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Original Article

Genotype-specific Response to 144-week Entecavir Therapy for HBeAg-positive Chronic Hepatitis B with a Particular Focus on Histological Improvement: A Prospective Study

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Abstract

Background and Aims: Chronic hepatitis B (CHB) poses a major global health burden, with China particularly affected. Effective antiviral therapy is crucial to prevent disease progression, but responses may vary by Hepatitis B virus (HBV) genotype. This prospective study aimed to compare genotype-specific responses to 144-week entecavir (ETV) therapy in HBeAg-positive CHB patients, with particular emphasis on histological improvement assessed through paired liver biopsies. ***Methods:*** We enrolled 49 treatment-naïve CHB patients (HBV DNA ≥ 20,000 IU/mL, alanine transaminase (ALT) > 2× ULN, and Scheuer system G ≥ 2) who received ETV 0.5 mg/day. HBV genotyping was performed using Polymerase Chain Reaction and fragment length analysis. The primary endpoint was histological improvement (*i.e.*, ≥ 2-grade reduction in necroinflammatory activity without fibrosis progression), evaluated via paired biopsies (baseline and week 144) by blinded pathologists. Secondary endpoints included virological response (*i.e.*, serum HBV DNA < 100 IU/mL), HBeAg seroconversion, and ALT normalization. ***Results:*** The cohort included 24 genotype B and 24 genotype C patients (one genotype A patient was excluded from genotype-specific analyses). Genotype B showed significantly higher histological improvement rates (91.3% *vs.* 63.2%, $P = 0.027$) and greater inflammation resolution (0 ≤ G < 1: 56.5% vs. 26.3%, $P = 0.048$). Virological suppression was excellent in both groups (100% *vs.* 100%). HBeAg seroconversion trended higher in genotype C (29.2% *vs*. 50.0%, $P = 0.140$). All patients achieved ALT normalization by week 48, with no safety concerns. ***Conclusions:*** HBV genotype B demonstrates superior histological responses to ETV therapy compared with genotype C, supporting the clinical value of HBV genotyping for personalized CHB management. These findings highlight the importance of considering viral genotype when evaluating treatment outcomes.

Keywords: Chronic Hepatitis B; genotype; Entecavir; Histological improvement; HBeAg-positive; Long-term antiviral therapy.

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Introduction

Hepatitis B virus (HBV) infection continues to pose a substantial global health burden, with an estimated 296 million chronic carriers worldwide.[1] Without effective treatment, chronic hepatitis B (CHB) can progress to liver fibrosis and cirrhosis and hepatocellular carcinoma (HCC) in a proportion of patients[2,3] and is thus a significant contributor to liver-related morbidity and mortality, particularly in China.[4,5] Worldwide, approximately two million liver-related deaths occur annually, but less than 10% of the global demand for liver transplants is met.[6] China bears the world's heaviest hepatitis B burden, with approximately 90 million CHB sufferers, accounting for nearly one-third of the global total infected population.[7]

At present, nucleos(t)ide analogues (NAs) such as entecavir (ETV), tenofovir disoproxil fumarate, and tenofovir alafenamide fumarate are recommended as first-line NAs for the treatment of HBV infection, as they are convenient to use, cheap, and safe, with good virological response. However, they cannot clear cccDNA and have low hepatitis B surface antigen (HBsAg) seroconversion rates; thus, virological recurrence may occur once the drug is stopped. Therefore, long-term administration of NAs is required.[8–11] Among the commonly recommended NAs, ETV is a nucleoside reverse transcriptase inhibitor used to treat CHB.[12] ETV is a potent antiviral drug that inhibits viral replication by disrupting the process of DNA synthesis.[13,14] Since its approval by the authority in 2005,[8] ETV has been extensively utilized as a preferred first-line drug for the treatment of CHB due to its high genetic barrier and ability to suppress the virus.[15,16]

Successful antiviral therapy, indicated by serum alanine transaminase (ALT) normalization, HBsAg loss, undetectable HBV DNA (< 100 IU/mL), HBeAg seroconversion (in HBeAg-positive patients), and improvement in non-invasive fibro-

sis markers (*e.g.*, liver stiffness measurement, FibroTest, *etc.*), provides profound and long-lasting viral suppression, thereby resulting in histological improvement (*i.e.*, reversing liver fibrosis and cirrhosis) and consequently preventing the development of HCC.[10] It has been reported that ETV not only effectively suppresses viral replication but also improves liver fibrosis and cirrhosis.[17,18] However, as an NA, ETV also shares therapeutic limitations as previously described, and the factors that influence the clearance of cccDNA, HBsAg seroconversion, virological recurrence, and histological changes of liver tissues have not been fully elucidated.

Recently, it has been revealed that both viral and host factors[19] contribute to persistent HBV infection and progression to severe chronic liver diseases.[9,10,19] Emerging evidence highlights the significant role of HBV genotypes in modulating infection persistence and disease progression; genotype-specific variations have been observed in the natural course of HBV infection and the consequences of CHB.[20–23] Moreover, a few preliminary studies have suggested correlations between genetic variants and clinical outcomes and treatment response.[24–26] For example, in a Taiwanese cohort, Kao *et al*.[24] reported that HBV genotype C was associated with more severe liver diseases, such as cirrhosis and HCC, in older individuals, whereas genotype B was more likely to be linked to HCC development at a younger age. However, this study was limited by its cross-sectional design (unable to establish causality), modest sample size for rare genotypes, unadjusted confounders (*e.g.*, viral load), and lack of longitudinal data. In a more recent study of HBsAg-positive pregnant women in Guizhou, Zhang *et al*. identified that HBV genotype was an independent factor related to the response of HBV DNA levels to antiviral therapy with telbivudine and tenofovir.[26] However, this study was limited by its retrospective nature and short duration (12 weeks). Therefore, large prospective studies are needed to confirm genotype-specific disease outcomes and treatment responses in terms of HBV DNA levels to antiviral therapy, especially with ETV. Moreover, whether HBV genotype is also associated with hepatic histological changes in response to antiviral therapy with ETV has not been explored.

Therefore, this prospective study with serial liver biopsies was conducted to evaluate genotype-specific responses to long-term ETV therapy, with a special focus on histological improvement in patients with HBeAg-positive CHB.

Methods

Patients and treatment procedures

This prospective study consecutively enrolled adult (18–65 years) patients with NA-naïve HBeAg-positive CHB at Guangdong Provincial Hospital of Traditional Chinese Medicine between December 2012 and August 2022. The inclusion criteria were the following: 1) documented HBsAg positivity for > 6 months; 2) HBV DNA ≥ 20,000 IU/mL; 3) elevated ALT (>2×ULN); and 4) significant histological activity (Scheuer system necroinflammatory activity G ≥ 2).[27] Patients with the following conditions were excluded: 1) liver cirrhosis (S = 4), severe inflammation (G = 4), or HCC; 2) coinfection with hepatitis C virus, hepatitis D virus, or human immunodeficiency virus; 3) other concomitant liver diseases, such as autoimmune liver disease and alcoholic liver disease; 4) previous NA treatment; 5) recent immunosuppressive therapy within 6 months; 6) pregnancy or lactation (female) or preparation for pregnancy (both female and male); or 7) absence of liver biopsies at baseline (prior to ETV treatment). The study protocol (Clinical Trial Registration: 2012ZX10005004) was approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (also known as Guangdong Provincial Hospital of Traditional Chinese Medicine), Guangzhou, China. All participants provided written informed consent, and the study was conducted in accordance with the Declaration of Helsinki (2013–2024).

All enrolled patients received oral ETV monotherapy at the standard dose of 0.5 mg once daily. Medication was taken each morning on an empty stomach (at least 2 h before or after a meal). The minimum treatment duration was 144 weeks (approximately three years), with dose adjustments prohibited per protocol. Concomitant use of other antiviral agents, hepatoprotective medications, or immunomodulators was not permitted during the study period.

Laboratory assays

Liver biochemistry and serum virological load levels were assessed at baseline and weeks 4, 8, 12, 16, 20, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, and 144. Viral markers were evaluated at baseline and weeks 24, 48, 60, 72, 96, 120, and 144. These measurements were performed to evaluate liver function, monitor disease progression, and assess treatment response. All liver biochemical indicators, including serum albumin, ALT, aspartate aminotransferase, total bilirubin, and complete blood count (platelets), were analyzed using a fully automated biochemical analyzer to assess hepatic injury, synthetic function, and potential fibrosis. Viral serological markers, including HBsAg, antibodies to HBsAg (anti-HBs), HBeAg, and antibodies to HBeAg (anti-HBe), were quantified using Cobas e601 (Roche Diagnostics, Basel, Switzerland) to determine viral antigen burden and infection status. HBV DNA viral load was measured by real-time quantitative fluorescence polymerase chain reaction (ABI 7500, Applied Biosystems, Foster City, CA) with a lower detection limit of 100 IU/mL to monitor viral replication activity and treatment efficacy. HBV genotyping was performed through sequence alignment of the preS/S region (nucleotides 2825–1019, approximately 1,410 bp) using the NCBI genotyping tool, with reference sequences obtained from GenBank. No primary or compensatory polymerase resistance-associated mutations (rtM204V/I, rtL180M, *etc*.) were identified in the overlapping region; HBV genotypes were classified into 10 major types (A to J) based on genomic sequence divergence.[28]

Liver biopsies were taken at baseline and were requested at week 144 of treatment, with all specimens processed using standardized protocols. Liver biopsies were evaluated by two independent pathologists according to the Scheuer scoring system.[27] Necroinflammatory activity was graded on a 5-point scale as G0 (none), G1 (mild), G2 (mild to moderate), G3 (moderate), and G4 (severe). Fibrosis was staged on a 5-point scale as S0 (no fibrosis), S1 (minimal fibrosis), S2 (moderate fibrosis), S3 (severe fibrosis), and S4 (cirrhosis). Discrepancies between pathologists (*i.e.*, > 1 stage/grade difference) were resolved through consensus review with a third senior pathologist.

Efficacy and safety assessments

Histological response was evaluated according to the Scheuer scoring system.[27] A histological response was defined as an improvement of ≥ 1 grade in necroinflammatory activity without fibrosis progression. For descriptive purposes, the extent of improvement was further classified as a very effective response (≥ 2-grade reduction without fibrosis progression), an effective response (1-grade reduction without fibrosis progression), and an ineffective response (no improvement in necroinflammatory activity or the presence of fibrosis progression). The primary efficacy endpoint of this study was histological response, including both very effective

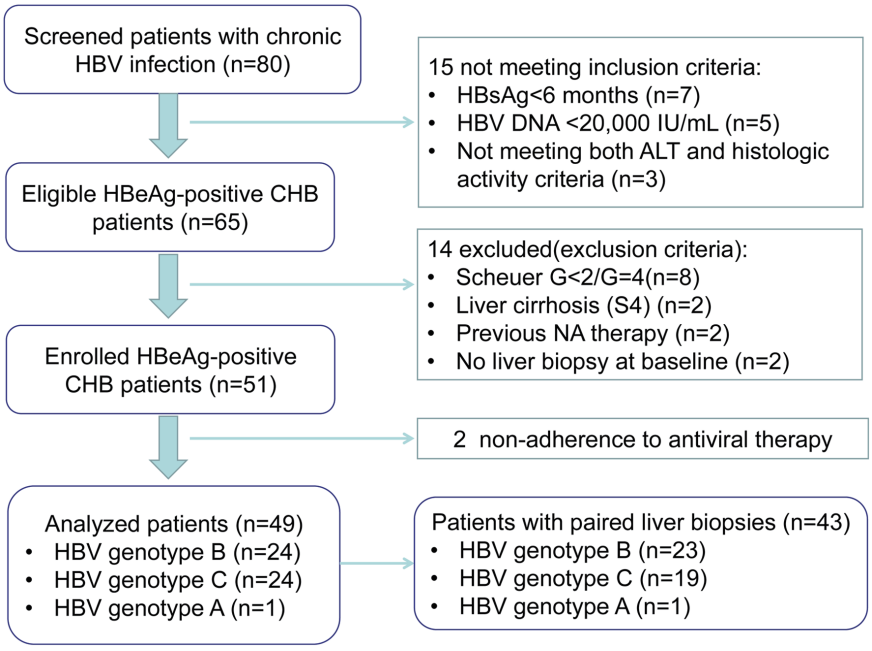

Fig. 1. Flow diagram of the study. CHB, chronic hepatitis B; ETV, entecavir.

and effective responses.[29,30] Secondary endpoints included the following: (1) virological response (*i.e.*, serum HBV DNA < 100 IU/mL, indicating viral suppression); (2) serological responses, including HBeAg loss (*i.e.*, disappearance of hepatitis B e antigen), HBeAg seroconversion (*i.e.*, development of anti-HBe antibodies), HBsAg loss (*i.e.*, clearance of surface antigen), and HBsAg seroconversion (*i.e.*, disappearance of serum HBsAg and the presence of anti-HBs); and (3) biochemical response (*i.e.*, ALT normalization ≤ 40 IU/mL, reflecting resolution of hepatic inflammation). These parameters were assessed longitudinally, with viral markers evaluated at baseline and weeks 24, 48, 60, 72, 96, 120, and 144, and virological load (HBV DNA) and ALT levels measured at baseline and weeks 4, 8, 12, 16, 20, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, and 144. Safety monitoring encompassed the entire 144-week treatment period, including documentation of adverse events (AEs, graded by CTCAE v5.0 criteria) and laboratory abnormalities, with particular attention to renal function and hematological parameters.

Statistical analysis

For this prospective study, patients who received regular ETV therapy and underwent a post-treatment liver biopsy were included in the analysis for the primary endpoint (*i.e.*, histological responses), and patients who received regular ETV therapy and had complete data were included in analyses for the secondary endpoints. When assessing alterations in serum HBV DNA, a log-normal distribution for HBV DNA levels was presumed. Categorical variables were expressed as proportions (%) and compared using the chi-square or Fisher's exact test; odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Continuous variables were presented as mean ± standard deviation or median (interquartile range) and assessed using Student's t-test or the Mann–Whitney U test, where appropriate. The Kaplan–Meier method was utilized to analyze the cumulative rates of ALT normalization and achievement of virological response. Statistical analyses were performed using SPSS version 19.0 (SPSS, Chicago, IL, USA). Statistical significance was determined at a *P* value of less than 0.05.

Results

Baseline characteristics

A total of 80 patients with chronic HBV infection were initially screened; 15 patients were not eligible for not meeting the inclusion criteria: seven with HBsAg positivity < 6 months, five with HBV DNA < 20,000 IU/mL, and three not meeting both the ALT and histological activity criteria. Of the 65 eligible HBeAg-positive CHB patients, 14 were excluded, including 10 without baseline liver biopsy (n = 2) or with baseline biopsies not meeting histological criteria (Scheuer G < 2/G = 4, n = 8), two with liver cirrhosis (S4), and two with previous NA therapy. Additionally, two enrolled patients were excluded from the analysis due to non-adherence to antiviral therapy. Thus, 49 patients, with a median age of 24.0 (22.5–31.0) years and 39 (79.6%) males, were initially recruited and received ETV therapy in the present study (Fig. 1).

Of the 49 patients, 1 (2.0%), 24 (49.0%), and 24 (49.0%) were identified with HBV genotypes A, B, and C, respectively (Table 1). Twenty-seven (55.1%) and 22 (44.9%) patients had necroinflammatory activity scores of 2 ≤ G < 3 and 3 ≤ G < 4, respectively, and 5 (10.2%), 34 (69.4%), and 10 (20.4%) patients had fibrosis scores of 0 ≤ S < 2, 2 ≤ S < 3, and 3 ≤ S < 4, respectively (Table 1). The HBeAg and HBV DNA levels were significantly higher in genotype B than in genotype C groups (934.3 [243.5–1,079.0] *vs.* 439.7 [88.4–767.1] S/CO, *P* = 0.007, and 7.8 [7.3–8.3] *vs.* 7.3 [6.6–7.7] $\log_{10}$ IU/mL, *P* = 0.003, respectively). There were no significant differences in baseline demographic characteristics, including age, gender ratio, and body mass index, or laboratory findings, including ALT, aspartate aminotransferase, albumin, total bilirubin, and platelets, or liver histology between the genotype B and genotype C groups (all *P* > 0.05) (Table 1).

Table 1. Baseline demographic, laboratory, histological, and genotypic characteristics of participating patients with HBeAg-positive chronic hepatitis B treated with entecavir for 144 weeks

Variables	All patients (n = 49)	Genotype A (n = 1)	Genotype B (n = 24)	Genotype C (n = 24)	*P*-value*
Demographic characteristics					
Age (years)	24.0 (22.5–31.0)	26.0	25.0 (22.0–29.0)	25.0 (22.3–32.8)	0.234
Males	39 (79.6)	1	19 (79.2)	19 (79.2)	1.000
Body mass index	21.0 (19.1–23.0)	18.4	20.9 (19.5–22.8)	21.2 (18.9–22.5)	0.680
Laboratory data					
ALT (U/L)	183.0 (138.5–311.0)	87.0	198.5 (143.0–362.0)	165.0 (122.0–238.0)	0.280
AST (U/L)	92.0 (55.0–146.0)	51.0	92.0 (55.0–146.0)	87.5 (52.5–149.0)	0.254
Albumin (g/L)	44.0 (40.8–46.1)	45.6	42.9 (40.1–45.1)	44.4 (41.1–46.4)	0.333
TBIL (μmol/L)	15.1 (10.8–19.7)	10.8	15.4 (10.7–20.0)	14.9 (10.9–18.4)	0.897
Platelet (10^9/L)	176.0 (149.0–206.0)	219.0	191.0 (153.0–209.0)	165.5 (142.0–196.0)	0.118
HBeAg (S/CO)	535.0 (74.7–925.1)	918.0	925.1 (361.3–1,043.0)	348.9 (26.7–743.7)	0.007
HBV-DNA ($\log_{10}$ IU/mL)	7.5 ± 0.8	7.4	7.8 ± 0.7	7.2 ± 0.8	0.003
*Liver histology***					
Necroinflammatory activity					
0 ≤ G < 2	0 (0%)	0	0 (0)	0 (0)	0.144
2 ≤ G < 3	27 (55.1)	0	11 (45.8)	16 (66.7)	
3 ≤ G < 4	22 (44.9)	1	13 (54.2)	8 (33.3)	
Fibrosis					
0 ≤ S < 2	5 (10.2)	0	3 (12.5)	2 (8.3%)	0.892
2 ≤ S < 3	34 (69.4)	1	16 (66.7)	17 (70.8)	
3 ≤ S < 4	10 (20.4)	0	5 (20.8)	5 (20.8)	

Data are expressed as median (interquartile range) or number (%), where appropriate. *, comparison between genotypes B and C groups. **, Scheuer criteria were used for grading liver histology. Necroinflammatory activity was graded on a 5-point scale: G0 = no inflammatory activity; G1 = mild inflammation (confined to portal areas); G2 = mild to moderate inflammation (focal necrosis or inflammation extending beyond the portal limit); G3 = moderate inflammation (noticeable piecemeal necrosis and/or lobular inflammation); and G4 = severe inflammation (extensive piecemeal necrosis and bridging hepatic necrosis). Fibrosis was staged on a 5-point scale: S0 = no fibrosis; S1 = minimal fibrosis (enlarged, fibrotic portal tracts); S2 = moderate fibrosis (periportal or portal–portal septa without architectural distortion); S3 = severe fibrosis (bridging fibrosis with architectural distortion without cirrhosis); and S4 = cirrhosis. ALT, alanine aminotransferase; AST, glutamic oxaloacetic transaminase; TBIL, total bilirubin; HBeAg, hepatitis B envelope antigen; IU, international unit; S/CO, signal-to-cutoff ratio.

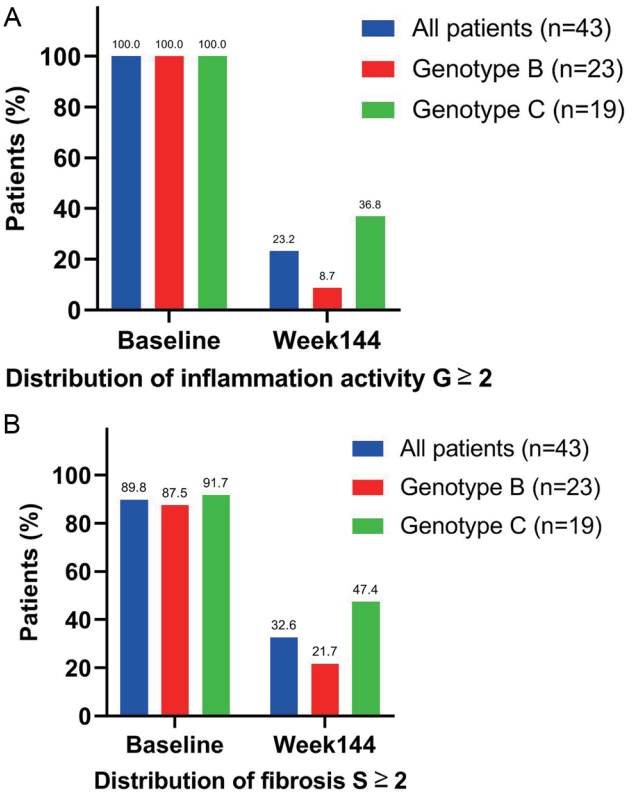

Fig. 2. Proportions of patients with inflammatory activity G ≥ 2 (A) and with fibrosis S ≥ 2 (B), based on the Scheuer scoring system,[27] in 43 patients with HBeAg-positive chronic hepatitis B at baseline and 144 weeks after treatment with entecavir in relation to genotypes. Data from one patient with genotype A with inflammatory activity G ≥ 2 but fibrosis 1 < S < 2 after 144-week entecavir treatment are not illustrated.

Histological response

At the 144-week endpoint, a secondary liver biopsy was performed in 43 patients (23, 19, and 1 with genotypes B, C, and A, respectively). The proportions of patients with necroinflammatory activity scores G ≥ 2 and fibrosis stages S ≥ 2 decreased from 100% to 23.2% and from 89.8% to 32.6%, respectively, in these 43 patients with paired biopsy data (Fig. 2A and B). Specifically, genotype B patients showed reductions in G ≥ 2 from 100% to 8.7% and in S ≥ 2 from 87.5% to 21.7%, whereas genotype C patients exhibited declines from 100% to 36.8% for G ≥ 2 and from 91.7% to 47.4% for S ≥ 2 (Fig. 2A and B). Notably, the overall histological response rate was achieved in 34 (79.1%) of the 43 patients; the rate was significantly higher in patients with genotype B than in those with genotype C (91.3% *vs.* 63.2%, OR = 6.125, 95% CI: 1.09–34.38, $P = 0.027$). Very effective and effective response rates were 78.3% and 13.0% in the genotype B group, compared with 21.1% and 42.1% in the genotype C group, respectively (Table 2). In addition, the proportion of patients with necroinflammatory activity G ≥ 2 at week 144 was significantly higher in the genotype C group than in the genotype B group (36.8% *vs.* 8.7%, OR = 0.163, 95% CI: 0.03–0.92, $P = 0.048$).

Virological response

At week 144, 48 (98.0%) patients achieved HBV DNA reduction to undetectable levels (i.e., < 100 IU/mL); one patient with genotype A failed to achieve a virological response to treatment. A virological response was achieved in 0%, 4.2%, and 4.2% of patients with genotype B at weeks 12, 16, and 20, respectively, whereas the corresponding rates were 29.2%, 29.2%, and 33.3%, respectively, for patients with genotype C ($P = 0.009$, $P = 0.045$, and $P = 0.026$, respectively, at the three time points; Fig. 3A), suggesting a fundamental difference in the response between genotypes during the initial treatment period. However, the rates were similar afterwards, with both groups reaching 100% at week 96 and thereafter. Kaplan–Meier analysis revealed no significant intergroup difference in the cumulative virological response over the entire treatment period (log-rank $\chi^2 = 2.800$, $P = 0.094$; Fig. 3B), indicating comparable long-term viral suppression despite the early-phase response difference.

Serological response

The overall HBeAg loss and cumulative HBeAg seroconversion rates were 46.9% and 38.8%, respectively, at week 144. The HBeAg loss rates at weeks 24, 48, 60, 72, 96, 120,

Table 2. Histological response to 144-week treatment with entecavir in terms of liver necroinflammatory activity in patients with genotypes B and C

	All patients (n = 43)*	Genotype B (n = 23)	Genotype C (n = 19)	*P*-value#
Histological response				0.027
Very effective (n = 22)	22 (51.2)	18 (78.3)	4 (21.1)	
Effective (n = 12)	12 (27.9)*	3 (13.0)	8 (42.1)	
Ineffective (n = 9)	9 (20.9)	2 (8.7)	7 (36.8)	
Necroinflammatory activity at week 144				0.048
0 ≤ G < 1 (n = 18)	18 (41.9)	13 (56.5)	5 (26.3)	
1 ≤ G < 2 (n = 15)	15 (34.9)	8 (34.8)	7 (36.8)	
G ≥ 2 (n = 10)	10 (23.2)*	2 (8.7)	7 (36.8)	
Fibrosis at week 144				0.098
0 ≤ S < 1 (n = 13)	13 (30.2)	10 (43.5)	3 (15.8)	
1 ≤ S < 2 (n = 16)	16 (37.2)*	8 (34.8)	7 (36.8)	
S ≥ 2 (n = 14)	14 (32.6)	5 (21.7)	9 (47.4)	

Data are expressed as number (%). *, including one patient with genotype A. #, comparison between genotypes B and C groups.

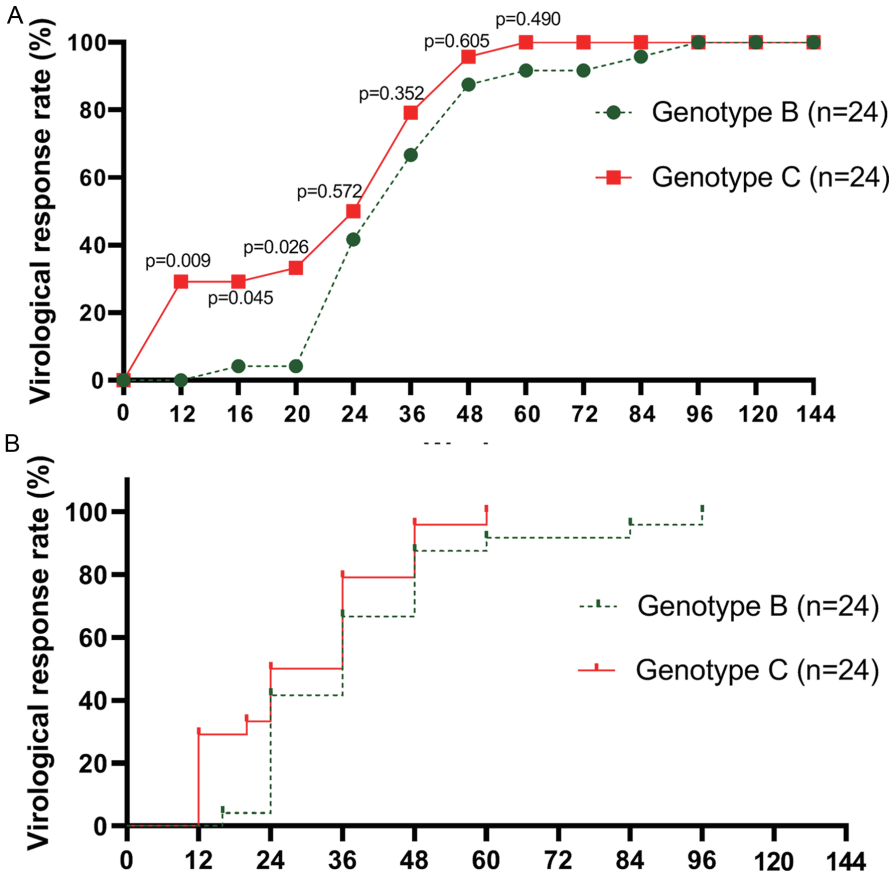

Fig. 3. Virological response (*i.e.*, HBV DNA < 100 IU/mL) to 144-week treatment with entecavir in HBeAg-positive patients with HBV genotypes B and C (A), and Kaplan–Meier analysis of HBV DNA detection with log-rank χ^2 = 2.800 and *P* = 0.094 (B). HBV, hepatitis B virus.

and 144 of treatment were 12.5%, 16.7%, 25.0%, 25.0%, 25.0%, 29.2%, and 37.5%, respectively, in patients with genotype B, and 12.5%, 33.3%, 41.7%, 41.7%, 41.7%, 50.0%, and 58.3%, respectively, in patients with genotype C (all $P > 0.05$). The cumulative HBeAg seroconversion rates at weeks 24, 48, 60, 72, 96, 120, and 144 were 12.5%, 16.7%, 20.8%, 20.8%, 20.8%, 20.8%, and 29.2%, respectively, in patients with genotype B, and 12.5%, 29.2%, 37.5%, 37.5%, 37.5%, 37.5%, and 50.0%, respectively, in patients with genotype C (all $P > 0.05$; Table 3). From week 60 until week 144, only one (2%) patient with genotype B achieved HBsAg loss with subsequent HBsAg seroconversion.

Biochemical response

The median baseline serum ALT level was 183.0 (138.5–311.0) U/L. ALT levels returned to normal in all patients after 48 weeks of treatment, and this was maintained at week 144. There was no significant difference in ALT normalization rates at weeks 4, 12, 24, 36, and 48 of treatment between the two groups (Fig. 4A). In addition, Kaplan–Meier analysis also found no significant difference in the ALT normalization rate between the two groups at any time points (log-rank $\chi^2 = 0.232$, $P = 0.630$; Fig. 4B).

Safety

Overall, seven AEs, all mild, were reported in 13 (26.5%) of the 49 patients: six patients with genotype B and seven patients with genotype C (Supplementary Table 1). Transient fatigue (n = 4) was the most common symptom, followed by headache (n = 3), dizziness (n = 3), and insomnia (n = 3). All AEs resolved spontaneously without specific medical intervention. Notably, there were no laboratory abnormalities, including abnormal complete blood counts, creatine kinase, lactic acidosis, or clinically significant elevations in serum creatinine or Estimated Glomerular Filtration Rate, throughout the treatment course. No serious AEs were reported.

Discussion

In the present study, we investigated the long-term (*i.e.*, 144 weeks) efficacy of ETV treatment and compared the therapeutic effects, mainly in terms of histological response, between genotypes B and C via paired liver biopsies in patients with HBeAg-positive HBV infection. The present study demonstrated a genotype-specific hepatic histological response (indicated by ≥ 1-grade reduction in necroinflammatory activity without fibrosis progression) to long-term ETV treatment; the histological response rate was significantly higher in patients with genotype B than in those with genotype C (91.3% *vs*. 63.2%, $P = 0.027$). A higher virological response rate was observed in patients with genotype B than in those with genotype C in the early stage (within 20 weeks) of treatment; however, the response rates were similar in both groups in the later stage, with response rates exceeding 90% by 60 weeks and 100% by 96 weeks. In addition, good serological (in terms of HBeAg loss and HBeAg seroconversion)

Table 3. Serological responses to 144-week treatment with entecavir in patients with genotypes B and C

	All patients (n = 49)*	Genotype B (n = 24)	Genotype C (n = 24)	*P*-value#
HBeAg loss				
Week 24	6 (12.2)	3 (12.5)	3 (12.5)	1.000
Week 48	12 (24.5)	4 (16.7)	8 (33.3)	0.183
Week 60	16 (32.7)	6 (25.0)	10 (41.7)	0.221
Week 72	16 (32.7)	6 (25.0)	10 (41.7)	0.221
Week 96	16 (32.7)	6 (25.0)	10 (41.7)	0.221
Week 120	19 (38.8)	7 (29.2)	12 (50.0)	0.140
Week 144	23 (46.9)	9 (37.5)	14 (58.3)	0.149
HBeAg seroconversion				
Week 24	6 (12.2)	3 (12.5)	3 (12.5)	1.000
Week 48	11 (22.4)	4 (16.7)	7 (29.2)	0.303
Week 60	14 (28.6)	5 (20.8)	9 (37.5)	0.204
Week 72	14 (28.6)	5 (20.8)	9 (37.5)	0.204
Week 96	14 (28.6)	5 (20.8)	9 (37.5)	0.204
Week 120	14 (28.6)	5 (20.8)	9 (37.5)	0.204
Week 144	19 (38.8)	7 (29.2)	12 (50.0)	0.140
HBsAg loss				
Week 24	0	0	0	-
Week 48	0	0	0	-
Week 60	1 (2.0)	1 (4.2)	0	-
Week 72	1 (2.0)	1 (4.2)	0	-
Week 96	1 (2.0)	1 (4.2)	0	-
Week 120	1 (2.0)	1 (4.2)	0	-
Week 144	1 (2.0)	1 (4.2)	0	-
HBsAg seroconversion				
Week 24	0	0	0	-
Week 48	0	0	0	-
Week 60	1 (2.0)	1 (4.2)	0	-
Week 72	1 (2.0)	1 (4.2)	0	-
Week 96	1 (2.0)	1 (4.2)	0	-
Week 120	1 (2.0)	1 (4.2)	0	-
Week 144	1 (2.0)	1 (4.2)	0	-

Data are expressed as number (%). *, one patient with genotype A failed to achieve a serological response at weeks 24–144. #, comparison between genotypes B and C groups.

and excellent biochemical (indicated by ALT normalization) responses, as well as an acceptable safety profile, were observed in both groups.

Recently, clinical practice guidelines and consensus conference statements have highlighted the clinical relevance of HBV genotypes in therapeutic decision-making for CHB.[31,32] In Asia, CHB patients are predominantly infected with HBV genotypes B and C,[33] and genotypes play certain roles in disease progression and treatment outcomes.[34] The present prospective cohort study systematically evaluated histological responses across multiple HBV genotypes (A, B, and C), mainly between genotypes B and C, providing novel insights into genotype-specific patterns of hepatic histological improvement during antiviral therapy. Histological improvement is achievable alongside effective HBV suppression with long-term ETV therapy. A Japanese study[35] evaluating 167 nucleoside-naïve patients who received ETV, 0.5 mg daily, demonstrated 100% histological improvement at week 148 of treatment. A Turkish study of 46 CHB patients who underwent ETV monotherapy for a minimum of three years reported a notable enhancement in histological activity index and fibrosis scores in 50.0% and 30.4% of patients, respectively.[36] Both studies highlighted substantial and continuous enhancement in hepatic pathology with long-term ETV treat-

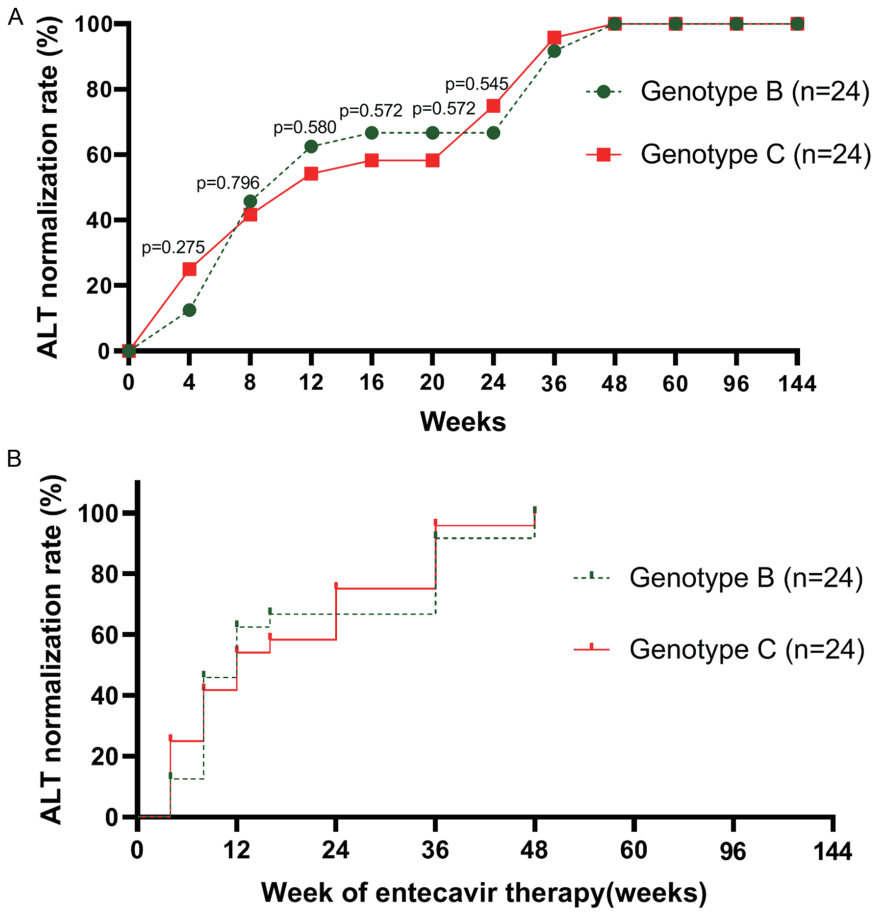

Fig. 4. Biochemical response, or ALT normalization (*i.e.*, reduction of ALT to ≤ 40 IU/mL), after 144-week treatment with entecavir in HBeAg-positive patients with HBV genotypes B and C (A), and Kaplan–Meier plot of ALT normalization with log-rank χ^2 = 0.232 and *P* = 0.630 (B). HBV, hepatitis B virus; ALT, alanine aminotransferase.

ment. However, these studies did not explore the association between HBV genotypes and histological improvement after long-term ETV treatment. The present study confirmed a significant histological response after 144-week ETV treatment in patients with HBeAg-positive CHB. Moreover, the histological response, as well as improvement in necroinflammatory activity, was more prominent in patients with genotype B than in those with genotype C, as evidenced by paired liver biopsy analyses. It has been shown that in Southern China, Guangdong, HBV genotypes B and C constitute the predominant circulating strains, accounting for 53% and 46%, respectively, of chronic HBV infections.[37] The prevalence of HBV genotypes (*i.e.*, 49% and 49%, respectively, for HBV genotypes B and C) is broadly comparable to the regional epidemiological pattern, suggesting minimal genotype-selection bias in the present study. Specifically, ETV was found to be more effective for patients with genotype B than for those with genotype C, although patients with genotype B exhibited significantly higher pretreatment levels of both HBV DNA and HBeAg compared with those with genotype C. These findings indicate that the observed difference in histological improvement between genotypes B and C is more likely attributable to HBV genotypes themselves, and that genotype-specific virological behavior is the underlying driver of divergent treatment responses, as patients in both groups were consistently HBeAg-positive and shared similar baseline characteristics. Genotypes B and C are two typical genotypes in China.[38] Indeed, studies have shown that patients with genotype C are more prone to developing cirrhosis and HCC compared with those with genotype B.[39] Emerging evidence has demonstrated that genotype C is associated with more extensive liver damage compared with genotypes A, B, and D,[40] and that genotype C HBV strains with T-1762 mutations cause more severe liver inflammation and fibrosis than genotype B, suggesting genotype-specific pathogenicity.[41]

Monitoring serum HBV DNA levels is currently considered the standard method for assessing virological response to therapy in clinical practice.[42] Evidence indicates that CHB patients with a high viral load at baseline tend to have better initial virological suppression when treated with NAs such as lamivudine, adefovir dipivoxil, tenofovir, and ETV.[43,44] The temporal dynamics of virological suppression observed in the present study revealed distinct genotype-specific patterns during the initial treatment phase. Specifically, the virological response rates at weeks 12, 16, and 20 for genotype B were relatively lower than those for genotype C. This early-phase difference is likely attributable to higher baseline HBV DNA and HBeAg levels in patients with genotype B than in those with genotype C, which may delay initial viral suppression.[45] Importantly, this early lag did not influence long-term antiviral outcomes, as both genotypes achieved complete

virological suppression by week 96. Therefore, the superior histological improvement observed in genotype B is unlikely to be fully explained by baseline viral load differences and may reflect intrinsic genotype-specific viral characteristics. These findings are consistent with previous studies confirming that genotype C is an independent risk factor for an unfavorable natural history, characterized by reduced HBeAg seroconversion and increased cirrhosis incidence.[46] While this genotype portends a poorer prognosis in untreated patients, evidence demonstrates that tenofovir-based regimens achieve comparable long-term virological outcomes across all genotypes.[47,48] This study thereby reconciles this apparent paradox by identifying genotype C patients as high-risk candidates; our findings underscore that they stand to benefit most from potent, long-term NA therapy, which mitigates their inherent genetic risk via complete viral suppression. The observed dissociation between early virological response and ultimate histological outcomes suggests that factors beyond an initial viral load decline may contribute to long-term treatment efficacy in terms of histological response. Notably, the early response advantage for genotype C did not translate into superior histological improvement, highlighting the complex interplay between viral and host factors in determining treatment outcomes. These findings underscore the importance of considering both short-term virological markers and long-term histological endpoints when evaluating antiviral efficacy.

Serum ALT levels are a valuable indicator of the host's immune response to viral infection.[49] Accompanying ALT normalization, virological response indicates reduced liver damage.[50] In the present study, genotype C demonstrated higher early response rates in ALT normalization than genotype B, which could potentially be attributed to the fact that, at baseline, the genotype B group had both higher viral loads and HBeAg levels, suggesting that higher initial viral activity might be associated with a slower early biochemical response in patients with genotype B compared with those with genotype C.

HBeAg loss and HBeAg seroconversion are important markers of effective treatment, suggesting a sustained immune response. HBeAg seroconversion is also a significant endpoint and a key indicator for withdrawing treatment in HBeAg-positive CHB patients.[51,52] In the present study, HBeAg seroconversion and HBeAg loss rates were 38.8% and 46.9%, respectively, at week 144, with the seroconversion rate notably higher than previously reported.[53] Further studies are needed to explain this discrepancy. HBeAg, a protein encoded by the pre-C gene,[54] is suppressed as immunomodulatory effects increase to inhibit HBV DNA replication.[55] Factors influencing HBeAg seroconversion include HBV genotype, pre-C mutations, and core promoter mutations.[56] Whereas one study with lamivudine treatment reported a higher sustained HBeAg seroconversion rate in genotype B patients compared with genotype C,[57] other studies did not find a consistent relationship between HBV genotype and response to antiviral therapies with adefovir, lamivudine, or ETV.[58,59] In clinical practice, the primary goal of NA therapy for CHB is to achieve sustained viral suppression, while a functional cure (defined as durable HBsAg loss with or without anti-HBs seroconversion) remains an ideal but less frequently attained endpoint.[30,60] However, achieving this goal remains uncommon with current antiviral therapies; previous studies have reported HBsAg loss rates ranging from 0–2% with NA monotherapy in HBeAg-positive patients.[30,61] Finally, different genotypes may exhibit varying responses to antiviral therapy in terms of HBsAg decline and loss, as a previous study reported that in patients receiving three-year ETV treatment, less time was required to achieve HBsAg seroclearance for genotypes A and D compared with genotype E.[62] However, that study did not include genotypes B and C. In the present study, spontaneous HBsAg seroclearance was observed in one (4.17%) of the 24 treatment-naïve patients with genotype B during the three-year follow-up period.

The present study had several limitations. First, despite an extended treatment duration (144 weeks) relative to previous studies in HBeAg-positive CHB patients, the sample size (n = 49) was relatively small, which may limit the generalizability of the findings; thus, studies with larger populations would enhance the statistical power for detecting subtle intergenotypic differences, particularly in subgroup analyses. Second, the exclusive focus on genotypes B and C in the present study precludes broader HBV genotypic comparisons, and future multicenter studies should be conducted with the geographic distribution of genotypes A, D, and E taken into consideration. Third, while paired liver biopsies provided robust histological data, their invasive nature restricted serial sampling frequency. Fourth, subgenotype analysis was not conducted in the present study, as only the partial preS/S sequencing region was used, which could not reliably differentiate HBV subgenotypes; therefore, further studies using full-length genome sequencing are warranted. Finally, the single-center design may introduce selection bias; validation in diverse ethnic populations would strengthen clinical applicability.

Conclusions

HBV genotype significantly influences liver histological response, with patients with genotype B exhibiting superior improvement in necroinflammation compared with those with genotype C. These findings provide compelling evidence to support the incorporation of HBV genotyping into routine clinical practice, as it can serve as a critical determinant for predicting histological responses and thus should be considered when formulating individualized therapeutic strategies for CHB patients. Standardization of follow-up and systematic endpoint assessment would further strengthen the validity and clinical relevance of the present study.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conception and design (LL), provision of study materials (all authors), figure drawing (LL), manuscript writing (LL, QX), and revisions and final approval of the manuscript (LL, SL). All authors approved the final version and publication of the manuscript.

Ethical statement

The study protocol (Clinical Trial Registration: 2012ZX10005 004) was approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (also known as Guangdong Provincial Hospital of Traditional Chinese Medicine), Guangzhou, China (approval number: BF2019-041-01). All participants provided written informed consent, and the study was conducted in accordance with the Declaration of Helsinki (as revised in 2024).

Data sharing statement

The datasets generated during the current study are available from the corresponding author upon reasonable request.

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