Impact of HCV viremia on HBV biomarkers in patients coinfected with HBV and HCV

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Research Article

Abstract

Backgrounds: Hepatitis B virus (HBV) biomarkers reflect the status of HBV infection; however, their role in patients with chronic hepatitis B and C (HBV/HCV) coinfection remains unknown. This study evaluated the characteristics of HBV biomarkers in patients with chronic HBV/HCV coinfection.

Methods: One hundred untreated HBV/HCV coinfected patients were enrolled. Active viral infection was defined as viral load above 2,000 U/L and 15 U/L for HBV and HCV, respectively. Blood samples were analyzed for HBV biomarkers, including hepatitis B surface antigen (HBsAg), hepatitis B core-related antigen (HBcrAg), HBV DNA, and HBV pregenomic RNA (HBV pgRNA). The impact of HCV viremia was also studied.

Results: A total of 15 patients were HBV-inactive/HCV-inactive, 63 patients were HBV-inactive/HCV-active, 14 patients were HBV-active/HCV-inactive and 8 patients were HBV-active/HCV-active. A total of 71 (71%) patients were active HCV and 22 (22%) were active HBV. HBsAg, HBcrAg, and HBV DNA correlated with each other ($P < 0.001$). HBV pgRNA displayed no correlations with HBV DNA, HBsAg, or HBcrAg. Patients with HCV viremia had significantly lower HBV DNA, HBsAg, and HBcrAg levels as well as higher HBV pgRNA levels and lower HBV DNA:pgRNA ratio than those without viremia (HBV DNA, $P < 0.001$; HBsAg, $P = 0.015$; HBcrAg, $P = 0.006$; HBV pgRNA, $P = 0.073$; and HBV DNA:pgRNA ratio, $P < 0.001$).

Conclusions: In patients coinfected with HBV and HCV, HBsAg, HBcrAg, and HBV DNA significantly correlated with each other. HBV and HCV coinfected patients with HCV viremia have lower HBV DNA, HBsAg, HBcrAg, and HBV DNA:pgRNA ratio as well as higher HBV pgRNA levels.

Background

The prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) coinfection is not uncommon in high epidemic areas because they share common routes of transmission [1, 2]. Patients with HBV/HCV coinfection have a higher risk of advanced liver disease, cirrhosis, and hepatocellular carcinoma (HCC) than those with monoinfection [1, 3]. This population also carries the risk of HBV DNA reactivation following HCV direct-acting antiviral treatment.[4] Hence, understanding the viral interactions and molecular aspects of HBV/HCV coinfection are important for treatment.

Patients with HBV/HCV coinfection show a large spectrum of virological profiles, which demonstrates the complexity of the interaction between the two viruses.[5, 6] The most common clinical scenario among coinfected patients is HCV predominance with high HCV RNA levels and low HBV DNA levels; some patients experience HBV predominance with high HBV DNA levels and low HCV RNA levels, while others present alternating phases of dominance of one virus over the other [3].

Since the presence of HCV viremia may suppress HBV DNA, the status of HBV infection cannot be evaluated with HBV DNA simply due to the persistence of covalently closed circular DNA (cccDNA) in the nuclei of infected hepatocytes [5, 6]. Although liver biopsy is the most accurate technique for

quantification of intrahepatic cccDNA, its utility is limited by its invasive nature, the potential for sampling error, and the lack of a standardized assay [7, 8]. Several non-invasive biomarkers, including hepatitis B surface antigen (HBsAg), hepatitis B core-related antigen (HBcrAg), and HBV pregenomic RNA (HBV pgRNA), have been developed to reflect the activity of intrahepatic cccDNA [7, 9]. Growing evidence supports the serum HBV DNA correlated with serum HBsAg, HBcrAg, and HBV pgRNA in patients without antiviral treatment [9–11]. These biomarkers also serve as surrogate markers to reflect the cccDNA activity in virally suppressed patients with low detectable HBV DNA under nucleos(t)ide analogues (NA) therapy [12–15]. However, the above evidence does not disclose the characteristics of HBV biomarkers in HBV/HCV coinfected patients.

The aim of this study was to investigate the presentation of and correlations among HBV biomarkers in patients with HBV/HCV coinfection. The impact of HCV viremia on HBV biomarkers was also evaluated.

Methods

Subjects

This study included 100 patients (> 20 years old) with chronic HBV/HCV coinfection who received regular follow-up at Dalin Tzu Chi Hospital and Chia-Yi Christian Hospital in Southern Taiwan from May 2017 to July 2019. The included patients were seropositive for HBsAg and the anti-HCV antibody (anti-HCV) for more than 6 months at the time of enrollment into the study. Patients aged < 20 years or with any antiviral treatment history, human immunodeficiency virus or hepatitis delta virus infection, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, Wilson's disease, α1-antitrypsin deficiency, overt hepatic failure, or evidence of HCC were excluded.

According to current guidelines, the cutoff vale using to define the HBeAg(-) patients with active CHB and those who required treatment was 2,000 IU/mL [16, 17]. Therefore, we used above 2,000 IU/mL as the definition of active HBV infection in this study. The HCV patients with detectable viremia required anti-HCV treatment [18, 19]. In this study, we used the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test v2.0 (Roche Diagnostics, Rotkreuz, Switzerland) with a lower limit of quantification of 15 IU/mL. Active HCV infection was defined as a virus load above 15 IU/mL [1]. According to these definitions, the cohort was divided into four different groups: HBV-inactive/HCV-inactive group (BICI), HBV-inactive/HCV-active group (BICA), HBV-active/HCV-inactive group (BACI), and HBV-active/HCV-active group (BACA).

Ethical considerations

The study was approved by the Ethics Committee of Dalin Tzu Chi Hospital (approval number B10901004). Its design and conduct conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Before commencement of the study, all patients signed informed consent forms.

Clinical monitoring

All subjects underwent examinations at the hospital's gastrointestinal outpatient department. Blood samples were collected at regular clinic visits. Blood samples were collected for evaluating levels of HBsAg, hepatitis B e antigen (HBeAg), HBcrAg, HBV pgRNA, HBV DNA, anti-HCV, HCV RNA, and HCV genotype. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, estimated glomerular filtration rate (eGFR), prothrombin time, and platelets were measured, and abdominal ultrasonography and alpha-fetoprotein (AFP) levels were evaluated. Fibrosis-4 (FIB-4) was used as a non-invasive test for liver fibrosis.[20] Liver cirrhosis was diagnosed by radiologic cirrhosis or FIB-4 > 3.25.[21] Radiologic cirrhosis was defined as coarse liver echotexture with nodularity and small liver size or the presence of features of portal hypertension (e.g., ascites, splenomegaly, and varices) noted on liver imaging.[22] A diagnosis of fatty liver was based on results from abdominal ultrasound, including features of hepatorenal echogenicity contrast, liver brightness, deep attenuation, and vessel blurring.[23] All ultrasonographic images were stored as photocopies.

HBV/HCV quantification and HCV genotyping

Serum HCV RNA levels were measured using the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test v2.0 (Roche Diagnostics, Rotkreuz, Switzerland) with a lower limit of quantification of 15 IU/mL. HCV genotyping was performed using the COBAS® HCV GT assay (Roche Diagnostics). Serum HBV DNA levels were measured using the COBAS® HBV quantitative nucleic acid test on the COBAS® 4800 System (Roche Diagnostics), with a lower limit of quantification of 5 IU/mL.

HBsAg quantification

HBsAg quantification was quantified using the two-step sandwich assay on a fully automated chemiluminescent enzyme immunoassay system (CLEIA) (Lumipulse G1200; Fujirebio, Inc., Tokyo, Japan) in a Lumipulse G1200 automated analyzer (Fujirebio, Inc.). The HBsAg-HQ reagents were provided by Fujirebio, Inc. The linear detection range of the HBsAg-HQ ranged from 5 to 150,000 mIU/mL. If the serum exceeded the upper detection limit, it was diluted 200-fold and remeasured.

HBcrAg quantification

This HBcrAg assay was performed using the Lumipulse G1200 CLEIA analyzer (Fujirebio, Inc.) with a lower limit of detection of 2.0 log U/mL and a linear range of 3.0–7.0 log U/mL (1 kU/mL is equal to 3 log U/mL). Samples with HBcrAg level above 7 log U/mL were diluted and retested to calculate the quantitative HBcrAg level. According to the Lumipulse® G HBcrAg Immunoreaction cartridges product inserts, the accuracy of the test was confirmed by assay values (calculated with the assay values in log U/mL) for 3 in-house controls ranging within ± 5% of their control values.

Extraction and reverse transcription of HBV pgRNA

HBV RNA was extracted from 50 µl of serum using the Total RNA Extraction Miniprep System Kit according to the manufacturer's instructions (Viogene, Taipei, Taiwan) and treated with DNase I (Thermo Fisher Scientific, Waltham, MA, USA). Isolated HBV RNA was reverse transcribed using RevertAid reverse

transcriptase (Thermo Fisher Scientific, Waltham, MA, USA) with an HBV specific RT primer for HBV pgRNA and total HBV RNA, respectively. Before commencing reverse transcription (RT), 20 μl of RNA, 1 μl of 10 μM primer and 1 μl of 10 mM dNTPs (Thermo Fisher Scientific, Waltham, MA, USA) were mixed and incubated at 70°C for 5 mins and then placed immediately on ice for 1 min. RT was then initiated with the addition of RT reaction mix to a 35 μl final volume at a final concentration 1x RT buffer, 1 μl of RNAse inhibitor (Life Technologies) and 1 μl of RevertAid reverse transcriptase. Cycling conditions were 42°C for 60 min, followed by 75°C for 5 min. cDNA samples were held at 4°C before proceeding to quantitative real-time polymerase chain reaction (qPCR).

Quantification of serum HBV pgRNA

The levels of serum HBV RNA were detected by qPCR in LightCycler 480 II Real-time PCR Detection System (Roche, Mannheim, Germany) with a SYBR Green method. We modified a transcript- specific quantitative PCR (qPCR) assay previously described.[24] The primers used to detect HBV 3.5 kb RNA were showed in Supplementary Table 1. The standards were constructed by PCR using each primer from HBV full genome (accession number: KJ790199), and the PCR products were subsequently ligated into the T&A™ Cloning Vector (Yeastern Biotech, Taiwan). The qPCR reaction mixture (20 μl) contained 10μl 2 x GoTaq® Green Master Mix (Promega Corp., Madison, WI), 0.5 μl forward primer (10 μM), 0.5 μl reverse primer (10μM), 1μl cDNA template and 8 μl double distilled water (ddH2O). The reaction mixture was denatured at 95°C for 5 min, followed by 40 cycles at 95°C for 20 s, 60°C for 40 s. The limit of detection (LOD) of serum HBV pgRNA was 1466 copies/mL, as calculated by probit analysis (Supplementary Table 2). For statistical analysis, those serum samples with HBV RNA below LOD or not detected were recorded as 1465 copies/mL (3.17 log copies/mL).

Statistical analysis

Statistical analysis was performed using SPSS Statistics version 19.0 software (SPSS Inc., Chicago, IL, USA). Variables were expressed as frequency count, percentage of total, and mean ± standard deviation. The chi-square test or Fisher's exact test was used for comparison of categorical data as appropriate. The Mann-Whitney *U* test was used to compare differences in continuous variables between groups. In addition, linear regression analysis was performed to evaluate the correlations between HBV biomarkers. A positive b coefficient indicated a positive association between HBV biomarkers. A *P* value < 0.05 was considered to be statistically significant.

Results

Demographic and laboratory features of the study subjects

A total of 100 HBV/HCV coinfected patients were enrolled in this study. There were 37 men and 63 women, and the mean patient age was 62.3 years (SD: 10.7 years) (Table 1). A total of 15 patients were HBV-inactive/HCV-inactive, 63 patients were HBV-inactive/HCV-active, 14 patients were HBV-active/HCV-inactive and 8 patients were HBV-active/HCV-active. A total of 71 (71%) patients were active HCV and 22

(22%) were active HBV. All of the included patients were seronegative for HBeAg, except one patient with HBeAg-positive CHB in the BICA group.

Table 1
Clinical and virological characteristics of the patients

	Total (n = 100)	BICI (n = 15)	BICA (n = 63)	BACI (n = 14)	BACA (n = 8)	P value
Age (years)†	62.3 ± 10.7	58.8 ± 12.4	63.6 ± 10.7	57.9 ± 9.1	65.5 ± 6.4	0.144
Male (n, %)	37 (37.0%)	4 (26.7%)	24 (38.1%)	4 (28.6%)	5 (62.5%)	0.337
Cirrhosis (n, %)	27 (27.0%)	0 (0.0%)	21 (33.3%)	2 (14.3%)	4 (50.0%)	0.018
Fatty liver (n, %)	35 (35.0%)	5 (33.3%)	22 (34.9%)	7 (50.0%)	1 (12.5%)	0.364
Alcoholism	14 (14.0%)	1 (6.7%)	9 (14.3%)	1 (7.1%)	3 (37.5%)	0.180
HCV RNA (log IU/mL)†	4.5 ± 2.3	1.0 ± 0.0	5.9 ± 0.9	1.0 ± 0.0	5.8 ± 0.5	< 0.001
Genotype (n, %)						< 0.001
Type 1	45 (45.0%)	0 (0.0%)	38 (60.3%)	0 (0.0%)	7 (87.5%)	
Type 2	22 (22.0%)	0 (0.0%)	21 (33.3%)	0 (0.0%)	1 (12.5%)	
Type 6	4 (4.0%)	0 (0.0%)	4 (6.3%)	0 (0.0%)	0 (0.0%)	
HBV DNA (log IU/mL) †	2.3 ± 1.5	1.9 ± 0.8	1.5 ± 0.7	4.6 ± 0.9	4.8 ± 1.5	< 0.001
HBsAg (Log IU/mL) †	1.0 ± 2.1	1.4 ± 1.9	0.5 ± 2.1	1.9 ± 1.8	2.3 ± 0.7	0.004
HBcrAg (Log IU/mL) †	3.2 ± 0.6	3.2 ± 0.5	3.1 ± 0.5	3.6 ± 0.7	3.5 ± 1.2	0.008

BICI, HBV-inactive/HCV-inactive group; BICA, HBV-inactive/HCV-active group; BACI, HBV-active/HCV-inactive group; BACA, HBV-active/HCV-active group (BACA); HCV, hepatitis C virus; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBcrAg, hepatitis B core-related antigen; pgRNA, pregenomic RNA; FIB-4, fibrosis-4 index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, alpha-fetoprotein; eGFR, estimated glomerular filtration rate.

†Data are expressed as mean ± standard deviation.

	Total (n = 100)	BICI (n = 15)	BICA (n = 63)	BACI (n = 14)	BACA (n = 8)	P value
HBV pgRNA (copies/mL) †	4.1 ± 1.2	3.7 ± 1.2	4.2 ± 1.2	3.9 ± 1.1	4.9 ± 1.6	0.119
FIB-4†	2.5 ± 2.1	1.4 ± 0.5	2.7 ± 2.4	2.1 ± 1.8	3.5 ± 1.7	0.008
Total bilirubin (mg/dL) †	0.7 ± 0.3	0.7 ± 0.3	0.7 ± 0.4	0.9 ± 0.3	0.7 ± 0.3	0.166
ALT (U/L) †	65.2 ± 55.0	35.7 ± 25.2	73.6 ± 55.2	29.8 ± 9.5	106.4 ± 79.5	< 0.001
AST (U/L) †	48.4 ± 40.5	28.3 ± 18.1	52.6 ± 36.6	26.9 ± 9.3	91.2 ± 80.2	< 0.001
Albumin (g/dL) †	4.3 ± 0.3	4.5 ± 0.2	4.3 ± 0.4	4.3 ± 0.2	4.2 ± 0.2	0.123
Prothrombin time (sec) †	10.8 ± 0.7	10.7 ± 0.3	10.7 ± 0.8	11.1 ± 0.6	10.7 ± 0.7	0.172
AFP (U/L) †	28.3 ± 192.8	3.2 ± 2.4	40.7 ± 241.4	3.9 ± 4.4	16.0 ± 24.7	0.035
Platelets (x 10^3/mm^3) †	186.2 ± 58.2	205.5 ± 45.6	183.0 ± 59.99	192.4 ± 58.4	163.9 ± 62.1	0.432
eGFR†	86.6 ± 24.7	82.5 ± 16.3	85.0 ± 25.5	100.7 ± 26.7	86.6 ± 24.7	0.194

BICI, HBV-inactive/HCV-inactive group; BICA, HBV-inactive/HCV-active group; BACI, HBV-active/HCV-inactive group; BACA, HBV-active/HCV-active group (BACA); HCV, hepatitis C virus; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBcrAg, hepatitis B core-related antigen; pgRNA, pregenomic RNA; FIB-4, fibrosis-4 index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, alpha-fetoprotein; eGFR, estimated glomerular filtration rate.

†Data are expressed as mean ± standard deviation.

Based on viral load, patients were placed into one of the following groups: BICI (n = 15; 15.0%), BICA (n = 63; 63.0%), BACI (n = 14; 14.0%), or BACA (n = 8; 8.0%). The demographic, clinical, and virological characteristics of each group are presented in Table 1. HBV DNA, HBsAg, HBcrAg, and HCV RNA showed statistically significant differences among the four groups while HBV pgRNA did not. Liver enzyme (ALT/AST), cirrhosis, FIB-4, and AFP levels in the BICA and BACA groups were higher than those in the BICI and BACI groups. There were no statistically significant differences among the four groups regarding age, sex, fatty liver, alcoholism, albumin, total bilirubin, eGFR, prothrombin time, and platelet count.

Hbv Biomarkers Of The Patients With Hbv/hcv Coinfection

Figure 1 shows the HBV biomarkers (including HBV DNA, HBsAg, HBcrAg, and HBV pgRNA) of each of the four groups. The HBV-active groups (BACA & BACI) had significantly higher level of HBV DNA (Fig. 1A; BACA vs. BICA, $P < 0.001$; BACA vs. BICI, $P < 0.001$; BACI vs. BICA, $P < 0.001$; BACI vs. BICI, $P < 0.001$). Levels of serum HBsAg in the HBV-active groups (BACA & BACI) were significantly higher than that in the BICA group (Fig. 1B; BACA vs. BICA, P = 0.013; BACI vs. BICA, P = 0.007). The serum HBcrAg level in the BACI group was higher than that in the BICA group (Fig. 1C; $P = 0.001$). In addition, the level of HBV pgRNA in the BACA group was significantly higher than that in the BICI group (Fig. 1D; $P = 0.001$); however, there was no significant difference in the HBV pgRNA level among the four groups (Table 1; $P = 0.119$). Interestingly, using HBV DNA divided by HBV pgRNA, the HBV DNA:HBV pgRNA ratio was significantly higher in BACA and BACI than in BICI and BICA (Fig. 1E; BACA vs. BICA, $P < 0.001$; BACA vs. BICI, $P = 0.023$; BACI vs. BICA, $P < 0.001$; BACI vs. BICI, $P < 0.001$).

Correlations between HBV biomarkers of the patients with HBV/HCV coinfection

Linear regression analyses demonstrated positive linear correlations between HBsAg, HBcrAg, and HBV DNA. HBV DNA significantly correlated with HBsAg (Fig. 2A; R = 0.476, R^2 = 0.227, β = 0.463, and $P < 0.001$) and HBcrAg (Fig. 2B; R = 0.474, R^2 = 0.225, β = 0.474, and $P < 0.001$). HBsAg also showed a positive correlation with HBcrAg (Fig. 2C; R = 0.392, R^2 = 0.153, β = 0.392, and $P < 0.001$). However, HBV pgRNA showed no correlations with HBV DNA, HBsAg, and HBcrAg (Fig. 2D-F; HBV DNA, R = 0.017, R^2 = 0.0003, β= − 0.017, and $P = 0.864$; HBsAg, R = 0.111, R^2 = 0.012, β = 0.111, and $P = 0.270$; and HBcrAg, R = 0.086, R^2 = 0.0037, β= − 0.086, and $P = 0.395$, respectively).

HBV biomarkers in HBV/HCV coinfected patients with and without HCV viremia

In HBV/HCV coinfected patients with HCV viremia, HBV DNA, HBsAg, and HBcrAg were significantly reduced than in those without viremia (Fig. 3A-C; HBV DNA, $P < 0.001$; HBsAg, $P = 0.015$; and HBcrAg, $P = 0.006$, respectively). Although the difference was not statistically significant, patients with viremia exhibited higher HBV pgRNA levels than those without viremia (Fig. 3D; $P = 0.073$). Interestingly, patients with viremia had a lower HBV DNA:HBV pgRNA ratio than those without viremia, indicating lower reverse transcription efficiency of pgRNA in patients with viremia (Fig. 3E; $P < 0.001$). The amount of HCV RNA had a negative correlation between HCV RNA and HBV DNA/ pgRNA ratio (Fig. 3F; R = 0.416, R^2 = 0.173, β = -0.416, and $P < 0.001$).

Discussion

The present study demonstrated that HBV DNA, HBsAg, and HBcrAg show linear positive correlations with each other in HBV/HCV coinfected patients. However, HBV pgRNA levels were not associated with HBV DNA, HBsAg, or HBcrAg. The presence of HCV viremia appeared to suppress HBV DNA, HBsAg, HBcrAg, and indicated an increasing level of HBV pgRNA. The lower HBV DNA:HBV pgRNA ratio in patients with HCV viremia indicated ineffective reverse transcription of HBV pgRNA.

In line with previous epidemiology reports, the current study determined that active HCV infection plays a dominant role in patients with HBV/HCV coinfection (Table 1).[2, 25] Active HCV status in patients with HBV/ HCV coinfection correlated with higher extent of liver injury (ALT/AST), higher fibrosis scores (FIB-4), and enhanced cirrhosis (Table 1). However, the association between high HBV DNA (active HBV) and patient characteristics (such as liver injury or cirrhosis) was not significant. This observation suggests that identification of non-invasive biomarkers, other than HBV DNA, is necessary to reflect the transcriptional activity of intrahepatic viruses and immune response.

Several reports have demonstrated that serum HBV DNA correlates with serum HBsAg, HBcrAg, and HBV pgRNA during the natural course of hepatitis B viral infection.[26–29] One recent meta-analysis including 5,591 patients without antiviral therapy found a close correlation between HBcrAg and HBV DNA.[10] The optimal cut-offs (log U/mL) were 3.6 and 4.5 to diagnose HBV DNA levels (IU/mL) of ≥ 2,000 and ≥ 20,000, respectively.[10] The levels of serum HBV pgRNA strongly correlate with serum HBV DNA in treatment-naïve patients, but the correlation becomes weaker upon receiving NAs.[30] As for presentation in patients with HBV monoinfection, our study showed that HBsAg and HBcrAg levels changed in parallel with the HBV DNA level in the HBV/HCV coinfected patients (Fig. 2). Positive linear correlations between HBsAg, HBcrAg, and HBV DNA were identified (Fig. 2). In contrast, lack of an association between serum HBV pgRNA and other HBV biomarkers was noted in our study (Fig. 2D-F). Theoretically, both serum HBV DNA and HBV pgRNA can be useful markers for assessing HBV cccDNA activity in treatment-naïve patients. However, HBV DNA is suppressed due to halted HBV pgRNA reverse transcription by HCV viremia [31, 32], making HBV pgRNA a more direct marker for cccDNA. Thus, serum HBV pgRNA may be a surrogate marker for intrahepatic HBV cccDNA in patients with HBV/HCV coinfection compared to serum HBV DNA, HBsAg, and HBcrAg. However, further studies are warranted to clarify this observation.

The persistence of serum HBV pgRNA levels raises interest in the role of HCV viremia in HBV/HCV coinfected patients. In general, HCV is the dominant virus that actively replicates and suppresses the replication of HBV.[5, 33] The mechanism of this interaction is not well-understood, although several hypotheses regarding direct or indirect (mediated by various host immune responses) viral interference have been proposed to explain the dominant role of HCV. A number of studies have suggested that HBV suppression is mediated by the HCV core protein [31, 32] In coinfection, the HCV core protein was found to form a complex with HBV polymerase and impede its function.[31, 32] Another hypothesis states that HCV infection activates interferon production within the hepatocytes, thereby suppressing HBV further due to its antiviral effects.[3, 5] Our study revealed that HCV viremia is associated with low HBV DNA, HBsAg, and HBcrAg. These results suggest that HCV may suppress HBV polymerase and impair mRNA translation, which reduces the production of HBV DNA, HBsAg, and HBcrAg in patients with HBV/HCV coinfection.

In contrast with the suppression of HBV DNA, HBsAg, and HBcrAg, our study also discovered a trend of higher HBV pgRNA associated with HCV viremia (including in the BACA and BICA groups) (Fig. 3D; $P =$ 0.073). In HBV replication cycle, circular DNA genome of mature HBV from the linear pgRNA template takes place inside the core. [34] After HBV polymerase protein binding to the epsilon (ε) loop at the 5' end

of pgRNA, assembly of HBV begins with packaging of pgRNA into immature nucleocapsids (NC), which are converted to mature NCs containing the genomic relaxed circular (RC) DNA as a result of reverse transcription. [35] However, a number of studies have investigated that HCV core protein could complex with HBV polymerase and impede its function. [5], [31], [36] Therefore, HBV pgRNA virion levels increased in BACA and BICA groups might result from blocking the reverse transcription activity of HBV DNA polymerase while HCV core protein capture HBV polymerase. In general, the levels of serum HBV pgRNA strongly correlate with serum HBV DNA in treatment-naïve patients.[30] The lower level of HBV pgRNA is reasonable in the BICI and BICA groups due to the low HBV replication activity.[37] However, HCV viremia increased the level of HBV pgRNA in the BICA group and caused the BICA group to have comparable HBV pgRNA levels to the BACA and BACI groups (Fig. 1D). Combined with the effects of HBV viremia and HCV viremia, the BACA group with both viremia effects had higher HBV pgRNA levels than the BICI group with both negative viremia effects (Fig. 1D; $P <$ 0.001). Further studies are necessary to address the correlation between HCV viremia and HBV pgRNA.

Combined serum HBV DNA and HBV pgRNA levels are better indicators of cccDNA activity and the HBV DNA:HBV pgRNA ratio reflects the HBV polymerase protein reverse transcription activity.[38] The current study demonstrated that HCV viremia leads to a lower HBV DNA:HBV pgRNA ratio that is compatible with the suppression of HBV polymerase by HCV.[31, 32] Several studies have reported that NAs or pegylated interferon alpha therapy can effectively block reverse transcription and suppress HBV viral replication, causing an inversion of the HBV DNA:HBV RNA ratio.[39, 40] As the presentation of anti-HBV therapy, the current study demonstrated that patients with HCV viremia present a lower HBV DNA level and show a higher serum HBV pgRNA level than those without viremia. Lower HBV DNA:HBV pgRNA ratio further indicated lower reverse transcription efficiency of pgRNA in patients with HCV viremia.

There were several limitations in this study. First, the case number of some groups was small due to the nature of virological profiles. Most patients were HCV-dominant. However, this study remains the largest cohort study on this subject. This report adds new information about the characteristics of HBV biomarkers in patients with HBV/HCV coinfection. Second, our results do not provide information regarding the molecular mechanism of the increase in HBV pgRNA levels in patients with HCV viremia. Further studies are needed to clarify these aspects. Third, we compared the dynamic change of most HBV biomarkers, including HBsAg, HBV DNA, HBcrAg and HBV pgRNA in the current study. However, the analysis of other circulating HBV biomarkers such as microRNA or HBV nucleic acid-related antigen were not done. Further study maybe required.

Despite these limitations, this study has several strengths. Although there are many studies about the HBV biomarkers in HBV patients, this is the first study with the comprehensive investigation of various HBV biomarkers in HBV/HCV coinfected patients. Second, it is interesting to note that HBV pgRNA levels increase, especially when HCV proliferation is active. This is not mentioned in previous reports. Third, the relatively high HBV pgRNA level, low HBV DNA and low HBV DNA:HBV pgRNA ratio suggested that HCV viremia may be responsible for the decrease of HBV pgRNA RT activity. The results suggested that those

HBV biomarkers have the potential as the predictive biomarkers of HBV reactivation following HCV direct-acting antiviral treatment in patients with HBV/HCV coinfection.

Conclusions

The current study revealed that HBV DNA, HBsAg, and HBcrAg significantly correlated with each other in patients coinfected with HBV and HCV. In addition, HBV and HCV coinfected patients with HCV viremia have lower HBV DNA, HBsAg, HBcrAg, and HBV DNA:pgRNA ratio as well as higher HBV pgRNA than those without viremia. The possible involvement of HCV RNA in HBV mRNA translation and pgRNA reverse transcription activity needs further investigation.

Abbreviations

hepatitis B virus (HBV); hepatitis C virus (HCV); hepatocellular carcinoma (HCC); covalently closed circular DNA (cccDNA); hepatitis B surface antigen (HBsAg); hepatitis B core-related antigen (HBcrAg); HBV pregenomic RNA (HBV pgRNA); nucleos(t)ide analogues (NA); anti-hepatitis C antibody (anti-HCV); hepatitis B e antigen (HBeAg); aspartate aminotransferase (AST); alanine aminotransferase (ALT); estimated glomerular filtration rate (eGFR); alpha-fetoprotein (AFP); fibrosis-4 (FIB-4); nucleocapsids (NC)

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Dalin Tzu Chi Hospital (approval number B10901004). Its design and conduct conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Before commencement of the study, all patients signed informed consent forms.

Consent for publication

Not applicable.

Availability of data and material

The datasets used in the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

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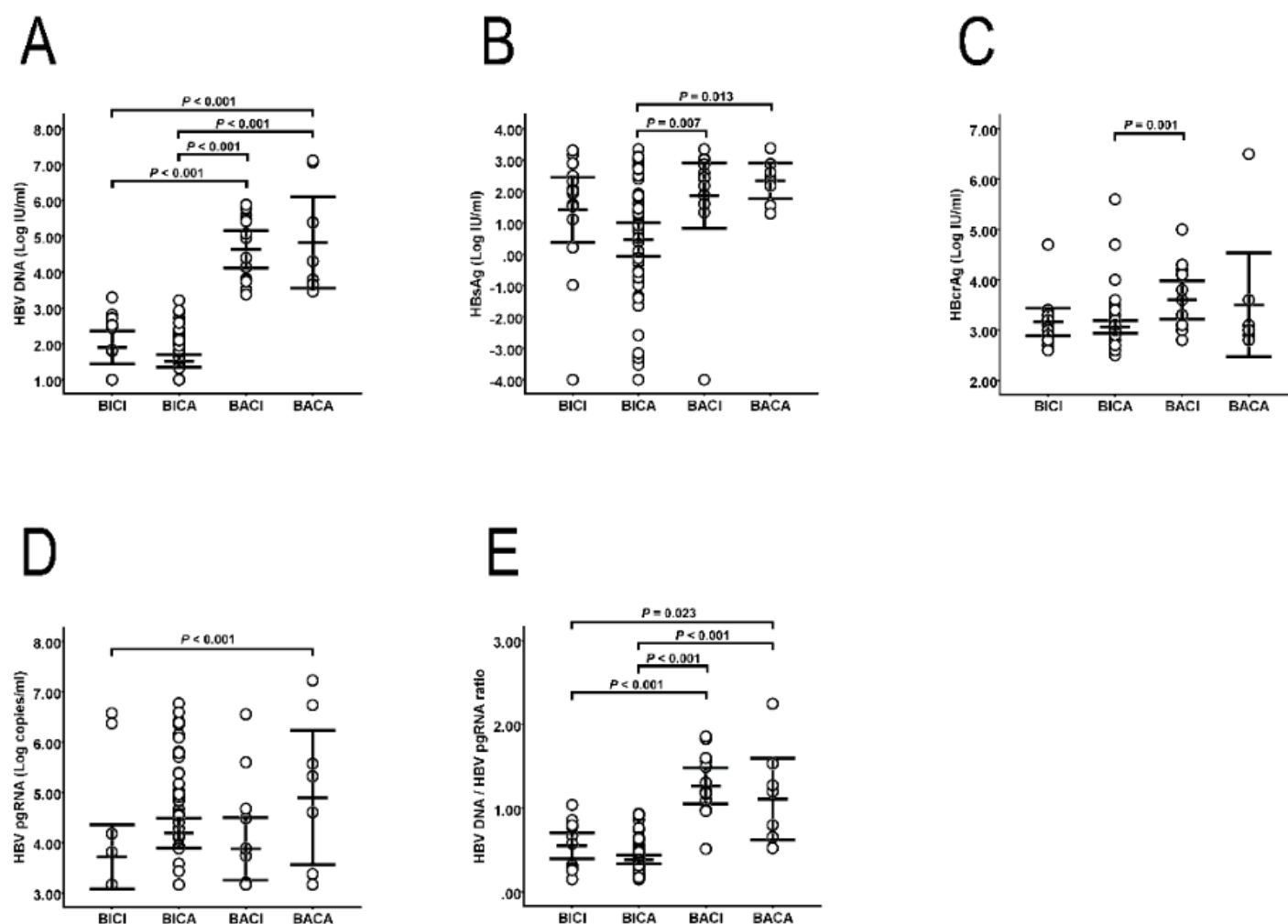
Figures

Figure 1

Expression of HBV DNA, HBsAg, HBcrAg, and HBV pgRNA in in patients coinfected with HBV/HCV (n = 100). A. HBV DNA was significantly higher in the HBV-active groups (Fig. 1A; BACA vs. BICA, $P < 0.001$; BACA vs. BICI, $P < 0.001$; BACI vs. BICA, $P < 0.001$; and BACI vs. BICI, $P < 0.001$). B. Serum HBsAg in the HBV-active groups was significantly higher than that in the BICA group (Fig. 1B; BACA vs. BICA, $P = 0.013$; and BACI vs. BICA, $P = 0.007$). C. Serum HBcrAg in the BACI group was higher than that in BICA group (Fig. 1C; $P = 0.001$). D. HBV pgRNA in the BACA group was significantly higher than that in the BICI group (Fig. 1D; $P < 0.001$). E. The HBV DNA:HBV pgRNA ratio was significantly higher in the BACA and BACI groups than in the BICI and BICA groups (Fig. 1E; BACA vs. BICA, $P < 0.001$; BACA vs. BICI, $P = 0.023$; BACI vs. BICA, $P < 0.001$; and BACI vs. BICI, $P < 0.001$).

HBV, hepatitis B virus; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; HBcrAg, hepatitis B core-related antigen; pgRNA, pregenomic RNA
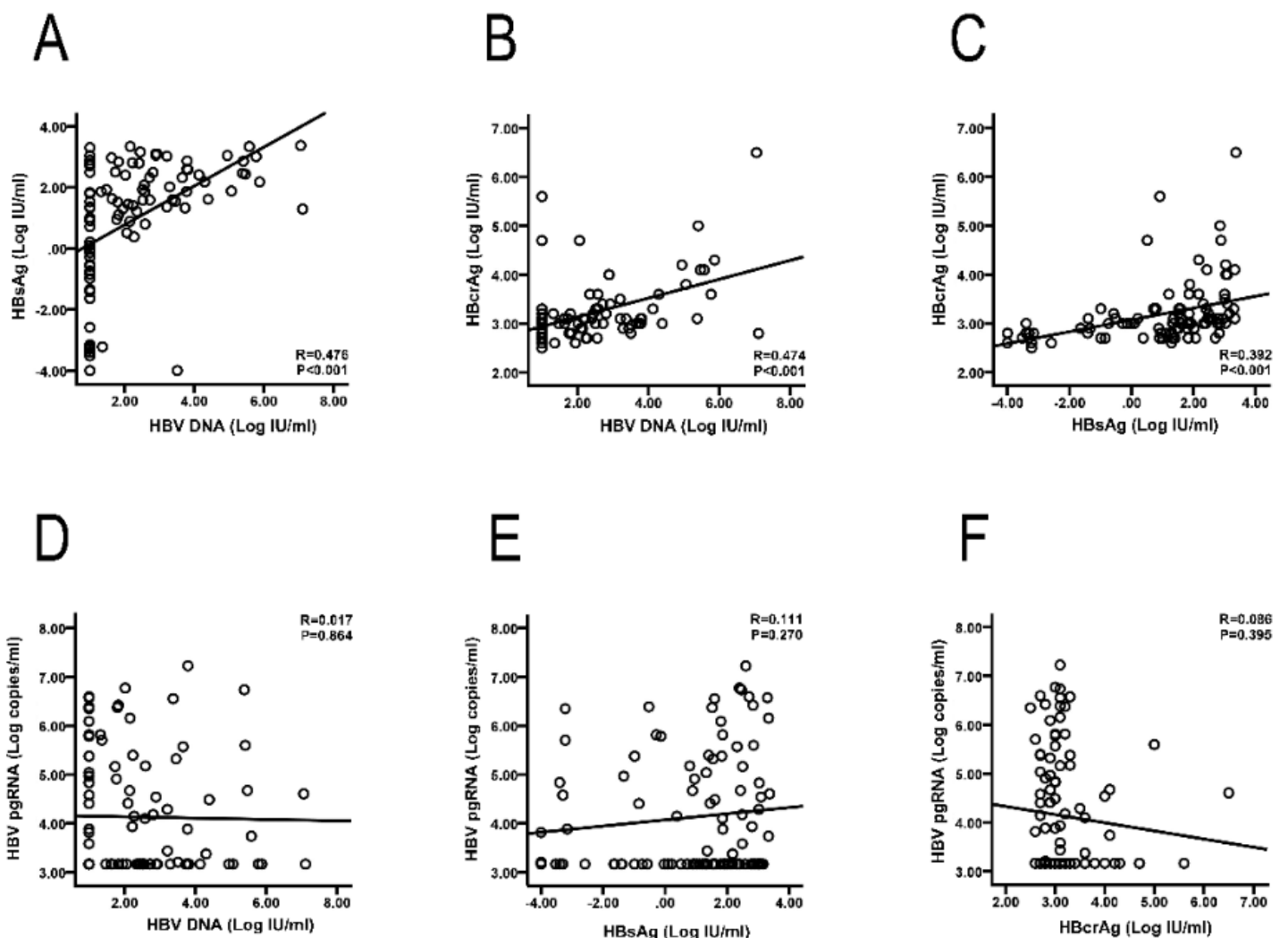

Figure 2

Linear regression analyses for the correlations between HBV biomarkers in patients coinfected with HBV/HCV. A. HBV DNA was significantly correlated with HBsAg (Fig. 2A; R = 0.476, R^2 = 0.227, b = 0.463, and $P < 0.001$) B. HBV DNA was significantly correlated with HBcrAg (Fig. 2B; R = 0.474, R^2 = 0.225, b = 0.474, and $P < 0.001$). C. HBsAg also showed a positive correlation with HBcrAg (Fig. 2C; R = 0.392, R^2 = 0.153, b = 0.392, and $P < 0.001$). D-F. HBV pgRNA had no correlations with HBV DNA, HBsAg, or HBcrAg (Fig. 2D-F; HBV DNA, R = 0.017, R^2 = 0.0003, b= - 0.017, and $P = 0.864$; HBsAg, R = 0.111, R^2 = 0.012, b= 0.111, and $P = 0.270$; and HBcrAg, R = 0.086, R^2 = 0.0037, b = - 0.086, and $P = 0.395$, respectively).

HBV, hepatitis B virus; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; HBcrAg, hepatitis B core-related antigen; pgRNA, pregenomic RNA
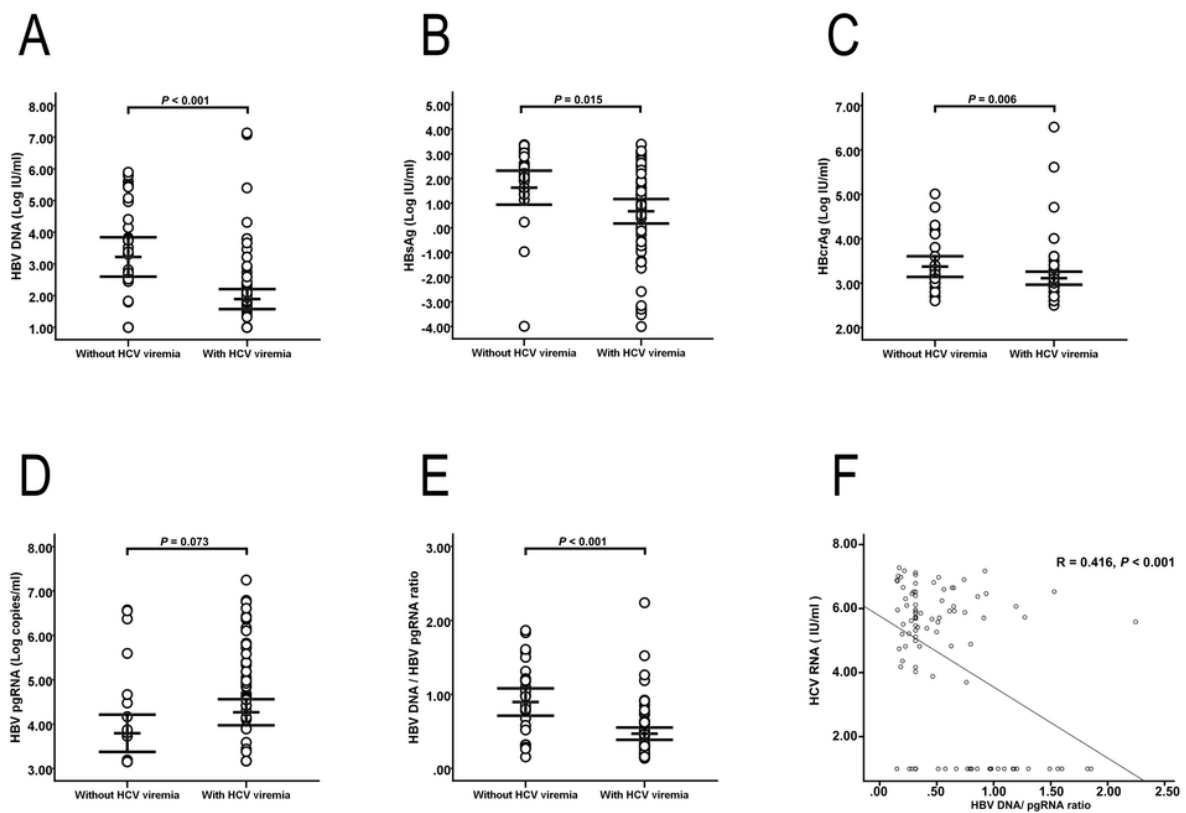

Figure 3

HBV biomarker expression levels in chronic HBV/HCV coinfected patients with (n = 71) and without (n = 29) HCV viremia. A-C. The HBV DNA, HBsAg and HBcrAg levels of patients with HCV viremia were significantly lower than those of patients without viremia (Fig. 3A-C; HBV DNA, $P < 0.001$; HBsAg, $P = 0.015$; and HBcrAg, $P = 0.006$, respectively). D. The HBV pgRNA level of patients with viremia trended higher than that of patients without viremia (Fig. 3D; $P = 0.073$). E. Patients with viremia had a lower HBV

DNA:HBV pgRNA ratio (Fig. 3E; *P* < 0.001). F. The amount of HCV RNA had a negative correlation between HCV RNA and HBV DNA/ pgRNA ratio (Fig. 2A; R = 0.416, R^2 = 0.173, b = -0.416, and *P* < 0.001).

HBV, hepatitis B virus; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; HBcrAg, hepatitis B core-related antigen; pgRNA, pregenomic RNA

Supplementary Files

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