

Correlations of Pathological Grade and HBV-DNA Level with Gallbladder Disease Occurrence in Patients with Chronic Hepatitis B

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Abstract

Objective: To investigate the correlations of pathological grade and HBV-DNA level with the occurrence of gallbladder diseases in patients with chronic hepatitis B, and to improve clinicians' understanding of this disease.

Methods: A total of 282 inpatients with chronic hepatitis B undergoing liver biopsy in Beijing Youan Hospital, Capital Medical University from January 2014 to April 2017 were enrolled. Data on liver pathological grade, HBsAg, HBeAg, HBV-DNA, and gallbladder disease conditions were retrospectively analyzed, and SPSS 19.0 software was used for statistical analysis.

Results: The incidence of gallbladder diseases in chronic hepatitis B patients were not significantly different among different HBsAg levels, between positive and negative HBeAg, or among different HBV-DNA groups ($P > 0.05$). The higher the G grade and S stage of the liver pathology, the higher the incidence of gallbladder wall thickening, with a statistically significant difference ($P < 0.05$).

Conclusion: Gallbladder wall thickening indicates that the degree of liver fibrosis in chronic hepatitis B patients is relatively high and can be used as one of the indexes to evaluate the progression of chronic hepatitis B.

Keyword: chronic hepatitis B; pathological grading; HBV-DNA level; gallbladder diseases

Background

Hepatitis B is a common infectious disease in China caused by an infection with the hepatitis B virus (HBV), with common symptoms such as asthenia, abdominal discomfort in the hepatic region, and nausea, and some patients may have manifestations of severe hepatic injury such as jaundice and coagulation disorders [1]. According to the WHO report in 2017, there were approximately 257 million people with chronic HBV infection distributed worldwide. It is estimated that the current prevalence rate of HBsAg in the general population in China is 5%-6%, and there are approximately 70 million people with HBV infection in China, including approximately 20 million-30 million people with chronic hepatitis B [2]. Currently, hepatitis B is still a serious problem threatening people's health in China. In the past, the medical community believed that the main target organ of HBV was the liver, but in recent years, the pantropic nature of HBV has been commonly recognized. Research has revealed that hepatitis B virus antigens exist in the myocardium, kidneys, salivary glands, and bile duct cells of patients with hepatitis B [3]. As the gallbladder is adjacent to the liver, it is extremely susceptible to HBV invasion. In order to understand the correlations of the pathological grading and HBV-DNA level with gallbladder diseases

in patients with chronic hepatitis B, the clinical data of 286 patients with chronic hepatitis B hospitalized in the Center for Integrated Traditional Chinese and Western Medicine of Beijing Youan Hospital in the past three years were analyzed and reported below.

1. Materials and Methods

1.1 Research participants

In this retrospective study, 286 inpatients with chronic hepatitis B who underwent liver biopsy in the Beijing Youan Hospital, Capital Medical University from January 2014 to April 2017 were enrolled. Among them, 3 cases with hepatitis C coinfection were excluded, and 3 cases who underwent cholecystectomy were excluded. Finally, 282 patients were enrolled as the participants in this study. Objective indicators within one week of hospital admission were taken as the baseline values.

1.2 Diagnostic Criteria

The *Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2015 Edition)* [4] formulated by the Chinese Society of Hepatology and Chinese Society of Infectious Diseases under the Chinese Medical Association was referred to for the diagnosis of chronic hepatitis B.

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1.3 Inclusion and Exclusion Criteria

1.3.1. Inclusion Criteria

- (1) Age: 18-80 years;
- (2) Gender: male or female;
- (3) The diagnostic criteria for chronic hepatitis B conformed to the *Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2015 Edition)* [4] formulated by the Chinese Society of Hepatology and Chinese Society of Infectious Diseases under the Chinese Medical Association.
- (4) With complete medical records and clinical data.

1.3.2 Exclusion Criteria

- (1) Cholecystectomy or ultrasonography showing that the gallbladder was not filled or that the reason was unknown;
- (2) Those with other liver diseases such as viral hepatitis, alcoholic liver disease, autoimmune liver disease, liver cirrhosis, and liver cancer;
- (3) Patients with massive hemorrhage of the digestive tract and severe underlying cardiovascular, cerebrovascular, pulmonary, renal, endocrine, and hematopoietic diseases;
- (4) Patients with severe immunodeficiency and neoplastic diseases;
- (5) Pregnant or lactating women;
- (6) Complicated by other serious systemic diseases and mental disorders;

1.4 Methods

1.4.1. Liver Pathological Examination

After the patients and their family members provided consent and signed the informed consent form, a liver biopsy was performed. The puncture point was located under the guidance of a B-mode ultrasonography, and the puncture was performed after disinfection and local anesthesia. Afterwards, another puncture point was selected. Under ultrasound guidance, a 16 G tissue biopsy needle was punctured into the liver parenchyma twice. Under real-time monitoring, two 2-cm liver tissue strips were aspirated with a biopsy gun, fixed with formaldehyde, embedded routinely, prepared into slides, and stained. The pathology slides were subjected to inflammation grading and fibrosis staging according to the inflammation activity and fibrosis degree of the liver tissue by pathologists in our hospital under a light microscope.

1.4.2. Ultrasonic Examination

After prohibition from eating and drinking for more than 8 hours, the patients laid in the supine position or the left lateral position. The liver, gallbladder, and spleen were routinely examined. The conditions of the gallbladder wall were observed, and its thickness was measured. Cholecystolithiasis [5]: Abnormal echoes such as spots and masses were observed in the gallbladder cavity, accompanied by acoustic shadows in the rear, and the displayed ultrasonic images changed with the patient's position. Gallbladder polyps [6]: Echo

uplift could be seen on the inner wall of the gallbladder, without acoustic shadows, and did not move with the changes in body position.

1.5 Data Processing Methods

SPSS 19.0 software was used to analyze the related data, and measurement data were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$). For measurement data satisfying the normal distribution and homogeneity of variance, the independent samples t-test was used when comparing mean values between the two groups. χ^2 test was adopted to compare the rates and enumeration data between two samples. A two-tailed $P < 0.05$ indicated a statistically significant difference.

2. Results

2.1 General Information

Among the 282 patients with chronic hepatitis B, there were 164 males (58.2%) and 118 females (41.8%). Their ages ranged from 18 to 67 years, with an average of 37.86 ± 10.84 years. There were 141 HbeAg-positive patients and 136 HbeAg-negative patients. The average disease course of chronic hepatitis B was 11.65 ± 9.22 years.

2.2 Clinical Test Indicators

Among the 282 patients with chronic hepatitis B, the WBC was higher than the normal range in 3 cases. There were 129 cases with high ALT and 50 cases with TBIL higher than the normal range. The details are shown in Tab. 1.

2.3 Conditions of Gallbladder Diseases

The abdominal ultrasound examination results of the patients within one week of admission were collected. The ultrasound examination results of the 282 patients with chronic hepatitis B complicated by gallbladder diseases showed that the gallbladder diseases mainly presented as a rough gallbladder wall and gallbladder polyps, and the incidence of gallbladder diseases under ultrasonography was 99.29%. The details are shown in Tab. 2.

2.4 Correlation between Occurrence of Gallbladder Diseases and HBsAg

In order to investigate whether the incidence of gallbladder diseases in patients with chronic hepatitis B was significantly different between high-level HBsAg patients ($\text{HBsAg} \geq 1000 \text{ IU/ml}$) and non-high-level HBsAg patients ($\text{HBsAg} < 1000 \text{ IU/ml}$), the 282 patients were divided into the high-level HBsAg group ($n = 204$ cases) and the non-high-level HBsAg group ($n = 78$ cases), and a Chi-square test was conducted by SPSS 19.0. The results revealed that there was no statistically significant difference between both groups in the frequency of developing gallbladder stones,

Tab. 1 Clinical indicators of 282 patients with chronic hepatitis B

Item	Mean $\pm$ standard deviation
WBC ($\times 10^9/L$)	5.73 ± 1.49
PLT ($\times 10^9/L$)	195.29 ± 57.19
ALT (U/L)	76.68 ± 117.14
TBIL ($\mu\text{mol/L}$)	18.10 ± 20.43
ALB (g/L)	44.92 ± 9.91
GGT (U/L)	52.76 ± 68.15
Total cholesterol (mmol/L)	4.41 ± 0.96

Reference values: WBC: $4-10 \times 10^9/L$, PLT: $100-300 \times 10^9/L$, ALT: $9-50$ U/L, TBIL: $5-21$ $\mu\text{mol/L}$, ALB: $40-55$ g/L, GGT: $10-60$ U/L, total cholesterol (CHO) < 5.18 mmol/L)

Tab. 2 Distribution of gallbladder diseases in the 282 patients with chronic hepatitis B

Gallbladder diseases	N
Rough gallbladder wall	279 (98.9%)
Gallbladder polyps	34 (12.1%)
Gallbladder wall thickening	17 (6.0%)
Gallbladder stones	14 (5.0%)
Gallbladder wall edema	1 (0.35%)

gallbladder polyps, rough gallbladder wall, or gallbladder wall thickening ($P > 0.05$).

2.5 Correlation between Occurrence of Gallbladder Diseases and HBeAg

In order to investigate whether the incidence of gallbladder diseases in patients with chronic hepatitis B was significantly different between HBeAg(+) and HBeAg(-) patients, the 282 patients with chronic hepatitis B were divided into the HBeAg(+) group ($n = 144$ cases) and the HBeAg(-) group ($n = 138$ cases). The Chi-square test showed that there was no statistically significant difference between HBeAg-positive patients and HBeAg-negative patients in the frequency of developing gallbladder stones, gallbladder polyps, rough gallbladder wall, or gallbladder wall thickening ($P > 0.05$).

2.6 Correlation between Occurrence of Gallbladder Diseases and HBV-DNA

With reference to the *Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2015 Edition)*, the 282 patients were divided into two groups according to their HBV-DNA levels: the low level group ($n = 70$ cases) with HBV-DNA < 2000 IU/ml, and the non-low level group ($n = 212$ cases) with HBV-DNA ≥ 2000 IU/ml. The conditions of gallbladder diseases were compared between the chronic hepatitis B patients with different degrees of virus activity, and SPSS was used for the Chi-square test.

The results showed that there was no statistically significant difference in the frequency of developing gallbladder stones, gallbladder polyps, rough gallbladder wall, or gallbladder wall thickening

between the low level and the non-low level HBV-DNA groups ($P > 0.05$).

2.7 Correlation between Occurrence of Gallbladder Diseases and Pathological Examination

According to the pathological examination results, the 282 patients with chronic hepatitis B were divided into the G grade < 2 group (G0, G1) and the G grade ≥ 2 group (G2, G3, G4); the S stage < 2 group (S0, S1) and the S stage ≥ 2 group (S2, S3, S4). The incidence of gallbladder diseases in patients with different inflammation and fibrosis grades in the liver pathological examination were compared, and SPSS was used for the chi-square test. The results showed that the incidence of gallbladder wall thickening in patients with chronic hepatitis B was higher in the G grade ≥ 2 group than in the G grade < 2 group, and was higher in the S stage ≥ 2 group than in the S stage < 2 group, with statistically significant differences ($P < 0.05$). The details are shown in Tabs. 3 and 4.

2.8 Correlation between HBV-DNA Level and Liver Pathological Grade in Chronic Hepatitis B

According to their HBV-DNA levels, the 282 patients were divided into two groups: the low-level group ($n = 70$ cases) with HBV-DNA < 2000 IU/ml, and the non-low level group ($n = 212$ cases) with HBV-DNA ≥ 2000 IU/ml. The correlation between different HBV-DNA levels and liver pathological grades was compared using the chi-square test on SPSS. The results showed that the HBV-DNA level in patients with liver pathological G grade ≥ 2 was higher than in patients with pathological G grade < 2 , with a statistically significant difference ($P < 0.05$). The details are shown in Tabs. 5 and 6.

Tab. 3 Occurrence of gallbladder diseases in chronic hepatitis B patients with different pathological G grades

Gallbladder diseases	Classification	N	Pathological G grade		Chi-square value	P value
			G grade < 2	G grade ≥ 2		
Gallbladder stones	Yes	14	7(50.0%)	7(5.2%)	0.027	0.87
	No	268	140(52.2%)	128(94.8%)		
Gallbladder polyps	Yes	34	18(52.9%)	16(47.1%)	0.01	0.919
	No	248	129(52.0%)	119(48.0%)		
Gallbladder wall thickening	Yes	17	2(11.8%)	15(88.2%)	11.81	0.001
	No	265	145(54.7%)	120(45.3%)		

Tab. 4 Occurrence of gallbladder diseases in chronic hepatitis B patients with different pathological S stages

Gallbladder diseases	Classification	N	Pathological S stage		Chi square value	P value
			S stage < 2	S staging ≥ 2		
Gallbladder stones	Yes	14	9(4.8%)	5(5.3%)	0.027	0.869
	No	268	178(95.2%)	90(94.7%)		
Gallbladder polyps	Yes	34	23(67.6%)	11(32.4%)	0.031	0.861
	No	248	164(66.1%)	84(33.9%)		
Gallbladder wall thickening	Yes	17	5(29.4%)	12(70.6%)	11.027	0.001
	No	265	182(68.7%)	83(31.3%)		

Tab. 5 Correlation between liver inflammation degree and HBV-DNA level in patients with chronic hepatitis B

Pathological G grade	N	HBV-DNA level		Chi square value	P value
		Low level group (N)	Non-low-level group (N)		
G grade < 2	147	52(35.4%)	95(64.6%)	18.32	0.000
G grade ≥ 2	135	18(13.3%)	117(86.7%)		

Tab. 6 Correlation between liver fibrosis degree and HBV-DNA level in patients with chronic hepatitis B

Pathological S stage	N	HBV-DNA level		Chi square value	P value
		Low level group (N)	Non-low-level group (N)		
S stage < 2	187	49(26.2%)	138(73.8%)	0.567	0.451
S stage ≥ 2	95	21 (22.1%)	74(77.9%)		

3. Discussion

Gallbladder diseases, such as cholecystitis, gallbladder stones and gallbladder polyps, are among the most common digestive system diseases in China. With the changes in people's dietary habits and lifestyles, the incidence of gallbladder diseases has been gradually increasing. By reviewing the relevant literature on the epidemiological survey of gallbladder diseases in China in recent years, it was found that the prevalence rate of cholecystitis in the general population in China ranged from 1.42% to 8.97% [7-10]; the prevalence rate of

gallbladder polyps ranged from 4.5% to 8.7%, with most of the cases being males [9-12]; the prevalence rate of gallbladder stones ranged from 2.3% to 7.02% [8, 10, 13-16].

In this study, it was found that the incidence of rough gallbladder wall and gallbladder polyps in patients with chronic hepatitis B was 98.9% and 12.1% respectively, and both were higher than those in the general population in China. At present, it has been found that HBV infection can damage various systems in the whole body. As the gallbladder is located in the gallbladder fossa of the liver, and bile is secreted in the

liver and stored in the gallbladder, damage to the gallbladder is more common compared to other organs [12].

Hepatitis B virus can invade the gallbladder wall, resulting in damage to the epithelial cells of the gallbladder wall and motion abnormalities of the gallbladder muscles, which leads to cholecystitis, as well as the formation of inflammatory polyps or gallbladder stones. Lang et al. [17] used nested polymerase chain reaction (PCR) to examine the tissues of 18 patients with hepatitis B virus infection and found that HBV-DNA could be detected in the gallbladder, kidney, spleen, and other tissues. Some researchers [18] carried out an immunofluorescence analysis and immunofluorescence assay on gallbladder tissue from 58 patients who underwent cholecystectomy, and the results showed that HBsAg was detected in the gallbladder tissues of 17.2% of the patients. In addition, 142 patients with hepatitis B were followed-up for 18 months, and it was found that the impact of hepatitis B virus could lead to changes in the gallbladder morphology and function. However, this study did not reveal any correlation between the occurrence of gallbladder diseases and HBV-DNA level, HBsAg level, or positive HBeAg, respectively. The reason may be that hepatitis B virus does not directly kill the epithelial cells of the gallbladder wall and muscle tissue of the gallbladder but leads to the occurrence of gallbladder diseases through the immune response caused by the virus.

In this study, it was found that the incidences of gallbladder wall thickening in the G grade ≥ 2 group and the S stage ≥ 2 group were higher than those in the G grade < 2 group and the S stage < 2 group. The incidence of gallbladder wall thickening showed a statistically significant difference among the different degrees of liver inflammation or liver fibrosis ($P < 0.05$). The higher the inflammation and fibrosis grades in the liver pathological examination, the higher the incidence of gallbladder wall thickening. This is consistent with the conclusion obtained by Lin et al. [19], who found by comparing the degrees of liver fibrosis in patients with chronic HBV infection complicated by gallbladder wall abnormalities that the degree of fibrosis in the abnormal gallbladder wall group was significantly higher than that in the normal gallbladder wall group. This suggests that the occurrence of gallbladder inflammation is associated with the degrees of hepatocyte damage and fibrosis. It has been reported [20] that abnormal bile secreted by hepatocytes during hepatitis is excreted into the gallbladder through the bile ducts and accumulated in the gallbladder, which stimulates the gallbladder wall and causes edema and necrosis of its mucosa. Long-term stimulation of the gallbladder wall can cause thickening of the gallbladder wall, and the longer the stimulation time, the more serious the gallbladder injury.

In this study, it was found that the higher the serum

HBV-DNA level, the higher the degree of inflammation in the liver pathological examination ($P < 0.05$), but the serum HBV-DNA levels had no statistically significant difference among the different fibrosis grades in the liver pathological examination ($P > 0.05$). The results suggest that HBV-DNA level can reflect the degree of liver inflammation to a certain extent but cannot reflect the degree of liver fibrosis. Pathological examination of the liver puncture biopsy is the gold standard to judge the degree of liver injury, but because it is an invasive procedure, patients suffer great pain, high cost, and certain risks; therefore, it is generally not used as an examination item for the routine follow-up of chronic HBV infection patients [21]. Serum HBV-DNA level cannot be used alone as a serological index to evaluate the progress of chronic hepatitis B. Clinicians should pay attention to gallbladder wall conditions in ultrasound examination in their work, and consider the possibility of liver fibrosis when finding gallbladder wall thickening in patients with chronic HBV infection. Further examination should be performed to determine the progression of liver tissue lesions in time, and intervention measures should be taken.

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