International Journal of General Medicine

Dovepress

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ORIGINAL RESEARCH

Prevalence of Dual-Positivity for Both Hepatitis B e Antigen and Hepatitis B e Antibody Among Hospitalized Patients with Chronic Hepatitis B Virus Infection

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Objective: The detection of dual-positivity for both hepatitis B e antigen (HBeAg) and hepatitis B e antibody (anti-HBe) is not typically performed for patients with hepatitis B virus (HBV). This cross-sectional study was designed to figure out the prevalence of dual-positivity for both HBeAg and anti-HBe (DEP) among hospitalized patients with chronic hepatitis B virus infection (C-HBVI).

Patients and Methods: Data from 2820 cases with C-HBVI from two centers in China were retrospectively analyzed. Univariate and multivariate logistic regression analyses were undertaken to identify the risk factors for liver fibrosis (LF) and acute-on-chronic liver failure (ACLF).

Results: There were 165 (5.9%), 688, and 1903 patients in DEP, HBeAg+/anti-HBe-, and HBeAg-/anti-HBe+ groups, respectively. The DEP patients' median age was 43.6 years old and 71.5% of them were male. They had higher levels of alanine transaminase, total bilirubin, and international normalized ratio. Furthermore, DEP cases had a higher proportion of liver cirrhosis, and it was associated with non-invasive testing of LF, including aspartate transaminase (AST)-to-platelet ratio index (APRI) >1.5 (odds ratio (OR) = 1.96, 95% confidence interval (CI): 1.27–3.03, $P = 0.002$) and fibrosis-4 (FIB-4) score >1.45 (OR = 2.07, 95% CI: 1.28–3.34, $P = 0.003$). DEP also contributed to the elevated risk of ACLF (OR = 4.80, 95% CI: 2.02–11.39, $P < 0.001$).

Conclusion: DEP cases are at higher risks of LF and ACLF than other patients with HBV infection. A fast diagnosis and an active monitoring of liver diseases for DEP patients are extremely vital.

Keywords: hepatitis B e antigen, hepatitis B e antibody

Introduction

Hepatitis B virus infection (HBVI) is a worldwide health concern, and nearly 240 million HBVI cases and 650 thousand HBV-related deaths occur annually.[1] In China, there are near 30 million chronic hepatitis B (CHB) cases, of whom 1 million cases have liver cirrhosis and 0.3 million have HBV-associated hepatocellular carcinoma (HCC).[2]

The detection of dual-positivity for both hepatitis B e antigen and hepatitis B e antibody (DEP) is not typically performed for CHB patients. To date, relevant researches have pointed out that the prevalence of DEP is 0.2–2.6%

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Received: 9 July 2021
Accepted: 25 August 2021
Published: 16 September 2021

in CHB cases and 10.4% in CHB cases in the immune-active phase (I-AP).[3–5] It has been speculated that during the transition between positivity for HBeAg and positivity for anti-HBe, the serologic markers might achieve an optimal rate, making them simultaneously detectable.

According to the existence of HBeAg, the viral load of HBV-DNA, and the extent of liver damage, CHB can be divided into the following phases: immune-tolerant phase, I-AP, and inactive phase.[6] DEP patients can theoretically be in the immune-tolerant phase and I-AP, while the majority of them are found to be in the I-AP.[3] A previous research demonstrated that, among CHB cases in the I-AP, DEP cases had higher levels of alanine aminotransferase (ALT) and total bilirubin (TBIL) than other CHB cases,[4] indicating the influence of DEP on the progression of liver diseases (LDs). However, the clinical and virological features and mechanisms underlying DEP among chronic HBVI (C-HBVI) cases have remained elusive.

In this cross-sectional study, we attempted to ascertain the prevalence of DEP among hospitalized patients with C-HBVI, and to figure out the clinical relevance of DEP and LDs in two medical centers in China.

Patients and Methods

Patients

A total of 3689 hospitalized cases with C-HBVI (age >18 years old) who were admitted to the Dongguan People's Hospital (Dongguan, China; from 2014 (January) to 2018 (December)) and Nanfang Hospital (Guangzhou, China; from 2016 (January) to 2016 (December)) were recruited. The existence of hepatitis B surface antigen (HBsAg) in serum (≥6 months) was utilized to define HBVI. Cases co-infected with other viruses (eg, hepatitis C virus, hepatitis D virus, and human immunodeficiency virus) (n = 109) were ruled out. Those cases with other causes of hepatitis (eg, drug-induced LDs, autoimmune hepatitis, alcoholic LDs, etc.) (n = 47) and HCC (n = 713) were not involved. In the remaining 2820 cases, 5.9% (165/2820) were DEP cases. Those cases with HBeAg-negative and anti-HBe-negative (n = 64) were excluded from additional analyses except for prevalence analyses during recruitment (Figure 1).

Definitions

Characterization of acute-on-chronic liver failure (ACLF) cases was carried out by serum bilirubin level ≥5 mg/dL and coagulopathy (international normalized ratio (INR) ≥1.5 or prothrombin activity <40%) complicated by

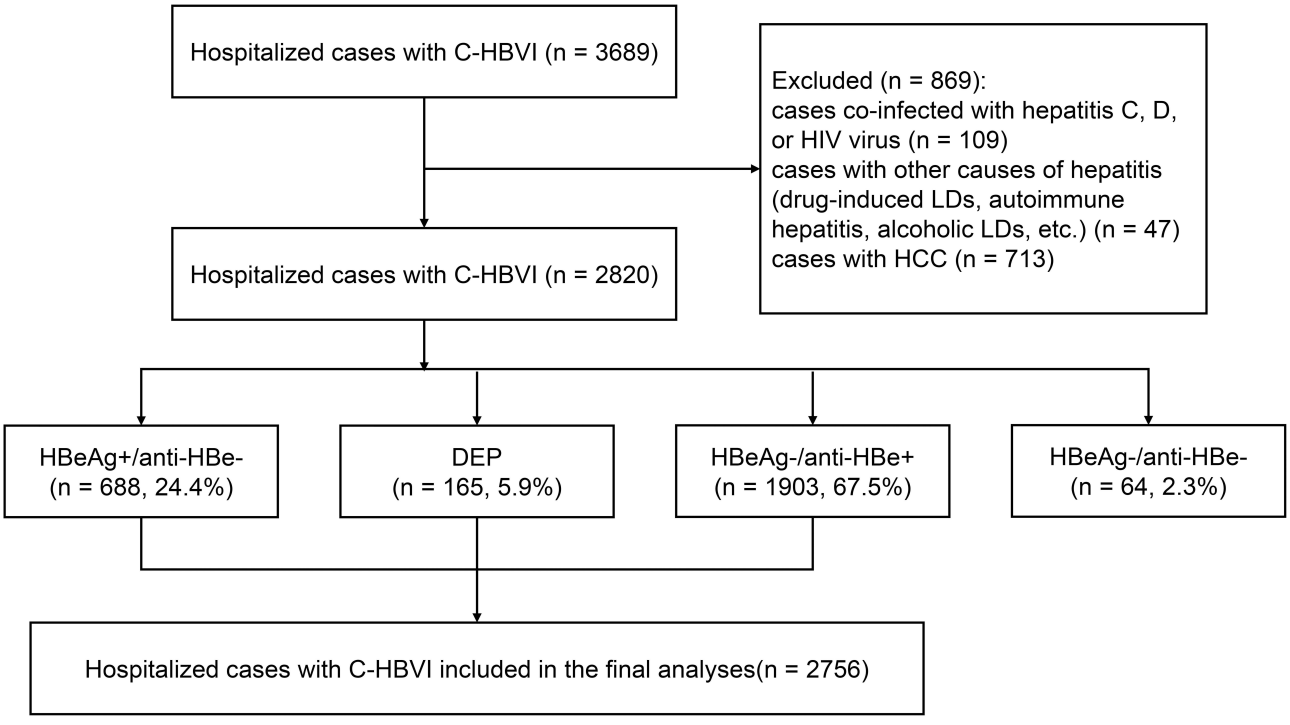

Figure 1 Flowchart of study design.
Abbreviations: DEP, dual-positivity for both hepatitis B e antigen and hepatitis B e antibody; C-HBVI, chronic hepatitis B virus infection; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; anti-HBe, hepatitis B e antibody; HIV, human immunodeficiency virus.

clinical ascites and/or encephalopathy for within 4 weeks.[7] The normal values of biochemical indices of liver function are as follows: ALT (male <50U/L, female <40U/L), aspartate transaminase (AST) (male <40U/L, female <35U/L), and absolute neutrophil count (6.4×10^9/L). Characterization of immune-tolerant phase was undertaken on the basis of HBeAg-positive with a high HBV DNA titer (typically $>10^6$ IU/mL) and a normal ALT level. I-AP was characterized by an intermittently or persistently elevated ALT level and a serum HBV DNA titer $>2\times10^4$ IU/mL in those cases with HBeAg-positive CHB and $>2\times10^3$ IU/mL in those cases with HBeAg-negative CHB, according to the 2018 HBV guidelines published by the American Association for the Study of Liver Diseases.[6] Virological response was defined as a serum HBV DNA titer lower than the lower limit of detection during treatment. Cirrhosis and decompensated cirrhosis were characterized by the results of ultrasonography, computed tomography (CT) and clinical criteria, indicating portal hypertension, such as ascites and esophageal and gastric varices. A moderate alcohol consumption level was defined as >40 g/day.

We used AST-to-platelet ratio index (APRI) and fibrosis-4 (FIB-4) scores to appraise liver fibrosis (LF), and these scores were calculated as described in advance.[8,9]

Serology Testing

Serologic markers for HBV, including HBsAg, anti-HBs, HBeAg, and anti-HBe, were measured using the DiaSorin Liaison-XL (DiaSorin, Saluggia, Italy) and ARCHITECT i2000SR (Abbott, Chicago, IL, USA) platforms. According to the upper limit of the instruments, HBsAg >150 IU/mL was used as the cut-off value for measuring high level of HBsAg because the HBsAg concentration in some cases had not been further diluted. The measurement of HBV DNA levels was undertaken using the Daan test (Daan Gene Co., Ltd. Affiliated to Sun Yat-sen University, Guangzhou, China) and Roche COBAS TaqMan HBV Test kit (Roche Diagnostics, Branchburg, NJ, USA) using the HBV DNA detection lower limits of 500 and 20 IU/mL, respectively. In addition, HBeAg titer >1.0 S/CO and anti-HBe titer <1.0 S/CO were considered as positive on the basis of protocols released by the manufacturers. Other laboratory tests were undertaken at local laboratories according to the standard procedures.

Statistical Analysis

The presentation of continuous variables was in the form of mean ± standard deviation (SD), and their analysis was undertaken using the Student's *t*-test or the Mann–Whitney *U*-test. The expression of categorical variables was in form of percentage, and their analysis was carried out using the Chi-square test or the Fisher's exact test. We also employed univariate and multivariate logistic regression analyses (termed as ULS and MLS analyses, respectively) with a stepwise backward procedure to examine the association of DEP with LF and ACLF. The cutoff values of FIB-4 > 1.45 and APRI > 1.5 were used to ascertain the absence of LF.[10] The prevalence of DEP was reported with 95% confidence interval (95% CI). We set the level of significance to $P < 0.05$. The SPSS 22.0 software (IBM Corp., Armonk, NY, USA) was utilized to statistically analyze the data.

Results

A total of 2756 cases with C-HBVI fulfilled the study criteria (Figure 1). Their demographic, serological, and clinical features are presented in Table 1 and S1.

Demographic Features of DEP Cases and Prevalence of DEP

Among C-HBVI cases, there were 165 DEP cases. After excluding 64 cases with HBeAg- and anti-HBe-negative, the remaining 2591 cases (control group) were assigned into 2 subgroups based on the results of a serology test: HBeAg+/anti-HBe- group (n = 688) and HBeAg-/anti-HBe+ group (n = 1903). Besides, 78 (10.8%), 281 (38.9%), and 363 (50.3%) cases (n = 722) in the I-AP were allocated to DEP, HBeAg+/anti-HBe-, and HBeAg-/anti-HBe+ groups, respectively (Table 2 and S2). In total, 71.5% (118/165) of the DEP cases were male, which was similar to the proportions of those who were in HBeAg +/anti-HBe- (64.7%) and HBeAg-/anti-HBe+ (67.9%) groups. DEP cases tended to be older than HBeAg+/anti-HBe- cases (43.6 ± 11.8 versus 41.7 ± 13.9 years old, $P = 0.085$) and were markedly younger than HBeAg-/anti-HBe + cases (43.6 ± 11.8 versus 52.3 ± 13.2 years old, $P = 0.001$).

The prevalence of DEP was 8.0% (95% CI: 6.4–9.6%) and 4.4% (95% CI: 3.4–5.4%) among cases who aged <45 and ≥45 years old, respectively ($P < 0.001$) (Table 3 and Figure 2). The prevalence of DEP among cases in the I-AP was 10.7% (95% CI: 8.5–13.0%). Additionally, 56.9%

Table 1 Demographic, Serological, and Clinical Features of DEP, HBeAg+/Anti-HBe-, and HBeAg-/Anti-HBe+ Cases with C-HBVI

	DEP	HBeAg+/Anti-HBe-	HBeAg-/Anti-HBe+
Total (n)	165	688	1903
Gender: male (n, %)	118 (71.5%)	445 (64.7%)	1292 (67.9%)
Age (years old)	43.6 ± 11.8	41.7 ± 13.9	52.3 ± 13.2
Treatment history (n, %)	45 (27.3%)	178 (25.9%)	439 (23.1%)
Alcohol consumption (n, %)	6 (3.6%)	18 (2.6%)	84 (4.4%)
ALT (U/L)	83.00 (31.30–314.80)	58.25 (27.08–224.93)	31.00 (19.40–65.33)
AST (U/L)	71.80 (38.95–220.40)	57.75 (34.00–142.23)	37.60 (26.20–69.23)
TBIL (μmol/L)	21.50 (13.80–70.85)	18.35 (12.60–32.20)	16.00 (11.50–26.33)
TBIL ≥ 34.2 μmol/L (n, %)	60 (36.4%)	160 (23.3%)	350 (18.4%)
ALB (g/L)	35.60 (31.40–39.80)	38.20 (34.01–41.69)	39.19 (33.61–42.97)
INR	1.15 (1.03–1.39)	1.07 (1.00–1.20)	1.04 (0.97–1.18)
INR ≥ 1.5 (n, %)	31 (18.8%)	57 (8.3%)	198 (10.4%)
PLT (10^9/L)	154.50 (107.00–213.75)	178.00 (122.75–227.00)	180.00 (121.00–232.00)
HBsAg > 150 IU/mL (n, %)	125 (75.8%)	617 (89.7%)	1205 (63.3%)
APRI	1.66 (0.61–4.95)	1.15 (0.46–2.82)	0.62 (0.34–1.57)
APRI ≥ 1.5 (n, %)	86 (52.1%)	269 (39.1%)	487 (25.6%)
FIB-4	2.56 (1.48–4.98)	1.83 (1.00–3.92)	2.11 (1.24–4.30)
FIB-4 > 1.45 (n, %)	126 (76.4%)	416 (60.5%)	1291 (67.8%)
Cirrhosis (n, %)	54 (32.7%)	157 (22.8%)	472 (24.8%)
Decompensated cirrhosis (n, %)	38 (23.0%)	89 (12.9%)	299 (15.7%)
ACLF (n, %)	13 (7.9%)	11 (1.6%)	56 (2.9%)
HBV DNA (n)	137	519	1232
HBV DNA Positive (n, %)	126 (92.0%)	444 (85.6%)	717 (58.2%)
HBV DNA (log_{10} IU/mL)	5.38 (4.03–6.59)	6.00 (3.94–7.27)	2.92 (2.70–4.70)
< 4 (n, %)	32 (23.4%)	133 (25.6%)	828 (67.2%)
≥ 4 and < 6 (n, %)	62 (45.2%)	127 (24.5%)	270 (21.9%)
≥ 6 and < 8 (n, %)	31 (22.6%)	199 (38.3%)	117 (9.5%)
≥ 8 (n, %)	12 (8.8%)	60 (11.6%)	17 (1.4%)
Immune-tolerant (n, %)	5 (3.6%)	82 (15.8%)	
Immune-active (n, %)	78 (56.9%)	281 (54.1%)	363 (29.5%)

Notes: P1-value for HBeAg+/anti-HBe+ group and HBeAg+/anti-HBe- group; P2-value for HBeAg+/anti-HBe+ group and HBeAg-/anti-HBe+ group; P-value for all groups.
Abbreviations: DEP, dual-positivity for both hepatitis B e antigen and hepatitis B e antibody; C-HBVI, chronic hepatitis B virus infection, HBeAg, hepatitis B e antigen; anti-HBe, hepatitis B e antibody; HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; AST, aspartate transaminase; TBIL, total bilirubin; ALB, albumin; INR, international normalized ratio; PLT, platelet; FIB, fibrinogen; APRI, AST-to-platelet ratio index; FIB-4, fibrosis-4; ACLF, acute-on-chronic liver failure.

(78/137) of the cases in the DEP group were in the I-AP, which was similar to the proportion of cases in the HBeAg +/anti-HBe- group (54.1%, P = 0.559), while it was remarkably higher than that in the HBeAg-/anti-HBe+ group (29.5%, P < 0.001).

Serological and Clinical Features of DEP Patients

The median ALT and AST levels in the DEP group (83.00 and 71.80 U/L) were remarkably higher than those in the HBeAg+/anti-HBe- (P = 0.024 and 0.014, respectively) and HBeAg-/anti-HBe+ groups (both P < 0.001). The TBIL level in the DEP group was notably higher than that in the HBeAg+/anti-HBe- (21.50 versus 18.35 μmol/L, P = 0.011) and HBeAg-/anti-HBe+ groups (16.00 μmol/L, P < 0.001). We figured out a greater proportion of cases with a TBIL level ≥34.2 μmol/L in the DEP group (36.4%) than that in the HBeAg+/anti-HBe- (23.3%) and HBeAg-/anti-HBe+ groups (18.4%) (P < 0.001). The median INR in the DEP group was 1.15, which was markedly higher than that in the HBeAg+/anti-HBe- (1.07, P < 0.001) and HBeAg-/anti-HBe+ groups (1.04, P < 0.001). The median platelet (PLT) count in the DEP group (154.50 × 10^9/L) was lower than that in the HBeAg+/anti-HBe- and HBeAg-/anti-HBe+ groups (P = 0.027 and 0.005, respectively). In the I-AP, the proportions of TBIL ≥ 34.2 μmol/L (48.7%) and INR ≥ 1.5 (23.1%) in the DEP group were noticeably higher than those in the HBeAg+/anti-HBe-

Table 2 Demographic, Serological, and Clinical Features of DEP, HBeAg+/Anti-HBe-, and HBeAg-/Anti-HBe+ Cases with C-HBVI in the Immune-Active Phase

	DEP	HBeAg+/Anti-HBe-	HBeAg-/Anti-HBe+
Total (n)	78 (10.8%)	281 (38.9%)	363 (50.3%)
Gender: male (n, %)	64 (82.1%)	206 (73.3%)	271 (74.7%)
Age (years old)	39.00 (33.75–47.00)	36.00 (30.00–44.00)	49.00 (41.00–59.00)
Treatment history (n, %)	19 (24.4%)	59 (21.0%)	68 (18.7%)
ALT (U/L)	261.65(107.25–698.83)	229.60 (85.40–508.20)	157.00 (71.80–412.00)
AST (U/L)	178.20 (88.48–414.10)	140.00 (71.00–261.05)	112.10 (64.00–268.60)
TBIL (μmol/L)	30.85 (14.50–136.35)	21.80 (14.80–49.80)	24.40 (14.00–87.30)
TBIL ≥ 34.2 μmol/L	38 (48.7%)	99 (35.2%)	146 (40.2%)
ALB (g/L)	34.85 (31.65–38.13)	37.25 (33.53–40.10)	36.45 (31.65–41.03)
INR	1.19(1.07–1.47)	1.10 (1.03–1.24)	1.15 (1.03–1.44)
INR ≥ 1.5 (n, %)	18 (23.1%)	24 (8.5%)	81 (22.3%)
PLT (10^9/L)	150.00(102.80–207.10)	171.00 (125.90–222.00)	162.00 (110.30–205.50)
HBsAg > 150 IU/mL (n, %)	55 (70.5%)	252 (89.7%)	276 (76.0%)
HBV DNA (log_{10} IU/mL)	6.14(5.28–7.27)	6.89 (5.91–7.74)	5.43 (4.38–6.39)
< 6 (n, %)	38 (48.7%)	81 (28.8%)	242 (66.7%)
≥ 6 and < 8 (n, %)	28 (35.9%)	148 (52.7%)	105 (28.9%)
≥ 8 (n, %)	12 (15.4%)	52 (18.5%)	16 (4.4%)
APRI	4.57 (1.94–8.97)	2.37 (1.18–5.00)	2.26 (1.02–5.46)
APRI ≥ 1.5 (n, %)	60 (76.9%)	181 (64.4%)	225 (62.0%)
FIB-4	2.86 (1.89–5.94)	2.11 (1.23–3.97)	3.07 (1.74–6.38)
FIB-4 > 1.45 (n, %)	67 (85.9%)	188 (66.9%)	300 (82.6%)
Cirrhosis (n, %)	24 (30.8%)	51 (18.2%)	107 (29.5%)
Decompensated cirrhosis (n, %)	16 (20.5%)	25 (8.9%)	65 (17.9%)
ACLF (n, %)	8 (10.3%)	6 (2.1%)	29 (8.0%)

Notes: P1-value for HBeAg+/anti-HBe+ group and HBeAg+/anti-HBe- group; P2-value for HBeAg+/anti-HBe+ group and HBeAg-/anti-HBe+ group; P-value for all groups.
Abbreviations: DEP, dual-positivity for both hepatitis B e antigen and hepatitis B e antibody; C-HBVI, chronic hepatitis B virus infection; HBeAg, hepatitis B e antigen; anti-HBe, hepatitis B e antibody; HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; AST, aspartate transaminase; TBIL, total bilirubin; ALB, albumin; INR, international normalized ratio; PLT, platelet; FIB, fibrinogen; APRI, AST-to-platelet ratio index; FIB-4, fibrosis-4; ACLF, acute-on-chronic liver failure.

group ($P < 0.05$), while no significant difference was identified in the mentioned proportions between the DEP and HBeAg-/anti-HBe+ groups.

The proportion of HBsAg > 150 IU/mL in the DEP group (75.8%, 125/165) was in the range of 63.3%–89.7%, as presented in the HBeAg+/anti-HBe- (89.7%, $P < 0.001$) and HBeAg-/anti-HBe+ groups (63.3%, $P = 0.001$). The HBV DNA level in the DEP group (median, 5.38 log_{10} IU/mL; range, 4.03–6.59 log_{10} IU/mL) was noticeably lower than that in the HBeAg+/anti-HBe- group and greater than that in the HBeAg-/anti-HBe+ group. In the majority of DEP cases, the HBV DNA level ranged from 4 to 6 log_{10} IU/mL (45.2%), while the HBV DNA level was in the range of 6 to 8 log_{10} IU/mL in the majority of HBeAg +/anti-HBe- cases (38.3%), and the HBV DNA level was dominantly <4 log_{10} IU/mL among HBeAg- cases (67.2%) (Figure 3A). In the I-AP, the median HBV DNA level in the DEP group (6.14 log_{10} IU/mL; range, 5.28–7.27 log_{10} IU/mL) was lower than that in the HBeAg+/anti-HBe- group (6.89 log_{10} IU/mL, $P = 0.001$) and higher than that in the HBeAg-/anti-HBe+ group (5.43 log_{10} IU/mL, $P < 0.001$) (Table 2 and S2; Figure 3B).

Proportions of LF and ACLF in DEP Patients

The median APRI score in the DEP group was 1.66 (range, 0.61–4.95), which was remarkably higher than that in the HBeAg+/anti-HBe- (median, 1.15; $P = 0.002$) and HBeAg-/anti-HBe+ groups (0.62, $P < 0.001$). The median FIB-4 score in the DEP group (2.56; range, 1.48–4.98) was noticeably higher than that in the HBeAg +/anti-HBe- group (median, 1.83; $P = 0.001$).

Among cases in the I-AP, the median APRI score in the DEP group (4.57; range, 1.94–8.97) was markedly higher than that in the HBeAg+/anti-HBe- (median, 2.37; $P = 0.002$) and HBeAg-/anti-HBe+ groups (median, 2.26; $P = 0.003$) (Table 2 and S2). The median FIB-4 score in the DEP group (2.86; range, 1.89–5.94) in the I-AP was

Table 3 Prevalence of DEP Among C-HBVI Cases

	Overall	DEP		HBeAg+/Anti-HBe-		HBeAg-/Anti-HBe+		HBeAg-/Anti-HBe-	
	N	n	Prevalence (%) (95% CI)	n	Prevalence (%) (95% CI)	n	Prevalence (%) (95% CI)	n	Prevalence (%) (95% CI)
Age (years old)									
< 45	1134	91	8.0 (6.4–9.6)	450	39.7 (36.8–42.5)	573	50.5 (47.6–53.4)	20	1.8 (1.0–2.5)
≥ 45	1686	74	4.4 (3.4–5.4)	238	14.1 (12.5–15.8)	1330	78.9 (76.9–80.8)	44	2.6 (1.8–3.4)
Gender									
Male	1898	118	6.2 (5.1–7.3)	445	23.4 (21.5–25.4)	1292	68.1 (66.8–70.7)	43	2.3 (1.6–2.9)
Female	922	47	5.1 (3.7–6.5)	243	26.4 (23.5–29.2)	611	66.3 (63.2–69.3)	21	2.3 (1.3–3.2)
Treatment									
Treatment naive	2129	120	5.6 (4.7–6.6)	510	24.0 (22.1–25.8)	1464	68.8 (66.8–70.7)	35	1.6 (1.1–2.2)
Treatment experienced	691	45	6.5 (4.7–8.4)	178	25.8 (22.5–29.0)	439	63.5 (59.9–67.1)	29	4.2 (2.7–5.7)
Immune statement									
Immune-tolerant	87	5	5.7 (0.9–10.6)	82	94.3 (89.4–99.1)				
Immune-active	727	78	10.7 (8.5–13.0)	281	38.7 (35.1–42.2)	363	49.9 (46.3–53.6)	5	0.7 (0.1–1.3)

Abbreviations: DEP, dual-positivity for both hepatitis B e antigen and hepatitis B e antibody; C-HBVI, chronic hepatitis B virus infection; HBeAg, hepatitis B e antigen; anti-HBe, hepatitis B e antibody.

higher than that in the HBeAg+/anti-HBe- group (median, 2.11; $P = 0.001$), while we did not identify a significant difference in FIB-4 score between the DEP and HBeAg-/anti-HBe+ groups.

The proportions of cirrhosis and decompensated cirrhosis in the DEP group (32.7% and 23.0%, respectively) were noticeably higher than those in the other two groups (HBeAg+/anti-HBe-, 22.8% and 12.9%, P = 0.008 and

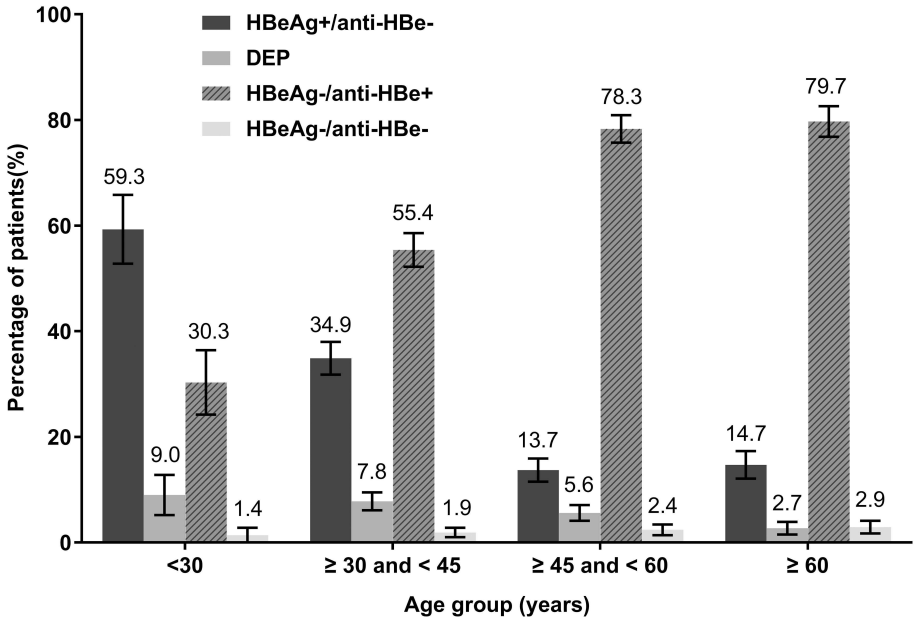

Figure 2 Prevalence of DEP among C-HBVI cases in age-dependent groups.
Abbreviations: DEP, dual-positivity for both hepatitis B e antigen and hepatitis B e antibody; C-HBVI, chronic hepatitis B virus infection; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; anti-HBe, hepatitis B e antibody.

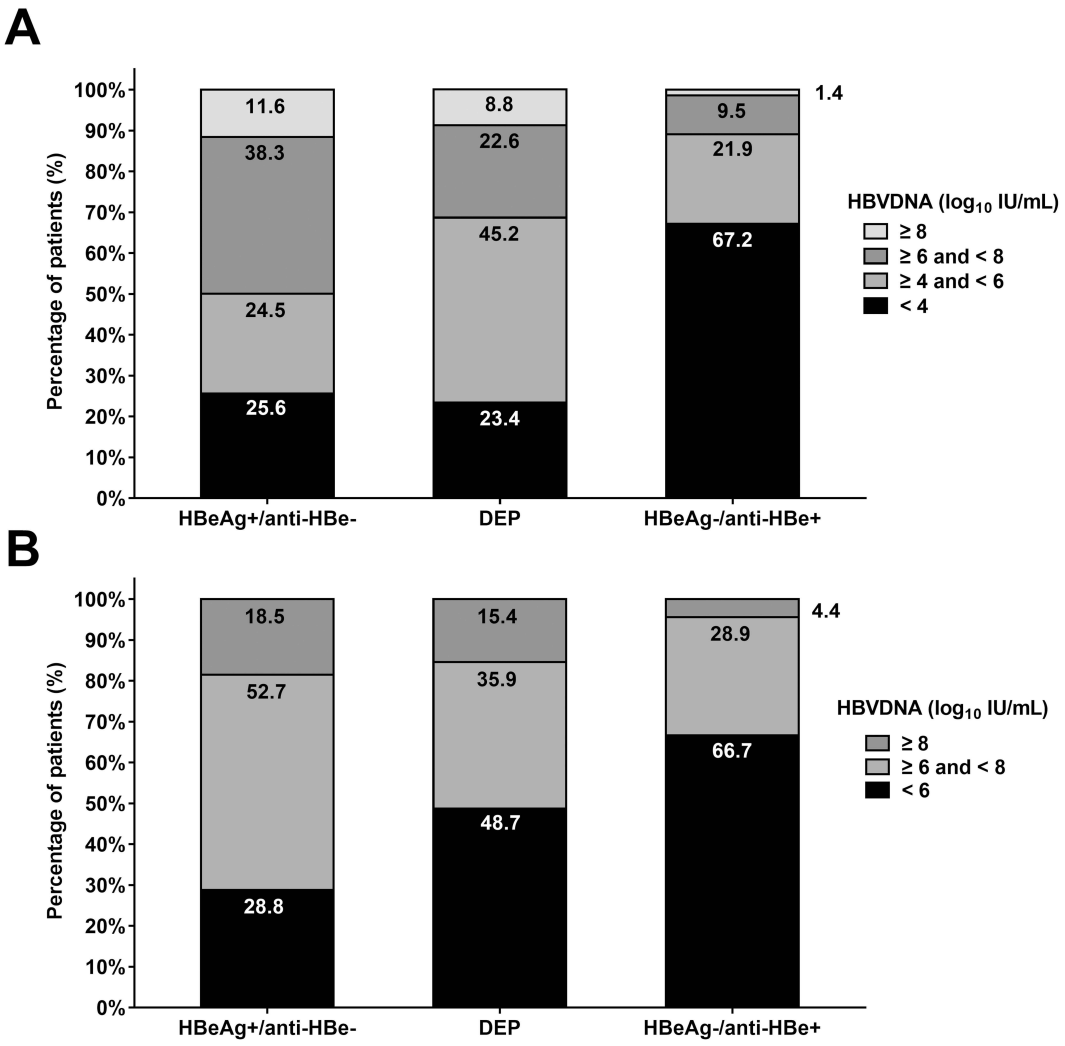

Figure 3 Distribution of DEP, HBeAg+/anti-HBe-, and HBeAg-/anti-HBe+ groups in association with HBV DNA level. (**A**) C-HBVI cases. (**B**) C-HBVI cases in the immune-active phase.

Abbreviations: DEP, dual-positivity for both hepatitis B e antigen and hepatitis B e antibody; C-HBVI, chronic hepatitis B virus infection; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; anti-HBe, hepatitis B e antibody.

0.001, respectively; HBeAg-/anti-HBe+, 24.8% and 25.7%, P = 0.025 and 0.015, respectively). Furthermore, the proportion of ACLF in the DEP group (7.9%, 13/165) was higher than that in the HBeAg+/anti-HBe- group (1.6%, P < 0.001) and HBeAg-/anti-HBe+ group (2.9%, P = 0.001).

In the I-AP, the proportions of cirrhosis (30.8%), decompensated cirrhosis (20.5%), and ACLF (10.3%) in the DEP group were greater than those in the HBeAg +/anti-HBe- group (P = 0.015, 0.004, and 0.003, respectively), while they were not significantly different between the DEP group and the HBeAg-/anti-HBe+ group (P = 0.821, 0.590, and 0.512, respectively).

Factors Associated with LF and ACLF

Evaluation of APRI and FIB-4 scores was conducted using ULS and MLS analyses. MLS analysis demonstrated that DEP was associated with both APRI > 1.5 (P = 0.002, odds ratio (OR) = 1.96, 95% CI: 1.27–3.03) and FIB-4 > 1.45 (P = 0.003, OR = 2.07, 95% CI: 1.28–3.34) (Table 4). Gender, higher HBV DNA level, history of alcohol consumption, history of antiviral treatment and I-AP were independently associated with APRI > 1.5, while age ≥45 years old, gender, history of alcohol consumption, history of antiviral treatment, and I-AP were independently associated with FIB-4 > 1.45.

Table 4 Factors Associated with APRI ≥ 1.5 and FIB-4 > 1.45 in C-HBVI Cases

	APRI ≥ 1.5				FIB-4 > 1.45			
	Univariate		Multivariate		Univariate		Multivariate	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
HBeAg/anti-HBe status								
HBeAg+/anti-HBe-	1	<0.001	1	0.001	1	<0.001	1	0.012
DEP	1.72(1.17–2.52)	0.005	1.96(1.27–3.03)	0.002	2.06(1.32–3.21)	0.001	2.07(1.28–3.34)	0.003
HBeAg-/anti-HBe+	0.59(0.48–0.73)	<0.001	0.88(0.68–1.14)	0.319	1.43(1.15–1.78)	0.001	1.12(0.86–1.44)	0.401
Age (years old)								
< 45	1				1		1	
≥ 45	0.70(0.59–0.85)	<0.001			4.28(3.47–5.28)	<0.001	5.64(4.44–7.16)	<0.001
Gender								
Female	1		1		1		1	
Male	1.61(1.30–1.99)	<0.001	1.35(1.06–1.71)	0.014	1.35(1.03–1.67)	0.006	1.30(1.02–1.65)	0.031
HBV DNA (log_{10} IU/mL)								
Undetectable	1	<0.001	1	0.018	1	0.031		
> undetectable and < 4	1.67(1.26–2.22)	<0.001	1.32(0.97–1.80)	0.078	0.87(0.66–1.13)	0.295		
≥ 4 and < 6	2.90(2.23–3.78)	<0.001	0.83(0.57–1.23)	0.354	1.34(1.02–1.76)	0.036		
≥ 6	5.04(3.85–6.60)	<0.001	1.19(0.77–1.83)	0.441	1.00(0.77–1.30)	0.993		
Alcohol consumption								
No	1		1		1		1	
Yes	2.97(1.91–4.61)	<0.001	3.64(2.23–5.94)	<0.001	4.12(2.06–8.27)	<0.001	4.15(1.97–8.75)	<0.001
HBsAg (IU/mL)								
≤ 150	1				1			
> 150	1.40(1.14–1.73)	0.002			1.07(0.86–1.33)	0.536		
Treatment history								
No	1		1		1		1	
Yes	1.23(1.00–1.50)	0.051	1.83(1.43–2.33)	<0.001	2.11(1.66–2.67)	<0.001	2.40(1.84–3.12)	<0.001
Immune-active								
No	1		1		1		1	
Yes	5.93(4.83–7.27)	<0.001	6.73(4.89–9.25)	<0.001	1.64(1.33–2.03)	<0.001	3.00(2.33–3.87)	<0.001

Abbreviations: DEP, dual-positivity for both hepatitis B e antigen and hepatitis B e antibody; C-HBVI, chronic hepatitis B virus infection; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; anti-HBe, hepatitis B e antibody; HBsAg, hepatitis B surface antigen; APRI, AST-to-platelet ratio index; FIB-4, fibrosis-4.

ACLF was also evaluated by ULS and MLS analyses (Table 5). ULS analysis indicated that ACLF was associated with DEP, gender (male), history of alcohol consumption, history of antiviral treatment, I-AP, and a higher HBV DNA level. MLS analysis revealed that DEP (P < 0.001, OR = 4.80, 95% CI: 2.02–11.39) (HBeAg+/anti-HBe- as reference group), gender (male) (P = 0.016, OR = 2.32, 95% CI: 1.17–4.60), a higher HBV DNA level, history of antiviral treatment (P = 0.003, OR = 2.10, 95% CI: 1.28–3.46), and I-AP (P = 0.008, OR = 2.57, 95% CI: 1.29–5.15) were independently associated with ACLF.

Discussion

In this cross-sectional study, the prevalence of DEP among C-HBVI cases was 5.9% (165/2820), which was higher than the previously reported rate.[3,5] The prevalence of DEP in the immune-tolerant phase and I-AP was 5.7% and 10.7%, respectively, and the latter was similar to the rate mentioned in a previous study (10.4%).[4] The prevalence of cases in the I-AP among DEP patients was 56.9% (78/137), which was higher than the rate (42%) in immune-clearance phase reported in advance.[3]

The age of dual-positive cases fell between that of the HBeAg+/anti-HBe- and HBeAg-/anti-HBe+ groups,

Table 5 Factors Associated with ACLF in C-HBVI Cases

	ACLF			
	Univariate		Multivariate	
	OR (95% CI)	P	OR (95% CI)	P
HBeAg/anti-HBe status				
HBeAg+/anti-HBe-	1	0.001	1	0.002
DEP	5.34(2.29–12.45)	<0.001	4.80(2.02–11.39)	<0.001
HBeAg-/anti-HBe+	2.24(1.13–4.45)	0.021	2.58(1.24–5.37)	0.011
Age (years old)				
< 45	1			
≥ 45	1.07(0.67–1.72)	0.765		
Gender				
Female	1		1	
Male	2.66(1.36–5.21)	0.004	2.32(1.17–4.60)	0.016
HBVDNA (log_{10} IU/mL)				
Undetectable	1	0.008	1	0.011
< 4	3.33(1.60–6.91)	0.001	3.22(1.49–6.97)	0.003
≥ 4 and < 6	2.95(1.43–6.10)	0.003	1.76(0.67–4.61)	0.25
≥ 6	2.17(1.01–4.69)	0.048	1.43(0.49–4.20)	0.515
Alcohol consumption				
No	1			
Yes	2.52(1.17–5.42)	0.018		
HBsAg (IU/mL)				
≤ 150	1			
> 150	0.73(0.45–1.18)	0.2		
Treatment history				
No	1		1	
Yes	1.71(1.06–2.74)	0.027	2.10(1.28–3.46)	0.003
Immune-active				
No	1		1	
Yes	2.24(1.41–3.58)	0.001	2.57(1.29–5.15)	0.008

Abbreviations: DEP, dual-positivity for both hepatitis B e antigen and hepatitis B e antibody; C-HBVI, chronic hepatitis B virus infection; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; anti-HBe, hepatitis B e antibody; HBsAg, hepatitis B surface antigen; ACLF, acute-on-chronic liver failure.

indicating that DEP might occur before the seroconversion of HBV. The levels of ALT, AST, and TBIL in the DEP group were higher than those in the HBeAg mono-positive and anti-HBe mono-positive groups, while the ALB level was lower than that in the control group. Compared with the control group, DEP cases had higher INR and D-dimer level, while lower PLT count and fibrinogen level, demonstrating that DEP had a certain degree of coagulation dysfunction and a low level of fibrinolysis, which might lead to organ failure.[11,12] The HBV DNA level in the DEP group fell between that in the HBeAg mono-positive and anti-HBe mono-positive groups. As a high ALT level, a low HBV DNA level, and advanced LF are predictive factors for HBeAg seroconversion,[13] the serological results may suggest that the period of DEP precedes serological conversion.

In the present study, we also found that DEP was closely associated with advanced LF and ACLF. Our data demonstrated that there was a remarkably higher incidence of LF, cirrhosis, and ACLF in DEP cases with C-HBVI than in HBeAg mono-positive cases, which is in agreement with the results of a previous research.[4] However, this higher incidence was also observed in DEP cases compared with that in anti-HBe mono-

positive cases, suggesting that the detection of DEP may be associated with the progression of LDs.

Among C-HBVI cases in the I-AP, DEP cases had higher values of biochemical indices of liver function, including ALT and TBIL titers, as well as APRI and FIB-4 scores, than HBeAg+/anti-HBe- cases. DEP cases in the I-AP mainly suffered from more severe liver dysfunction, while the FIB-4 scores and the proportions of cirrhosis and ACLF were similar to those in anti-HBe mono-positive cases in the I-AP, which is consistent with the findings of a previous research.[4] However, the APRI score was higher in DEP cases than that in anti-HBe mono-positive cases. This inconsistency might be due to the lack of consideration of age in the calculation of APRI score, which might increase the effects of the age-dependent differences on the prediction of LF between the two groups.

The mechanism underlying the association between DEP and more severe LDs might include the occurrence of HBeAg/anti-HBe and antigen–antibody complex[14] in antigen–antibody reaction, as well as the accumulation of core promoter mutations in the I-AP.

First, in the process of seroconversion, along with the enhancement of anti-HBe level, the antibody and HBeAg coexist and generate antigen–antibody complex. The deposition of the immune complex may result in the stimulation of immune response and extrahepatic manifestations.[15] Moreover, the secretion of cytokines by cytotoxic T-lymphocyte (CTL) intercepts viral replication and HBV gene expression, which accelerates spontaneous HBeAg seroconversion.[16] Meanwhile, the enhancement of CTL may destroy hepatocytes and lead to temporary exacerbation of hepatitis before HBeAg seroconversion.[17]

Second, the double-mutation A1762T/G1764A in the basal core promoter (BCP)/X overlap region was found to be one of the most common core promoter mutations,[18] which was positively correlated with the levels of ALT and AST,[19,20] the fluctuation of HBV DNA level,[20,21] and the progression of LDs,[22–25] and it can be a predictor of HBeAg-to-anti-HBe seroconversion.[23] A previous research showed that this mutation might be selected through CTL escape during the loss of immune tolerance.[26] It is speculated that in the I-AP, during the accumulation of core promoter mutations, the coexistence of BCP mutations and wild-type sequences might lead to the suppression of affinity between HBeAg and anti-HBe, thereby leading to the dual-positivity for HBeAg and anti-HBe. This might explain the high levels of ALT and TBIL in DEP cases during seroconversion, and the elevated degree of LF was found to be similar to that in anti-HBe mono-positive cases in the I-AP along with the accumulation of mutations. However, this hypothesis cannot explain the phenomenon that cases with a low HBV DNA level <2000 IU/mL, who may be in the inactive phase, may have dual-positivity during the progression of LDs. This may be due to the reactivation of HBV, and longitudinal studies may provide more definitive evidence.

The limitations of this study should be pointed out. First, it was a cross-sectional study and lacked follow-up data of hospitalized cases. The recruited patients were all hospitalized cases, and certain differences with outpatients could not be excluded. The severity of cirrhosis was not evaluated, and the detailed disease course and treatment process were not accurately recorded to evaluate their effects on the LF and ACLF. Furthermore, the prevalence of DEP in Guangdong province might not be representative of that in the whole country (China) because of the uneven distribution of HBV genotypes. Moreover, the number of ACLF and LF patients in immune-tolerant phase was limited, which hindered us to perform the ULS and MLS analyses. A multicenter longitudinal study including genotyping is therefore required to elucidate the correlation between different genotypes.

Conclusions

In conclusion, DEP is infrequent among C-HBVI cases, and it is associated with hepatocyte and coagulation dysfunction and higher risks of LF and ACLF. The screening of DEP in C-HBVI cases and subsequent active treatments are imperative.

Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki. The Institutional Review Board (IRB) of Dongguan People's Hospital reviewed the protocol (KYKT2021-007) and waived the requirement for informed consent because anonymous data were analyzed retrospectively.

Acknowledgments

This manuscript was edited by a prestigious company, TopEdit (www.topeditsci.com), professionally working on English language editing services.

Author Contributions

Reading and approving the submitted version of the manuscript could be undertaken by all the authors, who substantially participated in all the stages of the research. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This research was supported by the Local Innovation and Research Teams Project of Guangdong Pearl River Talents Program (Grant No. 2017BT01S131) and Sanming Project of Medicine in Shenzhen (Grant No. SZSM201911001).

Disclosure

The authors declare that there is no conflict of interest.

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