treatment (n=92)	After treatment (n=92)	χ^2	P
Stage 0	2 (2.17%)	3 (3.26%)	0.206	0.650
Stage 1	41 (44.57%)	43 (46.74%)	0.088	0.767
Stage 2	23 (25.00%)	36 (39.13%)	4.216	0.040
Stage 3	19 (20.65%)	9 (9.78%)	4.212	0.040
Stage 4	7 (7.61%)	1 (1.09%)	4.846	0.028

fibrosis progressed even after antiviral treatment. See **Table 4** and **Figures 2**, **3**.

Discussion

Hepatitis B virus (HBV) infection damages hepatocytes and, if not effectively controlled, carries a risk of progressing to liver cancer [16]. Early detection of HBV infection and assessment of liver damage are crucial. Clinical diagnoses of HBV infection are often based on blood tests. However, the results of blood tests may not fully reflect the condition of the liver, limiting the treatment options and the assessment of the clinical efficacy [7, 8, 17]. Despite the inclusion of more clinical indicators and predictive models in evaluating the efficacy and prognosis of hepatitis B as research progresses, liver biopsy remains irreplaceable in the diagnosis and efficacy evaluation of hepatitis B [18, 19]. In this study, liver biopsy was used as a definitive diagnostic method for patients with liver disease or liver damage. Among the 146 patients who underwent liver biopsy for a definitive diagnosis, 116 cases had concordant pathological and clinical diagnoses. The highest diagnostic concordance rate was for hepatitis B, which is related to the specific viral infection causing hepatitis B and its ease of detection [3].

Previous studies have shown that liver biopsy plays a crucial role in the diagnosis and differential diagnosis of various liver diseases. Histopathological examination of liver tissue after biopsy is considered the "gold standard" for the diagnosis and efficacy evaluation of hepatitis [20]. Liver tissue pathology not only allows for the classification and assessment of benign or malignant liver lesions and their extent but also offers unique advantages in diagnosing complex liver conditions. However, its invasiveness and associated complications limit its clinical application [21]. Clinical studies indicate that chronic hepatitis often progresses through three stages: prolonged liver damage, cirrhosis, and carcinogenesis. The sparse distribution of nerves in liver implies that physical pain responses to liver damage are often subtle, resulting in most symptomatic patients only being diagnosed at the advanced stages [22]. Both cirrhosis and liver cancer are irreversible, but the fibrosis stage, which occurs before cirrhosis or cancer, is a phase that can be intervened and reversed [23]. During the liver fibrosis stage, reliance solely on clinical indicators such as blood tests, CT scans, ultrasound (US), and MRI often fail to accurately reflect the extent of the disease. Additionally, the liver has a strong metabolic capacity, hence some patients may only experience mild liver dysfunction after HBV infection. Clinically, these patients often do not receive antiviral treatment according to hepatitis B prevention and treatment standards, leading to disease progression and poor prognosis. A liver biopsy and subsequent histopathological examination can not only differentiate chronic

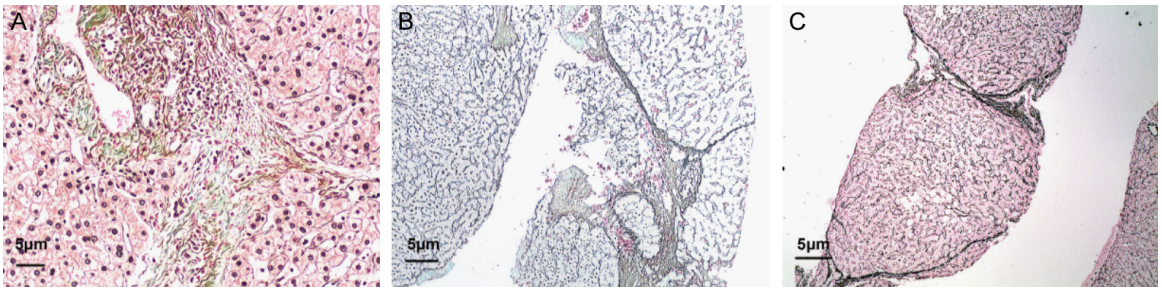

Figure 2. Pathological results from liver biopsy. A: Mild fibrosis; B: Moderate fibrosis; C: Severe fibrosis.

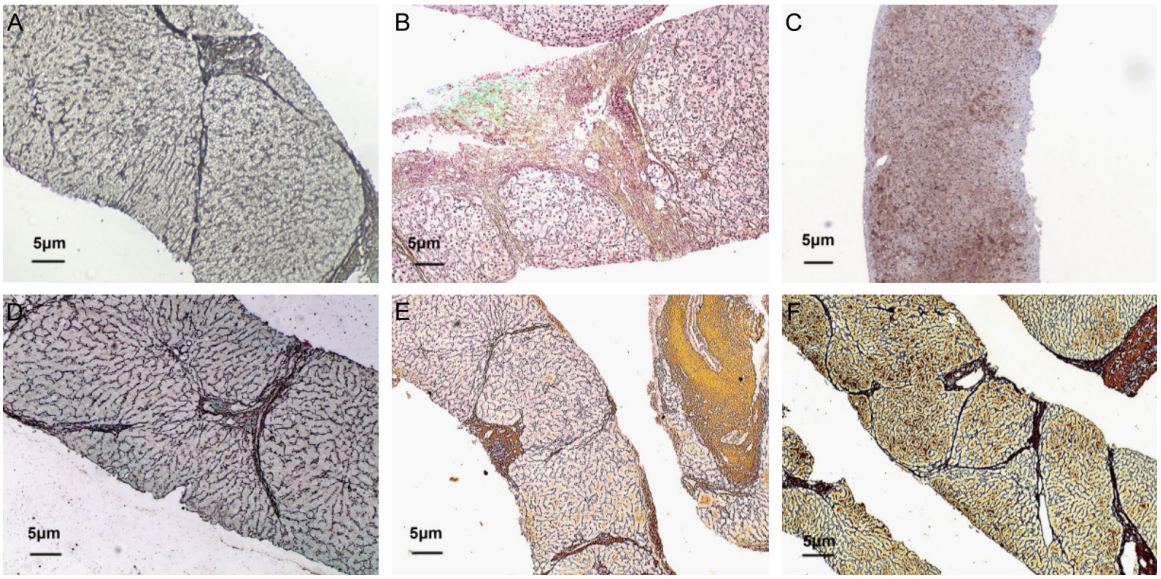

Figure 3. Comparison of pathological results before and after treatment. (A, D) Pre-treatment Stage 3 fibrosis (A) and post-treatment Stage 2 (D) in the same patient; (B, E) Pre-treatment Stage 4 fibrosis (B) and post-treatment Stage 2 (E) in the same patient; (C, F) Pre-treatment Stage 2 fibrosis (C) and post-treatment Stage 3 (F) in the same patient.

hepatitis B patients but also assess the extent of liver damage. This study shows that most patients developed liver fibrosis following HBV infection, with some advancing to stage of severe fibrosis. Early diagnosis and medical intervention are crucial for improving patients' prognoses.

Antiviral therapy is commonly used in patients diagnosed with hepatitis B in clinical settings. Entecavir, the most commonly used antiviral medication for the treatment of hepatitis B, exerts its antiviral effect by inhibiting HBV polymerase, fitting within the category of guanine nucleoside analogs [24]. This study showed that antiviral treatment significantly controlled viral replication in patients with hepatitis B. The proportion of HBeAg-positive and HBV-DNA-positive patients decreased markedly. Previous studies have also indicated that Entecavir has a favorable therapeutic effect on hepatitis B treatment [25].

For evaluating the efficacy of antiviral therapy for hepatitis B, clinical practice commonly uses serological markers (HBeAg and HBV-DNA quantification). Current clinical consensus suggests striving for a clinical cure when treating hepatitis B with antiviral therapy [26]. The definition of clinical cure includes the seroconversion of HBeAg and HBV DNA to negative and the sustained loss of HBsAg (for more than 6 months), with or without seroconversion of HBsAg [27]. The *2015 Hepatitis B Prevention and Treatment Guidelines in China* also emphasize the importance of histopathological

improvement in the liver tissue as part of the clinical cure [28]. The pursuit of a clinical cure is driven by several factors: First, it is beneficial to eliminating the psychological impacts of HBV infection, which affects not only physical but also psychological health of the patients. Despite regulations from Chinese administrative authorities prohibiting HBV screening during employee recruitment, school, and kindergarten registrations to eliminate discrimination, public bias and discrimination against chronic HBV carriers persist [29]. This issue extends beyond China. An Israeli study reported significant workplace discrimination and joblessness due to chronic HBV infection. This situation is more prominent among patients who do not undergo any therapy [30]. Achieving a clinical cure can effectively eliminate such biases, as evidenced by the negative conversion of HBsAg. Second, achieving a clinical cure allows for the safe discontinuation of medication. Long-term antiviral therapy with nucleoside analogs (NAs) significantly reduces the risks of cirrhosis and HCC in CHB patients [31], thereby improving their prognoses. Third, it enhances long-term patient outcomes. While long-term antiviral therapy with NAs could prevent or delay the occurrence of complications such as decompensated liver functions, HCC, and liver disease-related mortality, these complications cannot be fully avoided, especially in patients with liver cirrhosis [32]. A South Korean study demonstrated that CHB patients who received long-term antiviral therapy were more susceptible to HCC than the general population [33]. This study shows that while majority of patients experienced an improvement in the degree of liver fibrosis after proactive antiviral treatment, a small number of patients still exhibited worsening liver fibrosis despite aggressive antiviral therapy, highlighting the need for repeated liver biopsies.

Inevitably, there are several limitations in this study. The sample size of this study was relatively small; further expansion of the sample size could enhance the reliability of observations regarding the diagnostic and differential diagnostic value of liver biopsy. Increasing the follow-up time for patients could also provide deeper insight into the pathological changes after treatment.

In conclusion, liver biopsy holds significant value in the diagnosis and differential diagnosis of liver diseases, primarily because it effectively assesses the degree of liver fibrosis before and after antiviral treatment of CHB.

Disclosure of conflict of interest

None.

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