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Original Article

Correlations between serum hepatitis B surface antigen and hepatitis B core antibody titers and liver fibrosis in treatment-naïve CHB patients

Min-Ran Li [a], Huan-Wei Zheng [a], Shun-Mao Ma [b], Yun-Yan Liu [a], Lan-Xia Qie [a], Jun-Qing Li [a], De-Hua Wang [a], Xing-Li Sun [a], Gui-Fang Ren [a], Yan-Hua Zheng [a], Yu-Ling Wang [a], Er-Hei Dai [a,*]

[a] *Division of Liver Disease, The Fifth Hospital of Shijiazhuang, Hebei Medical University, Shijiazhuang, Hebei, China*
[b] *Department of General Surgery, North China Petroleum Bureau General Hospital, Renqiu, Hebei, China*

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Abstract

Background: Previous studies have revealed that quantitative hepatitis B surface antigen (HBsAg) or hepatitis B core antibody (qAnti-HBc) levels can be used as predictors of treatment response in both interferon-α and nucleoside analogue therapies. Few data have been published regarding the relationship between quantitative HBsAg or Anti-HBc levels and liver fibrosis stages in patients with chronic hepatitis B (CHB).
Methods: We conducted a cross-sectional study of treatment-naïve CHB patients. A total of 624 CHB patients were recruited. We assessed the serum HBsAg and qAnti-HBc levels, HBV DNA levels, HBV genotypes, BCP/PC mutations, histological fibrosis staging by Scheuer classification.
Results: In HBeAg (+) patients, the S0-1 subjects had significantly higher serum HBsAg and lower qAnti-HBc levels than the S2-4 subjects (both $p < 0.001$). A moderate inverse correlation was present between serum HBsAg levels and fibrosis scores ($r = -0.381$, $p < 0.001$), and a moderate positive correlation was found between qAnti-HBc levels and fibrosis scores ($r = 0.408$, $p < 0.001$). In the HBeAg (−) patients, the S0-1 subjects also had significantly lower qAnti-HBc levels than the S2-4 subjects ($p < 0.001$); however, no significant difference in the HBsAg levels was observed between the S0-1 and S2-4 subjects ($p > 0.05$). Serum qAnti-HBc levels showed a moderate positive correlation with fibrosis scores ($r = 0.383$, $p < 0.001$), while serum HBsAg levels exhibited a low inverse correlation with fibrosis scores ($r = -0.171$, $p < 0.001$). Multiple logistic regression analysis showed that the parameters for predicting significant fibrosis ($S \geq 2$) included age, PLT, qAnti-HBc levels, HBV genotype and BCP/PC mutations in HBeAg (+) group, and age, PLT, qAnti-HBc levels in HBeAg (−) group (all $p < 0.05$). The AUC of qAnti-HBc levels associated with the diagnosis of significant fibrosis abnormalities in HBeAg (+) and HBeAg (−) patients were 0.734 (95%CI 0.689 to 0.778) and 0.707 (95%CI 0.612 to 0.801), respectively.
Conclusion: Our study found an association between high serum qAnti-HBc levels and significant fibrosis in both HBeAg (+) and HBeAg (−) treatment-naïve CHB patients. However, low serum HBsAg levels were correlated with moderate to severe fibrosis in HBeAg (+) subjects only.

Keywords: Chronic hepatitis B; Hepatitis B surface antigen; Liver fibrosis; Quantitative anti-HBc

1. Introduction

Chronic hepatitis B (CHB) is a major global public health care problem.[1] Hepatitis B virus (HBV) is considered a major risk factor for disease progression to liver cirrhosis and hepatocellular carcinoma (HCC).[2] Previous studies confirmed that effective antiviral therapy may stop the progression of liver injury and can lead to the reversal of fibrosis.[3,4] At present, antiviral treatments for CHB patients include interferon (IFN) and oral nucleos(t)ide analogues (NAs). However, the rate of response to IFN was frequently low and the side effects were significant. NAs inhibit HBV replication and major of patients would require long-term treatment. While long-term NAs treatment increased the prevalence of drug-resistant HBV mutants and leaded to treatment failure. So, application of antiviral treatment drugs should be accordance with indications, which be important to avoid

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Dr. Er-Hei Dai, Division of Liver Disease, The Fifth Hospital of Shijiazhuang, Hebei Medical University, 42, Tanan Road, Yuhua District, Shijiazhuang, 050017, China.
E-mail address: daieh2008@126.com (E.-H. Dai).

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improper drug use and salvage treatment. Treatment of patients with CHB mostly relies on the stage of the liver inflammation/fibrosis. Antiviral treatment is recommended at least moderate inflammation/fibrosis.[5] Assessment of fibrosis stage is thus an important parameter in deciding treatment.

Currently, liver biopsy (LB) is still viewed as the gold standard for staging fibrosis. However, it is an invasive procedure with risk for potentially complications, difficult to repeat.[6] Hence, during the past decade, non-invasive modalities including transient elastography (FibroScan) and serum fibrosis markers have been developed quickly to reduce the need for liver biopsy. However, the diagnostic accuracy of FibroScan was high for cirrhosis, but poor for significant fibrosis.[7] Serum markers offer another attractive alternative to assess liver fibrosis stage, which are less invasive and could be performed repeatedly. These markers were classified as direct and indirect types. Direct markers represent extracellular matrix components, which include glycoproteins, collagens, collagenases and collagenase inhibitors. Indirect markers reflect the consequences of the liver damage, which include the platelet (PLT) count, the alanine transaminase (ALT) and aspartate transaminase (AST) levels, total bilirubin (TB), globina and so on. Direct and indirect markers may be used alone or in combination to produced composite scores which can be calculated based on formulas.

The quantification of hepatitis B surface antigen (HBsAg) serum levels as a marker for response to antiviral therapy is progressively being used in CHB patients.[8] Previous studies reported that serum HBsAg levels were inverse correlated with liver fibrosis stage in HBeAg− positive patients.[9,10] These findings highlight the prognostic value of quantitative HBV biomarkers levels in CHB patients. As another classical serologic HBV marker, recent evidence also shown that serum levels of quantitative hepatitis B core antibody (qAnti-HBc) to be useful in predicting favorable response in CHB patients with interferon-α or NAs therapy.[11] However, there are few studies to investigate the association between qAnti-HBc levels and liver fibrosis. Therefore, the aims of this study were to evaluate the use of quantitative HBV biomarkers (HBsAg and qAnti-HBc) levels in assessing liver fibrosis of treatment-naïve CHB patients.

2. Methods

2.1. Ethics statement

The present study was approved by the Medical Ethics Committee of The Fifth Hospital of Shijiazhuang. Written informed consent was obtained from all patients prior to liver biopsy and study entry with all clinical investigation conducted in compliance with the 1975 Declaration of Helsinki.

2.2. Patients

The present study included treatment-naïve CHB patients who were enrolled at the Division of Liver Disease, The Fifth Hospital of Shijiazhuang, Hebei Medical University between January 2012 and December 2015. All patients were HBsAg-positive for at least 6 months before study entry. Other inclusion criteria were as follows: treatment naïve and availability of relevant patient laboratory and clinical data. Patients with concomitant liver diseases, including hepatitis A, C, E or human immunodeficiency virus (HIV) co-infection, drug hepatitis, alcohol-related liver disease, Wilson disease, autoimmune hepatitis and decompensated liver cirrhosis or HCC were excluded.

2.3. Liver biopsy

Ultrasonographic-guided liver biopsy was performed according to a standardized protocol. Percutaneous liver biopsy was performed using a 16-gauge needle (Bard, Germany, 1620). Liver histology was assessed by a single experienced pathologist blinded to all other data. Liver biopsy specimens were formalin-fixed, paraffin-embedded, and prepared by hematoxylin-eosin staining for morphological evaluation, Masson's trichrome staining, and reticulin staining for pathological assessment. Specimens were staged for inflammatory/fibrosis according to the Scheuer classification.[12] "Insignificant fibrosis" was defined as a Scheuer fibrosis score equal to or less than 1. "Significant fibrosis" was defined as a Scheuer score more than or equal to 2. The severity of hepatic steatosis was evaluated with grades from 0 to 3 corresponding to the percentage of fatty change in <5%, 5%−30%, 30%−60%, and ≥60% of the liver parenchyma.

2.4. Laboratory assays

Blood samples used for measurements were obtained on the day of the biopsy. The complete blood cell counts were measured on XN-1000 (Sysmex, Kobe, Japan), and clinical biochemical tests were performed using a 7600-020 clinical analyzer (Hitachi High-Technologies, Tokyo, Japan). The upper limit of normal alanine transaminase (ALT) was 40 U/L. Markers of hepatitis virus including Hepatitis B e antigen (HBeAg) and antibodies to HBeAg (anti-HBe) were measured using commercially available immunoassays (Roche Diagnostics, Branchburg, NJ, USA). The serum HBV DNA levels were detected with real-time polymerase chain reaction system (Applied Biosystems 7500, ABI, Foster City, CA, USA). Serum HBV DNA concentrations of 500 IU/mL or more were referred to as HBV DNA positive. Serum HBsAg titers were quantified using the Elecsys HBsAg II quant assay (Roche Diagnostics, Branchburg, NJ, USA), with a diagnostic range from 0.05 to 130 IU/mL. Samples with HBsAg levels higher than 130 IU/mL were retested at serial dilutions of 1:10. The serum qAnti-HBc levels was measured using double-sandwich immunoassay (Wantai, Beijing, China) that was calibrated using the WHO standard (NIBSC, UK).[13]

2.5. HBV genotyping and determination of BCP/PC mutants

HBV DNA was extracted from patients' serum using QIAamp DNA Blood Mini Kit (QIAGEN USA) and subjected to a nested PCR. HBV genotypes were performed by PCR as previously described.[14] Amplification of BCP/PC region of the HBV genome was carried out by PCR with HBV-specific

primers, and then sequence data for mutations at nucleotides 1762 and 1764 of the BCP region and nucleotides 1858, 1896 and 1899 of the PC region were analysed. Vector NTI Suite software package (Informax, Frederick, MD) was used to analyse and assemble the sequencing data.

2.6. Statistical analysis

Categorical variables are expressed as counts and percentages, as appropriate. Continuous variables are presented as median (range) or mean ± (SD). Statistical analyses were performed using the chi-squared or Fisher's exact tests for categorical variable, while Student's t test or the Mann−Whitney U-test was used for statistical comparisons. Correlations of HBsAg and qAnti-HBc with liver fibrosis stages were assessed using Spearman's method. Multinomial (binary) logistic regression was applied to evaluate parameters predicting significant fibrosis based on histology. Receiver operating characteristic (ROC) curves and areas under the ROC curves (AUC) were calculated to evaluate the diagnostic accuracy of parameters for liver fibrosis activity. Statistical analyses were performed using SPSS ver. 17.0 software (SPSS, Chicago, IL, USA). p values of <0.05 were considered statistically significant. All p values were two-sided.

3. Results

3.1. Patient characteristics

Four hundred and eighty-nine HBeAg-positive (HBeAg (+)) and one hundred thirty-five HBeAg-negative (HBeAg (−)) patients were included in the present study. The baseline characteristics at the time of liver biopsy were summarized in Table 1. The mean age of the 624 patients (428 males, 196 females) was 32.79 ± 11.68 years. The HBeAg (+) patients were younger than the HBeAg (−) patients ($P < 0.001$). The HBeAg (+) individuals had significantly higher HBV DNA (7.16 ± 1.18 $\log_{10}$IU/mL) and HBsAg (4.00 ± 0.70 $\log_{10}$IU/mL) levels than those with HBeAg (−) (4.99 ± 1.40 $\log_{10}$IU/mL and 3.46 ± 0.57 $\log_{10}$IU/mL, respectively, $p < 0.001$). However, the HBeAg (+) group presented a significantly lower average qAnti-HBc level (4.14 ± 1.06 $\log_{10}$IU/mL) than HBeAg (−) group

Table 1
Patient characteristics.

	All (n = 624)	HBeAg (+) (n = 489)	HBeAg (−) (n = 135)	p[a]
Gender, M/F	428/196	330/159	98/37	0.30
Age, years	32.79 ± 11.68	30.94 ± 10.59	39.51 ± 12.95	<0.001
PLT, 10^9/L	187.49 ± 60.94	194.95 ± 58.34	160.79 ± 62.75	<0.001
ALT, U/L	75 (40−152)	72 (38−144)	99 (47−169)	0.15
AST, U/L	43 (26−85)	40 (25−80)	58 (34−97)	0.32
TBIL, μmol/L	19.80 ± 16.22	19.06 ± 15.12	22.51 ± 19.55	0.03
HBV DNA, $\log_{10}$IU/mL	6.69 ± 1.52	7.16 ± 1.18	4.99 ± 1.40	<0.001
HBsAg, $\log_{10}$IU/mL	3.88 ± 0.71	4.00 ± 0.70	3.46 ± 0.57	<0.001
qAnti-HBc, $\log_{10}$IU/mL	4.23 ± 0.99	4.14 ± 1.06	4.52 ± 0.61	<0.001
HBV genotype, (%)[b]				0.23
B	53 (8.95)	45 (9.51)	8 (6.72)	
C	535 (90.37)	425 (89.85)	110 (92.44)	
D	3 (0.51)	3 (0.63)	0 (0.00)	
B/C	1 (0.17)	0 (0.00)	1 (0.84)	
Wild type/BCP/PC mutation type[c]	271/277	245/187	26/90	<0.001
Hepatocyte steatosis, (%)				0.454
No	541 (86.70)	424 (86.71)	117 (86.67)	
Mild	66 (10.58)	49 (10.02)	17 (12.59)	
Moderate	11 (1.76)	10 (2.04)	1 (0.74)	
Severe	6 (0.96)	6 (1.23)	0 (0.00)	
Liver inflammation, (%)				<0.001
G0	21 (3.37)	20 (4.09)	1 (0.74)	
G1	214 (34.29)	185 (37.83)	29 (21.48)	
G2	269 (43.11)	201 (41.10)	68 (50.37)	
G3	116 (18.59)	82 (16.77)	34 (25.19)	
G4	4 (0.64)	1 (0.20)	3 (2.22)	
Liver fibrosis, (%)				<0.001
S0	7 (1.12)	7 (1.43)	0 (0.00)	
S1	328 (52.56)	287 (58.69)	41 (30.37)	
S2	158 (25.32)	111 (22.70)	47 (34.81)	
S3	84 (13.46)	59 (12.07)	25 (18.52)	
S4	47 (7.53)	25 (5.11)	22 (16.30)	

[a] HBeAg (+) vs. HBeAg (−).
[b] 32 patients could not be genotyped with our assay.
[c] 76 patients were not successful sequencing of the BCP/PC region of the HBV genome.

(4.52 ± 0.61 $\log_{10}$IU/mL). No significant differences were found for gender, ALT and AST levels between the two cohorts (all $p > 0.05$). There were 53 (8.95%) patients infected with genotype B HBV and 535 (90.37%) patients infected with genotype C HBV, and the HBV genotype composition difference was not statistically significant ($p > 0.05$). There were more patients with BCP/PC mutations in HBeAg (−) group than HBeAg (+) group ($p < 0.001$).

3.2. Liver histology

Histological analysis demonstrated that 65 (13.29%) patients showed hepatic steatosis in HBeAg (+) group, which was similar to HBeAg (−) group (13.33%) ($P = 1.00$). The inflammation grades were G0, G1, G2, G3 and G4 in 20 (4.09%), 185 (37.83%), 201 (41.10%), 82 (16.77%), 1 (0.20%) of HBeAg (+) subjects and 1 (0.74%), 29 (21.48%), 68 (50.37%), 34 (25.19%), 3 (2.22%) of HBeAg (−) subjects, respectively (Table 1). There were more patients with significant inflammation (G > 1) in HBeAg (−) group (77.78%) than HBeAg (+) group (58.08%). Seven patients (1.12%) were classified as S0, three hundred twenty-eight (52.56%) as S1, one hundred fifty-eight (25.32%) as S2, eighty-four (13.46%) as S3, and forty-seven (7.53%) as S4, including cirrhosis (Table 1). All of patients with fibrosis stage 0 were HBeAg positive. Among the HBeAg (+) patients, 294 (60.12%) patients had insignificant fibrosis (<S2), which was significantly higher than the proportion in the HBeAg (−) group (30.37%, $P < 0.001$).

3.3. Association between histological fibrosis stage and HBsAg or qAnti-HBc levels

Among the HBeAg (+) CHB patients, the mean levels of HBsAg for different stages of fibrosis were as follows: S0-1 (4.16 ± 0.70 $\log_{10}$IU/mL), S2 (3.85 ± 0.64 $\log_{10}$IU/mL), S3 (3.75 ± 0.62 $\log_{10}$IU/mL), and S4 (3.44 ± 0.58 $\log_{10}$IU/mL); the mean levels of qAnti-HBc for different stages of fibrosis were as follows: S0-1 (3.84 ± 1.14 $\log_{10}$IU/mL), S2 (4.54 ± 0.73 $\log_{10}$IU/mL), S3 (4.70 ± 0.68 $\log_{10}$IU/mL) and S4 (4.63 ± 0.74 $\log_{10}$IU/mL). The mean HBsAg level in the S0-1 subjects was significantly higher than that in the S2, S3, and S4 subjects ($p < 0.001$); however, the mean qAnti-HBc level in the S0-1 subjects was significantly lower than that in the S2, S3 and S4 subjects ($p < 0.001$) (Fig. 1A,B). Among the HBeAg (−) patients, the mean levels of HBsAg for different stages of fibrosis were as follows: S1 (3.54 ± 0.67 $\log_{10}$IU/mL), S2 (3.47 ± 0.60 $\log_{10}$IU/mL), S3 (3.42 ± 0.49 $\log_{10}$IU/mL), and S4 (3.33 ± 0.41 $\log_{10}$IU/mL); the mean levels of qAnti-HBc for the different stages of fibrosis were as follows: S1 (4.19 ± 0.64 $\log_{10}$IU/mL), S2 (4.55 ± 0.53 $\log_{10}$IU/mL), S3 (4.76 ± 0.51 $\log_{10}$IU/mL), and S4 (4.83 ± 0.50 $\log_{10}$IU/mL). The mean HBsAg levels in subjects with different stages of fibrosis were similar ($p > 0.05$); however, the mean qAnti-HBc level in the S1 subjects was significantly lower than those in the S2, S3, and S4 subjects ($p < 0.05$) (Fig. 1C,D).

The mean serum HBsAg and qAnti-HBc levels showed significant differences in patients with insignificant fibrosis (4.16 ± 0.70 $\log_{10}$IU/mL and 3.84 ± 1.14 $\log_{10}$IU/mL) compared to patients with significant fibrosis (3.77 ± 0.63 $\log_{10}$IU/mL and 4.60 ± 0.72 $\log_{10}$IU/mL) in the HBeAg (+) group (all $p < 0.001$). The mean qAnti-HBc levels were also

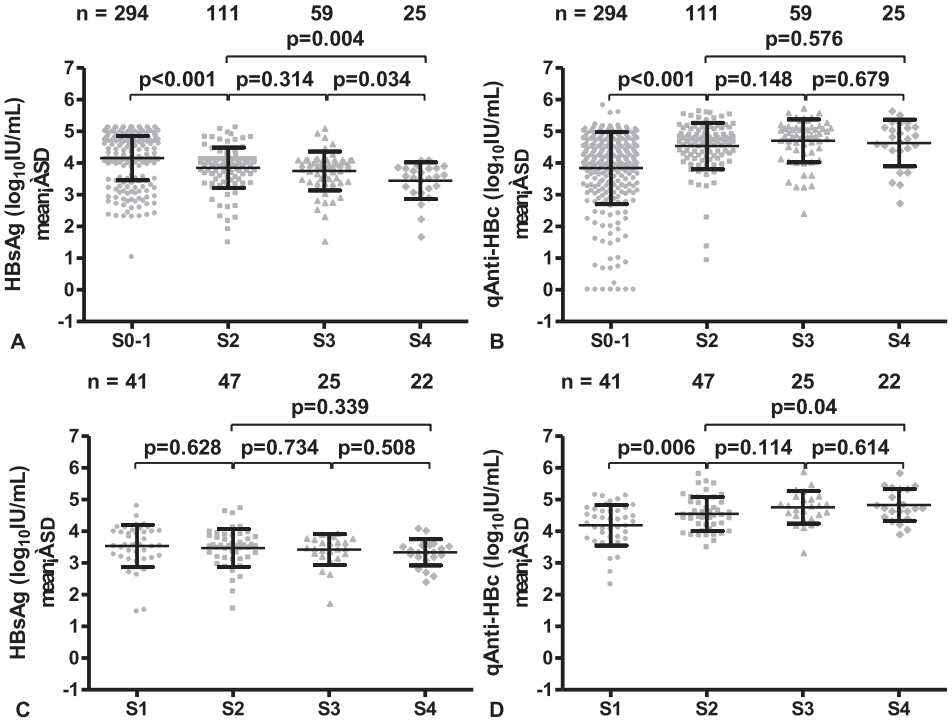

Fig. 1. Correlation between serum HBsAg or qAnti-HBc levels and liver fibrosis stages in HBeAg (+) (A/B) and HBeAg (−) CHB patients (C/D).

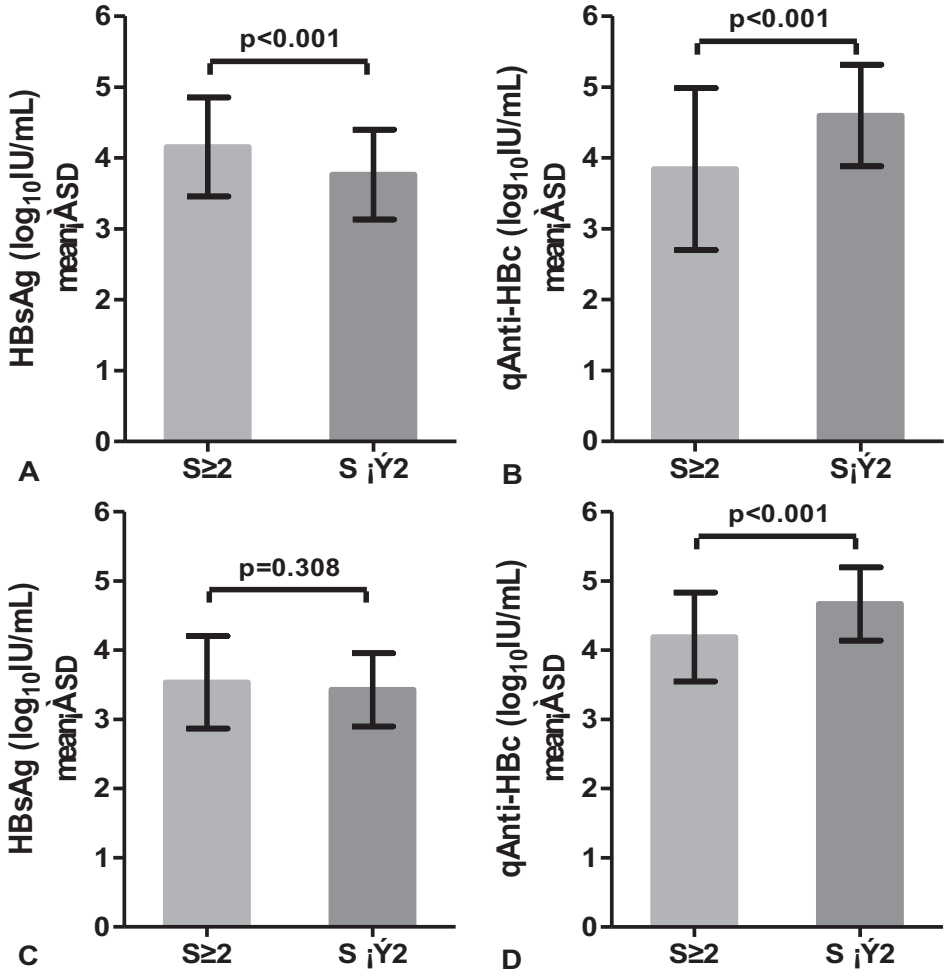

Fig. 2. Distribution of serum HBsAg and qAnti-HBc levels in HBeAg (+) (A and B) and HBeAg (−) patients (C and D) stratified by fibrosis stage.

significantly different in patients with insignificant fibrosis (4.19 ± 0.64 $\log_{10}$IU/mL) compared to patients with significant fibrosis (4.67 ± 0.53 $\log_{10}$IU/mL) in the HBeAg (−) group ($p < 0.001$). However, no significant difference was found in the mean HBsAg levels between patients with insignificant fibrosis (3.54 ± 0.67 $\log_{10}$ IU/mL) and those with significant fibrosis (3.43 ± 0.53 $\log_{10}$IU/mL) in the HBeAg (−) group ($p = 0.31$) (Fig. 2).

The serum qAnti-HBc levels showed a moderate positive correlation with fibrosis scores in both the HBeAg (+) (r = 0.408, $p < 0.001$) and HBeAg (−) groups (r = 0.383, $p < 0.001$). The serum HBsAg levels showed a moderate inverse correlation with fibrosis scores in the HBeAg (+) group (r = −0.381, $p < 0.001$), but had a low inverse correlation with the fibrosis scores in the HBeAg (−) group (r = −0.171, $p < 0.001$).

Table 2
Univariate analysis of variables associated with the presence of significant fibrosis in HBeAg (+) patients.

Parameter	No significant (n = 294)	Significant (n = 195)	*p*
Gender, M/F	195/99	135/60	0.554
Age, years	27.72 ± 8.83	35.79 ± 11.19	**<0.001**
PLT, 10^9/L	211.00 ± 51.48	171.56 ± 60.14	**<0.001**
ALT, U/L	76.00 ± 94.29	292.45 ± 386.03	**<0.001**
AST, U/L	43.82 ± 51.58	186.44 ± 282.54	**<0.001**
TBIL, μmol/L	16.27 ± 9.29	23.26 ± 20.37	**<0.001**
HBV DNA, $\log_{10}$IU/mL	7.42 ± 1.05	6.77 ± 1.26	**<0.001**
HBsAg, $\log_{10}$IU/mL	4.16 ± 0.70	3.77 ± 0.63	**<0.001**
qAnti-HBc, $\log_{10}$IU/mL	3.84 ± 1.14	4.60 ± 0.72	**<0.001**
HBV genotype, B/C	37/245	8/180	**0.002**
Wild type/BCP/PC mutation type	204/69	41/118	**<0.001**
Hepatocyte without/with steatosis	249/45	176/19	0.077

p-values in bold are considered significant.

Table 3
Multiple logistic regression analysis of factors associated with significant fibrosis in HBeAg (+) patients.

Parameter	Multivariate			
	OR	95%CI	Wald	*p*
Age, years	1.06	1.04−1.09	21.62	**<0.001**
PLT, 10^9/L	0.99	0.99−1.00	16.46	**<0.001**
ALT, U/L	1.00	1.00−1.01	1.43	0.231
AST, U/L	1.01	1.00−1.01	2.53	0.112
TBIL, μmol/L	1.01	0.98−1.04	0.56	0.456
HBV DNA, $\log_{10}$IU/mL	0.86	0.68−1.08	1.66	0.198
HBsAg, $\log_{10}$IU/mL	0.87	0.58−1.30	0.47	0.492
qAnti-HBc, $\log_{10}$IU/mL	1.65	1.19−2.30	8.96	**0.003**
HBV genotype, B/C	2.15	1.22−3.79	7.02	**0.008**
Wild type/BCP/PC mutation type	2.26	1.52−3.35	16.31	**<0.001**

p-values in bold are considered significant.

3.4. Factors associated with significant fibrosis

Variables associated with the presence of significant fibrosis were first assessed by univariate analysis. All variables except for gender and hepatic steatosis were identified as predictors of significant fibrosis in HBeAg (+) group (Table 2). Gender, age, PLT, AST, HBV DNA and qAnti-HBc were identified as predictors of significant fibrosis in HBeAg (−) group (Table 4). Significant variable from the univariate analysis were subjected to multiple logistic regression analysis (Tables 3 and 5). Age, PLT, qAnti-HBc levels, HBV genotype and BCP/PC mutations were identified as independent predictors of significant fibrosis in HBeAg (+) group, and age, PLT, qAnti-HBc levels in HBeAg (−) group (all $p < 0.05$).

3.5. Predictive value of qAnti-HBc for significant fibrosis

Serum qAnti-HBc levels, APRI and FIB-4 were used to predict the probability of being diagnosed with significant fibrosis abnormalities in HBeAg (+) and HBeAg (−) patients (Table 6 and Fig. 3). The AUC of serum qAnti-HBc levels associated with the diagnosis of significant fibrosis abnormalities in HBeAg (+) patients was 0.734 (95%CI 0.689 to 0.778). Based on the AUC analysis, both the APRI (0.847, 95%CI 0.810 to 0.884) and FIB-4 (0.836, 95%CI 0.797 to 0.875) scores predicted significant fibrosis were better than serum qAnti-HBc levels (both $p < 0.001$). In HBeAg (−) patients, the AUC of serum qAnti-HBc associated with the diagnosis of significant fibrosis abnormalities was 0.707 (95% CI 0.612 to 0.801), which similar to APRI (0.824, 95%CI 0.750 to 0.899) and FIB-4 (0.821, 95%CI 0.742 to 0.900) ($p = 0.06$ and $p = 0.07$).

4. Discussion

We preformed this cross-sectional study in a group of 624 consecutive CHB patients. This study population represents a well-characterized CHB cohort, which had not received previous therapy for HBV infection and had not been preselected in any way. 99.32% of patients were infected with HBV genotype C or B. The study provides the first detailed description of the relationship between quantitative HBV biomarkers and liver significant fibrosis across a large cohort of patients with CHB.

HBsAg is the surface antigen of HBV and is translated from pre-S1 mRNA and pre-S2/S mRNA, which are transcribed from the covalently closed circular DNA (cccDNA) and integrated HBV DNA sequence. The presence of this antigen indicates current hepatitis B infection. Our data confirmed the negative correlation between serum HBsAg levels and stages of fibrosis in HBeAg (+) patients, as previously reported,[9,10,15] and lower serum HBsAg levels were associated

Table 4
Univariate analysis of variables associated with the presence of significant fibrosis in HBeAg (−) patients.

Parameter	Not significant (n = 41)	Significant (n = 94)	*p*
Gender, M/F	23/18	75/19	**0.006**
Age, years	33.56 ± 12.73	42.11 ± 12.22	**<0.001**
PLT, 10^9/L	199.66 ± 52.90	146.94 ± 61.81	**<0.001**
ALT, U/L	112.68 ± 253.23	190.89 ± 209.30	0.064
AST, U/L	60.95 ± 114.17	115.44 ± 146.46	**0.036**
TBIL, μmol/L	19.85 ± 19.52	23.67 ± 19.56	0.298
HBV DNA, $\log_{10}$IU/mL	4.51 ± 1.52	5.20 ± 1.30	**0.008**
HBsAg, $\log_{10}$IU/mL	3.54 ± 0.67	3.43 ± 0.53	0.308
qAnti-HBc, $\log_{10}$IU/mL	4.19 ± 0.64	4.67 ± 0.53	**<0.001**
HBV genotype, B/C	2/31	6/79	1.000
Wild type/BCP/PC mutation type	10/23	16/67	0.222
Hepatocyte without/with steatosis	32/9	85/9	0.060

p-values in bold are considered significant.

Table 5
Multiple logistic regression analysis of factors associated with significant fibrosis in HBeAg (−) patients.

Parameter	Multivariate			
	OR	95%CI	Wald	*p*
Gender, M/F	0.47	0.17−1.27	2.23	0.136
Age, years	1.04	1.00−1.09	3.95	**0.047**
PLT, 10^9/L	0.99	0.98−1.00	7.90	**0.005**
AST, U/L	1.00	1.00−1.01	1.18	0.277
HBV DNA, $\log_{10}$IU/mL	1.32	0.94−1.85	2.49	0.115
qAnti-HBc, $\log_{10}$IU/mL	3.02	1.24−7.39	5.87	**0.015**

p-values in bold are considered significant.

Table 6
Area under the receiver operating characteristic curve, sensitivity, specificity, and predictive values of serum qAnti-HBc levels for significant liver fibrosis.

Group	Baseline value	AUROC	95% CI	Cut-off	Sensitivity	Specificity	PPV	NPV
HBeAg (+)	qAnti-HBc, $\log_{10}$IU/mL	0.734	0.689−0.778	4.58	63.08%	74.83%	62.44%	75.34%
	APRI	0.847	0.810−0.884	0.25	81.03%	77.89%	70.85%	86.09%
	FIB-4	0.836	0.797−0.875	1.30	65.13%	93.54%	86.99%	80.17%
HBeAg (−)	qAnti-HBc, $\log_{10}$IU/mL	0.707	0.612−0.801	4.37	75.53%	56.10%	79.78%	50.00%
	APRI	0.824	0.750−0.899	0.43	64.89%	87.80%	92.42%	52.17%
	FIB-4	0.821	0.742−0.900	1.33	76.60%	82.93%	91.14%	60.71%

with a greater likelihood of having moderate to severe fibrosis (S2-S4). Our study also investigated the relationship in HBeAg (−) CHB patients. However, no significant difference in serum HBsAg levels was observed among different fibrosis stages in these patients, and there was only low inverse correlation between serum HBsAg levels and fibrosis stages. The exact mechanism is not fully understood. Thompson AJ et al.[16] reported that the HBsAg titers were positively correlated with intrahepatic HBV cccDNA only in HBeAg (+) CHB patients, but not in HBeAg (−) CHB patients. This may occur if HBsAg is produced from an integrated HBV DNA sequence other than the intranuclear cccDNA in HBeAg (−) patients. Few studies have investigated the association between intrahepatic HBV cccDNA and liver fibrosis until recently. Liu Hui-yuan et al.[17] examined 48 treatment-naïve CHB patients. Their study showed that levels of HBV cccDNA in hepatocytes were negatively correlated with the severity of liver fibrosis. However, Wang Q et al.[18] reported that a lower ratio of cccDNA/intrahepatic HBV DNA is associated with a greater degree of liver fibrosis in HBeAg (+) patients, while the amount of intrahepatic cccDNA exhibits no association. Therefore, the association among serum HBsAg levels, intranuclear cccDNA, and liver fibrosis requires further validation.

Anti-HBc, as another classical serological marker of HBV infection, is an indicator of both past and persistent HBV infection. Our previous work categorizing qAnti-HBc levels according to the grade of liver inflammation in treatment-naïve CHB patients revealed that qAnti-HBc levels were positively associated with the liver inflammation grade and could serve as a new marker for the prediction of inflammation activity.[14]

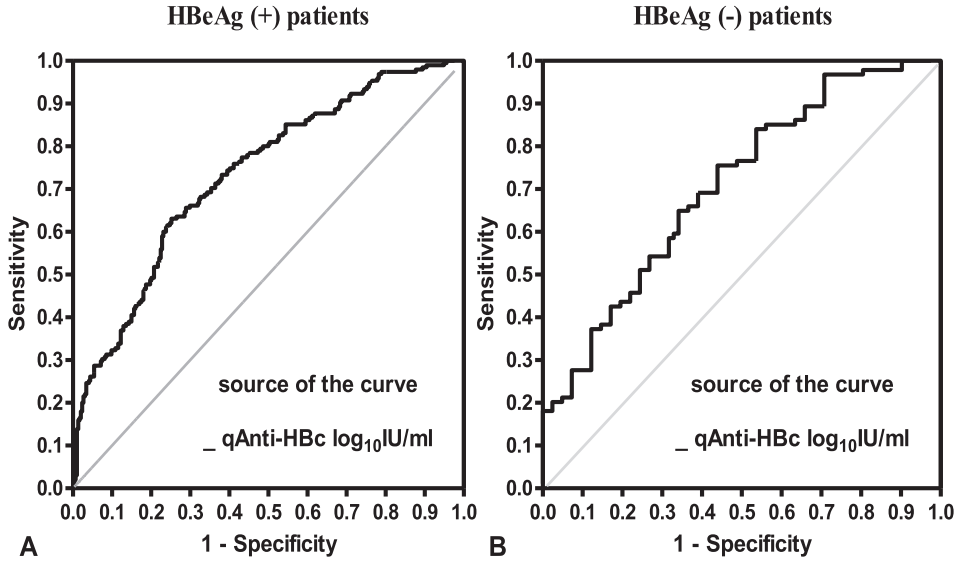

Fig. 3. Receiver operating characteristic curves of serum qAnti-HBc levels used to distinguish moderate to severe fibrosis in HBeAg (+) and HBeAg (−) CHB patients.

Chronic necroinflammation, hepatocyte injury, and liver regeneration result in fibrosis. In the present report, we demonstrated a correlation between qAnti-HBc levels and liver fibrosis. Among both HBeAg (+) and HBeAg (−) patients, a positive correlation was found between serum qAnti-HBc levels and liver fibrosis stage. No significant differences were found in the qAnti-HBc titers between the S3 and S4 subjects. The lack of any difference between the S3 and S4 subjects may be due to a reduction in the ability of viable hepatocytes to maintain viral replication as fibrosis progresses.

In present study, multivariable logistic regression and receiver operating characteristic (ROC) analyses were used to investigate routine parameters for predicting significant fibrosis. Multivarisble logistic regression analysis showed that routine parameters for predicting significant fibrosis included age, PLT, qAnti-HBc, HBV genotype and BCP/PC mutations in HBeAg (+) subjects, and age, PLT, qAnti-HBc in HBeAg (−) subjects. The AUC values of serum qAnti-HBc level for predicting significant fibrosis were 0.734 in HBeAg (+) group, which were inferior to APRI (0.847) and FIB-4 (0.836). In HBeAg (−) group, our study indicated the AUC value of serum qAnti-HBc level was not less accurate than APRI or FIB-4. However, serum HBsAg was not an independent parameter associated with significant fibrosis in the two groups, which implying a difference in the generation and clinical significance between these two HBV serological markers.

In conclusion, our data suggested that serum HBsAg levels were moderately related to liver fibrosis stage in HBeAg (+) CHB patients, but not in HBeAg (−) patients. However, qAnti-HBc levels were moderately related to liver fibrosis stage in all CHB patients. And qAnti-HBc can predict significant fibrosis in both HBeAg (+) and HBeAg (−) subjects with great accuracy. Quantitative detection of HBsAg and Anti-HBc may potentially reduce the need for liver biopsies and help guide clinical decision making in the management of CHB patients.

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