Liver Inflammation and Fibrosis of HBeAg-Negative Patients in the Grey Zone

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Abstract

Background Few studies have reported the disease severity of HBeAg negative patients with chronic hepatitis B (CHB) in the grey zone (GZ). This study aimed to investigate the degree of liver injury in the HBeAg negative GZ patients.

Methods One hundred and thirty-two treatment-naïve HBeAg negative GZ patients with HBV DNA $\geq 2,000$ IU/ml and ALT < 80 U/L who underwent liver biopsy were included. Liver histology was evaluated using the Scheuer classification system. Logistic regression analysis was used to identify independent risk factors of significant liver inflammation and fibrosis.

Results The median age of patients was 43.0 years and the median levels of HBV DNA was $4.3 \log_{10}$ IU/mL. The majority of patients (65.2%) were male. The distributions of liver fibrosis stages of patients were as follows: S0, 7 (5.3%); S1, 44 (33.3%); S2, 32 (24.2%); S3, 20 (15.2%); and S4, 29 (22.0%). The distributions of liver inflammation grades were: G0, 0 (0%); G1, 40 (30.3%); G2, 65 (49.2%); G3, 32 (24.2%); and G4, 20 (15.2%). The serum AST (1.051, 95%CI 1.008-1.096, $P=0.019$) and HBV DNA (1.579, 95%CI 1.023-2.438, $P=0.039$) levels were independently risk factors of significant liver fibrosis. The serum AST (1.072, 95%CI 1.023-1.124, $P=0.004$) was the only independently risk factor of significant liver inflammation. Serum HBV DNA and AST had moderate accuracy of predicting significant liver fibrosis and inflammation.

Conclusion Substantial patients exhibited significant liver injury in HBeAg negative GZ patients. High HBV DNA and AST levels were two independent risk factors of significant liver injury.

Background

Chronic hepatitis B virus (HBV) infection remains to be one of the most serious health burden, and more than 350 million people are chronically infected with hepatitis B virus (HBV) worldwide.¹ Chronic hepatitis B (CHB) can result in a series of clinical manifestations, including liver fibrosis, liver cirrhosis, liver failure and hepatocellular carcinoma (HCC).² Chronic HBV infection is a dynamic disease course, which can be divided into several different immune states based on the current guidelines (add the ref). Hepatitis B e antigen (HBeAg) status, HBV DNA and alanine transaminase (ALT) levels are three crucial factors in dividing the diseases phases of patients with chronic HBV infection.³⁻⁵ However, since a substantial portion of CHB patients could not be categorized into any phase as defined by the current guidelines,³⁻⁵ these disease states were described as grey zone (GZ).^{6,7} Among these, much attention has been paid to HBeAg negative GZ patients.

The definition of GZ remains inconsistent for HBeAg negative patients.^{6,7} Both HBV DNA $\geq 2,000$ IU/ml and normal or mild elevated ALT levels were proposed as common criteria in HBeAg negative GZ patients in previous studies.^{6,7} According to the international guidelines, HBeAg negative CHB patients with detectable HBV DNA and significant liver histology injury should be treated regardless of ALT level.³⁻⁵

Therefore, the severity of liver damage and identifying the risk factors is critical for the management of HBeAg negative GZ patients. However, few studies reported the severity of liver injury in HBeAg negative GZ patients. A retrospective study revealed that HBeAg negative GZ? patients with high level HBV DNA and normal ALT was associated with significant liver inflammation and fibrosis.⁸ However, more than half of the patients combined with fatty liver disease and the sample size is relatively small.⁸

Additional study is required to clarify the diseases severity of HBeAg negative GZ patients to guide the clinical practice. Therefore, this study aimed to investigate the severe degree of liver inflammation and fibrosis in the HBeAg negative GZ patients.

Methods

Study population

One thousand and five consecutive treatment-naïve cases of chronic HBV infection who underwent liver biopsy in two medical centers (Nanjing Drum Tower Hospital and Huai'an No. 4 People's Hospital) during April 2004 and July 2019 were included. The diagnostic criteria of CHB was hepatitis B surface antigen (HBsAg) being positive for at least 6 months. The enrolled criteria were ALT < 80 U/L, HBV DNA $\geq$ 2000 IU/ml and HBeAg negative.^{6,9} Patients who meet the following conditions were excluded: (1) HCC or/and other malignancy; (2) Other causes of chronic liver diseases: other viral hepatitis, nonalcoholic fatty liver disease, immune-related liver disease and hereditary metabolic liver disease, acquired immune deficiency syndrome and syphilis; (3) alcohol abuse per day? (> 40 g for men and > 20 g for women).

This study protocol was accorded with Declaration of Helsinki and was conducted with the approval of the Ethics Committee of the Nanjing Drum Tower Hospital and Huai'an No. 4 People's Hospital (protocol number?).

Laboratory assays and liver biopsy

Biopsy specimens were fixed in 10% formalin and embedded in paraffin. The specimens were evaluated by two pathologists regardless of clinical characteristics. Liver fibrosis and inflammation activity were assessed by Scheuer scoring system.¹⁰ Liver fibrosis was staged on a scale from S0 to S4, and S2-S4 was defined as significant fibrosis.¹⁰ Liver inflammation was graded on a scale from G0 to G4, and G2-G4 was defined as significant inflammation.¹⁰ Liver function and blood routine tests were detected within two weeks before liver biopsy. The upper limit of normal (ULN) of ALT was 40 U/L. HBsAg and HBeAg were measured by the enzyme-linked immunosorbent assay. The detection limitation of HBV DNA was 500 IU/ml in our study.

Statistical analysis

Categorical variables and continuous variables were presented as percentage and median (interquartile range (IQR)), respectively. Baseline characteristics were compared by Chi-square test for categorical variables and Mann-Whitney U test was used to compare continuous variables. The related risk factors of significant fibrosis and inflammation were identified by logistic regression analysis. The correlation between clinical parameters and pathological damage degree was performed by Spearman’s correlation analysis. Statistical significance was taken as $P < 0.05$. SPSS software version 22.0 was used for the statistical analyses (SPSS Inc., Chicago, IL, United States).

Results

Characteristics of the study patients

One thousand and five consecutive treatment-naïve cases of CHB who underwent liver biopsy were included in our study. A total of eight hundreds seventy three patients was excluded based on the above inclusion and exclusion criteria. Among them, two hundred and sixty-seven patients were excluded based on exclusion criteria, and 606 patients with HBeAg positive and/or HBV DNA $\leq 2,000$ IU/ml and/or ALT ≥ 80 U/L were also excluded. Eventually, 132 patients were included for the analysis (Fig. 1). The median age was 43.0 (IQR 37.0–49.0) years, and the majority of patients (65.2%) were male. The median of PLT, ALT, AST, ALB and HBV DNA levels were 163.0 (IQR 126.5 – 197.5) $\times 10^9$ /L, 40.0 (IQR 26.5 – 53.9) U/L, 31.0 (IQR 25.0 – 39.5) U/L, 45.0 (IQR 40.6 – 47.5) g/L and 4.3 (IQR 3.8 – 5.2) $\log_{10}$ IU/ml, respectively (Table 1).

Table 1
Baseline characteristics of study population.

Variables	n (%) or median (IQR)
Age (yr)	43.0 (37.0, 49.0)
Male (%)	86 (65.2)
PLT ($\times 10^9$ /L)	163.0 (126.5, 197.5)
TB (μ mol/L)	13.8 (10.9 18.2)
ALT (U/L)	40.0 (26.5, 53.9)
AST (U/L)	31.0 (25.0, 39.5)
GGT (U/L)	26.0 (18.0, 47.0)
ALB (g/L)	45.0 (40.6, 47.5)
GLB (g/L)	27.2 (24.8, 30.8)
HBV DNA ($\log_{10}$ IU/mL)	4.3 (3.8, 5.2)
PLT: platelets; TB: total bilirubin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; ALB: albumin; GLB: globulin.	

Liver inflammation and fibrosis of HBeAg-negative GZ patients

The distributions of fibrosis stages were listed as follows: 7 (5.3%) patients in S0, 44 (33.3%) patients in S1, 32 (24.2%) patients in S2, 20 (15.2%) patients in S3, and 29 (22.0%) patients in S4, respectively. More than half of the patients ($n = 81$, 61.4%) had significant liver fibrosis. The distributions of inflammation grades were as follows: none patients in G0, 40 (30.3%) patients in G1, 65 (49.2%) patients in G2, 32 (24.2%) patients in G3, and 20 (15.2%) patients in G4, respectively. A total of 92 (69.7%) patients had significant liver inflammation (Fig. 2).

Risk factors of significant liver fibrosis and inflammation

The PLT (0.992, 95%CI 0.986–0.998, $P = 0.01$), AST (1.063, 95%CI 1.021–1.107, $p = 0.003$) and HBV DNA (1.811, 95%CI 1.193–2.750, $P = 0.005$) were associated with significant liver fibrosis by univariate logistic regression analysis. Further multivariate logistic regression analysis showed that the AST (1.051, 95%CI 1.008–1.096, $P = 0.019$) and HBV DNA (1.579, 95%CI 1.023–2.438, $P = 0.039$) were independent risk factors of significant liver fibrosis (Table 2). Univariate logistic regression analysis revealed that ALT (1.025, 95%CI 1.001–1.049, $P = 0.039$), AST (1.070, 95%CI 1.022–1.121, $P = 0.004$) and GGT (1.023, 95%CI 1.004–1.042, $P = 0.017$) were correlated with significant liver inflammation, while AST (1.072, 95%CI 1.023–1.124, $P = 0.004$) was the only independent risk factor of significant liver inflammation (Table 3). In addition, we further analyzed the correlation in several clinical indexes, liver fibrosis stages and inflammation grades. The AST ($r = 0.273$, $P = 0.002$) and HBV DNA ($r = 0.248$, $P = 0.004$) levels were positively correlated with liver fibrosis stages, while ALT ($r = 0.189$, $P = 0.030$), AST ($r = 0.280$, $P = 0.001$) and HBV DNA ($r = 0.179$, $P = 0.04$) levels were positively correlated with liver inflammation grades (Table 4).

Table 2

Logistic regression analysis of clinical parameters associated with significant liver fibrosis in HBeAg-negative patients in the grey zone.

Variables	Univariate		Multivariate	
	OR (95% CI)	P value	OR (95% CI)	P value
Age (yr)	0.991 (0.956, 1.027)	0.617		
Sex	0.897 (0.428, 1.876)	0.772		
PLT ($\times 10^9/L$)	0.992 (0.986, 0.998)	0.01		
TB ($\mu\text{mol/L}$)	1.001 (0.957, 1.047)	0.965		
ALB (g/L)	0.935 (0.852, 1.026)	0.154		
GLB (g/L)	1.049 (0.969, 1.136)	0.234		
ALT (U/L)	1.021 (0.999, 1.042)	0.059		
AST (U/L)	1.063 (1.021, 1.107)	0.003	1.051 (1.008, 1.096)	0.019
GGT (U/L)	1.006 (0.996, 1.017)	0.238		
HBV DNA ($\log_{10}$ IU/mL)	1.811 (1.193, 2.750)	0.005	1.579 (1.023, 2.438)	0.039
PLT: platelets; TB: total bilirubin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; GLB: globulin; OR: odds ratio.				

Table 3

Logistic regression analysis of clinical parameters associated with significant liver inflammation in HBeAg-negative patients in the grey zone.

Variables	Univariate	Multivariate		
	OR (95% CI)	P value	OR (95% CI)	P value
Age (yr)	0.969 (0.932, 1.007)	0.113		
Sex	1.010 (0.463, 2.199)	0.981		
PLT (× 10 ⁹ /L)	0.996 (0.990, 1.002)	0.244		
TB (μmol/L)	1.017 (0.967, 1.070)	0.503		
ALB (g/L)	1.016 (0.928, 1.113)	0.734		
GLB (g/L)	1.084 (0.991, 1.186)	0.079		
ALT (U/L)	1.025 (1.001, 1.049)	0.039		
AST (U/L)	1.070 (1.022, 1.121)	0.004	1.072 (1.023, 1.124)	0.004
GGT (U/L)	1.023 (1.004, 1.042)	0.017		
HBV DNA (log ₁₀ IU/mL)	1.522 (0.995, 2.328)	0.053		
PLT: platelets; TB: total bilirubin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; GLB: globulin; OR: odds ratio.				

Table 4

Correlation between different clinical parameters and liver inflammation and fibrosis of HBeAg-negative patients in the grey zone

Correlation analysis	Fibrosis stages		Inflammation grades	
	R	P	R	P
Age	-0.060	0.492	-0.135	0.122
ALT	0.161	0.066	0.189	0.030
AST	0.273	0.002	0.280	0.001
HBV DNA	0.248	0.004	0.179	0.040
HBsAg	0.032	0.747	0.033	0.742
ALT: alanine aminotransferase; AST: aspartate aminotransferase; HBsAg: hepatitis B surface antigen.				

Diagnostic accuracy of HBV DNA and AST for the prediction of significant fibrosis and inflammation.

The area under the receiver operating characteristic curves (AUROCs) of HBV DNA and AST for predicting significant fibrosis were 0.647 (95%CI 0.553–0.741, $P = 0.005$) and 0.662 (95%CI 0.567–0.756, $P = 0.002$), and the optimal cut-off value was 5.0 log₁₀ IU/ml and 28.3 U/L, respectively. For predicting significant inflammation, the AST exhibited the AUROCs of 0.676 (95%CI 0.578–0.773, $P = 0.001$), with an optimal cut-off value of 28.0 U/L (Fig. 3, Table 5).

Table 5.
Diagnostic accuracy of HBV DNA and AST for the prediction of significant fibrosis and inflammation in HBeAg-negative patients in the grey zone

	Optimized cutoff	Sensitivity (%)	Specificity (%)	AUC (95%CI)	LR +	LR -	P value
Significant fibrosis							
HBV DNA (log ₁₀ IU/mL)	5.0	40.74	90.20	0.647 (0.553, 0.741)	4.157	0.657	0.005
AST (U/L)	28.3	69.14	58.82	0.662 (0.567, 0.756)	1.679	0.525	0.002
Significant inflammation							
AST (U/L)	28.0	75.00	62.50	0.676 (0.578, 0.773)	2.000	0.400	0.001

AUC: area under the receiver operating characteristic curve; CI: confidence interval; LR-: negative likelihood ratio; LR+: positive likelihood ratio; AST: aspartate aminotransferase.

Discussion

The assessment of severity of liver injury is an important step for the clinical management of HBeAg negative GZ patients to identify those who require antiviral treatment and avoid severe complications.^{3,11} However, few studies reported the distributions of inflammation and fibrosis stages and the risk factors of significant liver injury in HBeAg negative GZ patients. Our study found that 92 (69.7%) patients showed significant liver inflammation and 81 (61.4%) patients presented significant liver fibrosis, suggesting that about two thirds of patients had significant liver damage. According to recommendations of international guidelines, these patients should be treated as soon as possible.^{3–5} However, only a few patients received liver biopsy in clinical practice. Thus, the majority of HBeAg negative GZ patients with significant liver injury were not treated timely. A similar study was reported by Park et al. revealed that 60% patients had significant liver fibrosis and 61.9% patients had significant liver inflammation in CHB patients with ALT < 2 ULN and high HBV DNA level (> 20,000 IU/ml).¹² However, the majority of patients (81.2%) were HBeAg positive in that study. Kumor et al. demonstrated that high HBV DNA levels and persistently normal ALT

was an independently risk factor of significant liver fibrosis and inflammation in HBeAg negative CHB patients.⁸ Therefore, these results suggested that the absence of significant ALT elevation does not indicate the absence of significant liver injury in HBeAg negative GZ patients.

Furthermore, the present study further analyzed the risk factors of significant liver inflammation and fibrosis in HBeAg negative GZ patients. Our results revealed that high levels of AST and HBV DNA were associated with severe liver fibrosis and AST level was also positively correlated with advanced level of liver inflammation grade. Though the ALT level was considered as the most common used indicator of liver disease activity and severity in CHB patients, numerous studies have demonstrated that ALT was not a significant factor associated with liver inflammation and fibrosis in HBeAg negative CHB patients.^{13,14} Our study also found that ALT was not associated with liver inflammation grades nor fibrosis stages in HBeAg negative GZ patients. In contrast, our results suggested that AST can be a substitute of ALT for reflecting disease activity and severity in HBeAg negative GZ patients. Consistent with previous studies^{15,16}, high HBV DNA level was the most significant factor associated with significant liver fibrosis in our study. A prospective study revealed that the high level of HBV DNA was the strongest predictor for liver disease progression to cirrhosis in the future.¹⁶ Mohamadnejad et al. reported that HBV DNA could predict significant liver fibrosis with a sensitivity of 82% and specificity of 70% in HBeAg negative patients.¹⁵ Taken together, these results suggested that patients with high level HBV DNA should be closely monitored regardless of ALT level.

This study had several limitations. Firstly, the sample size was relatively small and patients were only enrolled from two medical centers. Thus, the results should be further validated by multi-center study with large samples. Secondly, the long-term prognosis of HBeAg negative GZ patients was not analyzed. Further study is needed to investigate the incidence of liver-related adverse events among these patients.

Conclusion

In summary, our study showed that more than half of the patients exhibited significant liver injury in HBeAg negative GZ patients. High HBV DNA and AST levels were two independently risk factors of significant liver injury for HBeAg-negative GZ patients.

Abbreviations

CHB, chronic hepatitis B; GZ, grey zone; HBV, hepatitis B virus; CHB, Chronic hepatitis B; HCC, hepatocellular carcinoma; HBeAg, Hepatitis B e antigen; ALT, alanine transaminase; HBsAg, hepatitis B surface antigen; ULN, upper limit of normal; IQR, interquartile range; AUROCs, area under the receiver operating characteristic curves.

Declarations

Acknowledgements

Not applicable.

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Availability of data and materials

The dataset generated or analyzed during the current study is available from the corresponding author on reasonable request.

Authors' contributions

CW and RH designed the study and revised the manuscript; RY, JW and JW analyzed the data and wrote the manuscript; XY, YC, JX, YY, KY, WW, YL, JL, ZZ, WD collated the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committees of the Nanjing Drum Tower Hospital and Huai'an No. 4 People's Hospital. The ethics committee waived the need for a written informed consent because this study was retrospective.

Consent for Publication

Not applicable

Competing interests

The authors have declared that no conflicts of interest exist.

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Figures

Figure 1

Flow diagram describing the selection of our study cohort.

Figure 2

Distribution of liver fibrosis stages (A) and inflammation grades (B) in HBeAg negative patients with CHB in the grey zone.

Figure 3

Diagnostic accuracy of HBV DNA and AST for the prediction of significant fibrosis (A) and inflammation (B).