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Original article

Serum hepcidin levels are related to serum markers for iron metabolism and fibrosis stage in patients with chronic hepatitis B: A cross-sectional study

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ABSTRACT

Background and study aims: The clinical significance of serum parameters of iron metabolism and hepcidin in liver disease remains unknown. Therefore, this study aimed to evaluate the association of serum hepcidin levels with fibrosis stage and serum iron parameters in patients with chronic hepatitis B (CHB).
Patients and Methods: This cross-sectional study included 126 treatment-naïve patients with CHB (median age, 39.0 years; 64.3% males) who were positive for hepatitis B surface antigen and 23 healthy controls (median age, 33.0 years; 52.2% males). Data on patient demographics, serum hepcidin levels, liver function tests and serum iron parameters and liver biopsy findings including fibrosis grade, histological activity index (HAI) and liver iron level were recorded.
Results: The median (minimum–maximum) serum hepcidin levels were significantly lower in the CHB group than in the control group [71.2 (13.3–672.7) vs. 657.5 (201.7–2714.2) pg/mL, $p < 0.001$]. Higher fibrosis stage was associated with higher transferrin saturation ($p = 0.029$), serum ferritin level ($p < 0.001$) and viral load ($p < 0.001$). Fibrosis stage and HAI were positively correlated with ferritin ($r = 0.407$, $p < 0.001$ and $r = 0.415$, $p < 0.001$, respectively) and transferrin saturation ($r = 0.219$, $p = 0.026$ and $r = 0.290$, $p = 0.003$, respectively) levels, whereas hepcidin level was negatively correlated with fibrosis stage ($r = -0.175$, $p = 0.051$), viral load ($r = -0.209$, $p = 0.020$) and ferritin level ($r = -0.244$, $p = 0.006$) level. There were no significant differences in serum iron level, total iron binding capacity and liver iron level among patients with different stages of fibrosis.
Conclusion: Reduced hepcidin levels and elevated transferrin saturation and ferritin levels are linked to fibrosis severity and HAI in patients with CHB.

Introduction

Chronic hepatitis B (CHB) is a common chronic liver disease (CLD) that often progresses to cirrhosis and is associated with increased risk of hepatocellular carcinoma (HCC) as well as high rates of morbidity and mortality [1–3]. Liver biopsy remains the gold standard for the diagnosis of fibrosis; however, this method shows several drawbacks such as sampling error risk, intra-observer variations and major associated complications [4,5]. Therefore, development of practical, noninvasive biomarkers for the surveillance of hepatitis B (HBV)-related diseases and their progression is of great clinical significance [3,4].

Iron overload, which is common in chronic HBV-related diseases, is associated with oxidative stress and subsequent tissue damage and chronic inflammation in the liver [3,6,7]. This association emphasises the potential role of hepcidin—a key regulator of iron homeostasis primarily produced by liver cells—as an indicator of changes in iron metabolism in HBV-related diseases [8–11]. A better understanding of the link between viral hepatitis, iron overload and disease progression is required, and improved clinical surveillance and treatment modalities are warranted [3,8–12]. In this regard, many indices of iron metabolism such as hepatic iron levels, serum iron and ferritin levels and transferrin saturation have been commonly used as diagnostic tools for iron overload as a risk factor for liver fibrosis [4,11,13,14]. Notably, the recently discovered hepcidin has emerged as a potential marker for fibrosis and cirrhosis owing to its role as a master regulator of systemic

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iron homeostasis by limiting serum iron levels through the control of iron efflux from cells [4,15,16].

However, most of studies investigating the utility of hepcidin levels in patients with HBV-related diseases were based on the measurement of the hepcidin precursor pro-hepcidin [17–21], and the impact of pro-hepcidin on iron metabolism regulation and its correlation with hepcidin levels remain unclear [3,22,23]. Moreover, the results of studies on hepcidin levels in CLDs of various aetiologies are inconsistent [3,11]. Increased serum hepcidin levels have been reported in patients with CHB and HCC [24], whereas decreases in serum pro-hepcidin levels have been reported in patients with CHB [18,19]. Furthermore, reduced hepcidin levels have been reported in patients with alcoholic liver disease (ALD), CHB, chronic hepatitis C (CHC) infection, nonalcoholic fatty liver disease [25,26] and HBV-related cirrhosis [27]. Therefore, the exact mechanism underlying the association of serum hepcidin levels with the progression of liver diseases and the clinical significance of the association between hepcidin and serum parameters of iron metabolism remain unexplained [3,4,11].

Therefore, this study aimed to compare serum hepcidin levels between patients with CHB and healthy controls to elucidate the association of serum hepcidin levels with fibrosis stage and serum iron parameters.

Patients and methods

Study population

This cross-sectional study, which was conducted in a tertiary care gastroenterology outpatient clinic, included 126 treatment-naïve patients with CHB [median age (minimum–maximum), 39.0 (17–75) years; 64.3% males] who were hepatitis B surface antigen-positive for at least 6 months and 23 healthy control subjects [median age (minimum–maximum), 33.0 (22–76) years; 52.2% males]. Controls were healthy volunteers with no abnormalities as determined through physical examination, medical history and laboratory tests. Exclusion criteria were non-HBV aetiology for liver disease; history of antiviral treatment or immunomodulatory therapy within the last 12 months; presence of severe systemic disease or infection, hemochromatosis, Wilson's disease, toxic or alcoholic hepatitis, decompensated liver cirrhosis, malignancy, anaemia and haematological disease or history of organ transplantation.

Written informed consent was obtained from all subjects following the detailed explanation of the objectives and protocol of the study, which was conducted in accordance with the ethical principles of the Declaration of Helsinki and approved by the ethics committee of authors' university.

Study parameters

Data on patient demographics, serum hepcidin levels, liver function tests and serum iron parameters were recorded for the patient and control groups. Liver biopsy findings including fibrosis grade, histological activity index (HAI) and liver iron level were obtained for all patients with CHB. Study parameters were compared among subgroups of patients with CHB categorised according to fibrosis stage; blood biochemistry data were compared between patients with CHB and controls.

Blood biochemistry

Complete blood count; erythrocyte sedimentation rate; total iron binding capacity and levels of C-reactive protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase, total bilirubin, albumin, iron, ferritin, HBV DNA, hepatitis B e antigen (HBeAg) and anti-hepatitis B e antibody were determined by routine laboratory tests.

For hepcidin measurement, serum aliquots were frozen at −80 °C immediately following blood collection. At the time of analysis, serum hepcidin (pg/mL) levels were determined using a commercial enzyme-linked immunosorbent kit (Hangzhou Eastbiopharm, China), according to the manufacturer's protocol.

Liver biopsy

All patients underwent ultrasonography-guided percutaneous liver biopsy with an automatic Tru-Cut needle using a lateral intercostal approach. Formalin-fixed and paraffin-embedded sections were stained with haematoxylin and eosin and Masson's trichrome. The slides were reviewed by the same experienced haematopathologist to determine HAI according to Ishak's modified HAI method [28]. Modified HAI grading was based on the necroinflammatory score (piecemeal necrosis, confluent necrosis, focal necrosis and focal inflammation) with a maximum possible score of 18 [29]. Modified HAI staging was based on architectural changes, fibrosis and cirrhosis and ranged from 0 (no fibrosis) to 6 (probable or definitive cirrhosis) [28].

Liver biopsy specimens were also assessed by the same haematopathologist to determine iron levels by histological staining with Perls' Prussian Blue (Perls for ferric iron; Bio Optica, Milan, Italy). Iron levels were graded from 0 (normal iron level) to 4 (very severe increase) in accordance with McSween's modified hepatic iron overload grading system [29].

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM, Armonk, NY). Categorical variables were analysed using Pearson's chi square test with the Monte Carlo simulation technique. Numerical variables were analysed using independent-samples *t*-test, Mann–Whitney *U* test, one-way analysis of variance with *post hoc* Fisher's least significant difference test, Games Howell test, Jonckheere–Terpstra test with Monte Carlo simulation technique and *post hoc* Dunn's test. Correlation analyses were performed using Spearman's correlation analysis. Data were expressed as means ± standard deviation, medians (minimum–maximum) and percentages (%) as appropriate. A p value of < 0.05 was considered to indicate statistical significance.

Results

Demographic characteristics and laboratory findings of the CHB and control groups

Comparison of the biochemical liver profiles between the CHB and control groups revealed that the levels of AST, ALT and ALP and prothrombin time were significantly higher and that the platelet counts were significantly lower in the CHB group than in the control group (Table 1). Importantly, the median (minimum–maximum) serum hepcidin level was significantly lower in the CHB group than in the control group [71.2 (13.3–672.7) vs. 657.5 (201.7–2714.2) pg/mL, $p < 0.001$] (Table 1).

In the CHB group, stage 0/1, 2/3 and 4–6 fibrosis were identified in 49 (31.7%), 56 (25.9%) and 30 (23.9%) patients, respectively. The mean HAI score was 4.3 ± 2.3 (range, 1.0–12.0). HBeAg positivity was evident in 16 (12.7%) patients, whereas increased iron deposition was found in 26 (20.6%) patients (23 males and 3 females); the

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Table 1
Demographic characteristics and laboratory findings in patient and control groups.

	CHB patients (n = 126)	Controls (n = 23)	p value
Demographics			
Age (year), median (min/max)	39.0 (17.0/75.0)	33.0 (22.0/76.0)	0.223
Gender, n (%)			
Male	81 (64.3)	12 (52.2)	0.340
Female	45 (35.7)	11 (47.8)	
BMI (kg/m^2), mean ± SD	25.7 ± 4.52	24.4 ± 3.54	0.053
Laboratory findings, median(min/max)			
AST(U/L)	27.5 (12.0/763.0)	19.0 (14.0/32.0)	**<0.001**
ALT(U/L)	32.5 (9.0/801.0)	16.0 (8.0/57.0)	**<0.001**
ALP(U/L)	76.5 (36.0/153.0)	62.0 (30.0/98.0)	**0.001**
GGT(U/L)	23.0 (4.0/284.0)	21.0 (9.0/58.0)	0.728
Total bilirubin (mg/dL)	0.6 (0.2/3.5)	0.6 (0.3/1.1)	0.656
Albumin (g/dL)	4.4 (2.0/5.0)	4.5 (3.5/5.1)	0.055
Hemoglobin (g/dL)	15.1 (9.5/17.8)	14.7 (12.6/16.8)	0.509
Platelet (cell/μl)	194500(43000/423000)	255000(156000/328000)	**0.001**
Prothrombin time (sec)	14.0 (11.0/22.0)	12.0 (10.0/14.0)	**<0.001**
ESR (mm/h)	7.0 (1.0/76.0)	5.0 (1.0/26.0)	0.207
CRP (mg/dL)	3.1 (0.1/37.0)	3.2 (3.2/4.9)	0.205
Iron parameters, mean ± SD			
Serum Fe (μg/dL)	94.0 (26.0/192.0)	106.0 (39.0/153.0)	0.464
TIBC (μg/dL)	344.4 ± 61.57	362.3 ± 81.14	0.322
Transferrin saturation (%)	0.3 (0.1/1.0)	0.3 (0.1/0.5)	0.536
Ferritin (ng/ml)	69.5 (3.9/994.0)	48.1 (4.2/270.0)	0.146
Hepcidin (pg/ml), mean ± SD	71.2 (13.3/672.7)	657.5 (201.7/2714.2)	**<0.001**

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamaglutamyl transferase; TIBC: total iron binding capacity (TIBC).
Bold values indicates statistically significant.

rates of mild, moderate and severe iron deposition were 69.2%, 26.9% and 3.9%, respectively.

Demographic characteristics and laboratory findings according to the stage of fibrosis in the CHB group

Higher fibrosis stages were associated with increased patient age; higher serum levels of AST, ALT, ALP, gamma-glutamyl transferase and total bilirubin; prolonged prothrombin time; lower serum albumin level and lower platelet count (Table 2). Higher fibrosis stages were associated with higher transferrin saturation and serum ferritin levels; however, there were no significant differences in serum iron, total iron binding capacity and liver iron level among patients in different stages of fibrosis (Table 2).

Hepcidin levels according to the stage of fibrosis and HBeAg positivity in the CHB group

A non-significant tendency for lower hepcidin levels was observed in patients with a fibrosis stage of 0/1 compared to those with advanced-stage fibrosis, whereas hepcidin levels were significantly lower in HBeAg-positive patients than in HBeAg-negative patients (Table 3).

Correlations between hepatic fibrosis, HAI and serum hepcidin levels

Hepcidin levels were negatively correlated with fibrosis stage; levels of AST, ALT, ALP and ferritin; HBV DNA levels and prothrombin time and positively correlated with serum albumin level and platelet count (Table 4).

Discussion

In the present cross-sectional study, hepatic iron overload was evident in 20% patients with CHB, whereas the serum iron parameters did not indicate iron overload in these patients. Advanced fibrosis was significantly associated with the biochemical liver profile, viral load, hepcidin levels and serum iron parameters. In addition, higher ferritin levels and transferrin saturation were observed in patients with advanced fibrosis than in those with mild fibrosis. These results are consistent with the reported association between increased serum iron levels and increased risk for steatosis progression to more severe liver pathologies such as steatohepatitis, fibrosis and HCC [30–32]. In fact, while a correlation between iron parameters and hepcidin is often reported in patients with CHC [33,34], the relationship between hepcidin and CHB remains inconclusive [3,18,19,24]. Previous studies have demonstrated that hepcidin levels were significantly lower in patients with CHB accompanied by advanced fibrosis and cirrhosis compared to controls. Furthermore, ferritin levels and transferrin saturation were higher in patients with CHB; however, there was no relationship between serum pro-hepcidin levels and iron parameters, suggesting that pro-hepcidin levels do not reflect the levels of the active form hepcidin [17,18,24]. Literature review showed that these studies usually used pro-hepcidin, whereas the present study utilised hepcidin, which is considered to be biologically active.

A previous study on the changes in serum markers of iron metabolism in 186 non-cirrhotic patients with CLD and 60 healthy controls found that serum iron, transferrin saturation and ferritin levels were elevated and that serum hepcidin levels were decreased in patients compared to controls; these differences were more remarkable in the presence of ALD, nonalcoholic fatty liver disease and CHC compared to CHB with autoimmune liver diseases. The authors reported the changes in serum markers of iron metabolism only in patients with CHB, whereas a significant positive correlation was found among serum iron, ferritin, transferrin saturation and hepcidin in all patients, which was stronger in those with metabolic steatohepatitis or alcoholic aetiologies than in those with steatosis [11]. Similarly, chronic viral hepatitis-induced suppression of hepcidin synthesis via oxidative DNA damage is considered to be relatively mild in HBV infection compared to that in hepatitis C virus infection [35].

In a previous study of 46 patients with HBV-related diseases and 20 healthy controls, the serum hepcidin levels, which were higher in those without cirrhosis than in controls, were comparable between the patients with cirrhosis and control subjects [3]. In another study assessing hepcidin levels in 228 patients with CLD of varying aetiologies in comparison with 45 healthy controls and 50 patients with non-liver diseases, CLD was associated with lower hepcidin levels; the lowest hepcidin levels were found in patients with cirrhosis [36].

The positive correlation of fibrosis stage and HAI with both serum ferritin and transferrin saturation levels and the negative correlation between fibrosis stage and serum hepcidin levels in the patients with CHB in the present study suggest a significant role for iron metabolism in the progression to cirrhosis in these patients. Therefore, our findings support the reported association of CLD with reduced hepcidin compared with healthy individuals [11] and the observed reduction in serum hepcidin levels with increasing fibrosis in patients with CLD [36,37]. Notably, hepcidin (or prehepcidin) levels were reported to be significantly lower in patients with HBV-related cirrhosis than in those without cirrhosis as well as healthy subjects [3,21,27].

Iron loading, viral infection and liver dysfunction are considered as major regulators of hepcidin in patients with CLD [3]. Given that liver is the primary source of hepcidin production [38], the reduced

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Table 2
Demographic characteristics and laboratory findings in CHB patients according to fibrosis stage.

	Hepatic fibrosis stage						p value*
	0 (n = 15)	1 (n = 25)	2 (n = 32)	3 (n = 24)	4 (n = 18)	5–6 (n = 12)	
Demographics							
Gender (M/F)	10/5	11/14	19/13	19/5	13/5	9/3	0158
Age (year)	35.3 ± 15.3 V	33.5 ± 9.5 IV V	38.8 ± 12.2 V	39.3 ± 13.2 V	43.6 ± 11.0	52.0 ± 11.8	**0.001**
BMI (kg/m²)	24.1 ± 4.1	26.1 ± 4.8	25.2 ± 3.5	27.0 ± 5.1	26.4 ± 4.3	24.9 ± 5.8	0.467
Laboratory findings							
AST(U/L)	21(12/112) III IVV	21(14/32) III IV V	22.5(13/92) III IV V	43.5(18/145)	44.5(19/763)	67(27/288)	**<0.001**
ALT(U/L)	24(9/74)) III IV V	23(12/64)) II III IV V	29(11/207) III IV V	77(16/355)	50(10/801)	65(16/572)	**<0.001**
ALP(U/L)	70.3 ± 17.0 V	67.2 ± 13.6 III V	79.7 ± 26.8 V	88.2 ± 22.2	91.2 ± 31.8	109.3 ± 20.7	**<0.001**
GGT(U/L)	19(10/39) I III IV V	16(7/76) II III IV V	19(4/82) III IV V	32.5(9/166) V	26.5(10/284) V	51.5(29/262)	**<0.001**
TBil (mg/dL)	0.57 (0.32/2.05)	0.52 (0.2/1.03)	0.54 (0.19/1.77)	0.57 (0.32/1.31)	0.66 (0.26/1.99)	1.035 (0.36/3.45) O I IIIII	0.011
Albumin (g/dL)	4.5 ± 0.2 IV V	4.4 ± 0.3 V	4.4 ± 0.3 V	4.4 ± 0.3 V	4.2 ± 0.4	3.6 ± 0.7	**<0.001**
PT (sec)	13.6 ± 1.0 V	13.8 ± 0.9 V	14.0 ± 1.1 V	14.2 ± 1.2 V	14.5 ± 1.3	17.3 ± 3.0	**<0.001**
Hemoglobin (g/dL)	15.3 ± 1.4	14.1 ± 1.7	14.6 ± 1.9	15.3 ± 1.3	15.3 ± 1.2	14.2 ± 2.4	0.069
Platelet (cell/μl)	216200 ± 64779 V	233320 ± 621267 IV V	212281.3 ± 67475.8 V	207750 ± 53819.5 V	180166.7 ± 44663.8	119416.7 ± 53707	**<0.001**
Iron parameters							
Fe (mg/dL)	94.08 ± 9.84	89.70 ± 8.09	92.29 ± 6.45	101.62 ± 8.94	107.93 ± 10.4	108.10 ± 14.1	0.634
TIBC (μg/dL)	360.42 ± 12.81	343.75 ± 10.0	351.56 ± 11.48	360.11 ± 12.9	331.67 ± 16.9	296.00 ± 30.06	0.151
TS (%)	0.25 ± 0.02 V	0.26 ± 0.02 V	0.26 ± 0.01 V	0.28 ± 0.02 V	0.33 ± 0.04	0.42 ± 0.08	**0.029**
Ferritin(ng/mL)	48(7.26/172) III IV V	36.5(7.46/124) II III IV V	63(4.25/435) IV V	93(8/725)	158(3.9/420)	116.2(8.21/994)	**<0.001**
Liver iron	0.20 ± 0.10	0.16 ± 0.09	0.38 ± 0.12	0.29 ± 0.15	0.33 ± 0.11	0.18 ± 0.12	0.760
HBVDNA (IU/ml)	4150(43/126000) II III IV V	3495(144/5500000) II III IV V	30000(107/170000000) V	97111(114/289765263) V	929,948 (5970/170000000)	3500000(17000/170000000)	**<0.001**
HbeAg negative	15 (100.0)	24 (96.0)	28 (87.5)	17 (70.8)	16 (88.9)	10 (83.3)	0.082
positive	0 (0.0)	1 (4.0)	4 (12.5)	7 (29.2)	2 (11.1)	2 (16.7)	

Data are expressed as mean ± SD and median (minimum/maximum) ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gammaglutamyl transferase; PT: prothrombin time; TIBC: total iron binding capacity (TIBC); TS: transferrin saturation.
I, II, III, IV and V indicates significance according to hepatic fibrosis stages of 1, 2, 3, 4 and 5, respectively.
Bold values indicates statistically significant.
* Jonckheere-Terpstra Test (Monte Carlo), Post Hoc Test (Dunn's Test), One-Way ANOVA Test; Post Hoc Test (LSD-Least Significant Difference, Games Howell), Pearson Chi-Square Test (Monte Carlo).

Table 3
Hepcidin levels in CHB patients according to fibrosis stage and HbeAg positivity.

		Hepcidin level Median (Minimum/Maximum)	p value
Fibrosis stage			
Stage 0 (n = 15)		102.3 (19.2/672.7)	0.051
Stage 1 (n = 25)		80.1 (17.1/361.8)	
Stage 2 (n = 32)		67.0 (13.3/212.1)	
Stage 3 (n = 24)		60.4 (18.9/238.6)	
Stage 4 (n = 18)		70.2 (14.1/210.4)	
Stage 5–6 (n = 12)		64.8 (16.2/148.2)	
Fibrosis stage			
Stage 0–1 (n = 40)		88.7 (17.1/672.7)	0.077
Stage 2–3 (n = 56)		66.2 (13.3/238.6)	
Stage 4–6 (n = 30)		68.1 (14.1/210.4)	
HbeAg	negative (n = 110)	76.2 (14.1/672.7)	**0.008**
	positive (n = 16)	57.1 (13.3/140.5)	
HAI	Low (<5) (n = 76)	81.05 (13.3/672.7)	0.167
	High (≥5) (n = 45)	65.2 (14.1/212.1)	
ALT (U/L)	Low (n = 68)	106.8 (14.1/2714.2)	**0.003**
	High (>40 in males, >35 in females) (n = 58)	67.3 (13.3/1632.1)	

Jonckheere-Terpstra Test (Monte Carlo), Mann Whitney U Test (Monte Carlo).
Bold values indicates statistically significant.

synthetic capacity of the liver due to tissue damage has been considered to be associated with the reduction in hepcidin levels in patients with cirrhosis [21]. In this regard, the observed tendency for a higher viral load accompanied with significantly lower hepcidin levels in patients with advanced fibrosis and the negative correlation of hepcidin with viral load as well as with ferritin levels in the current study cohort implicate viral infection and iron loading as important simulators of hepcidin synthesis in patients with CHB. Additionally, these findings emphasise the role of impaired synthetic capacity of the liver following the destruction of liver architecture during fibrosis in reducing hepcidin synthesis and suggest a possible association between lower hepcidin levels and fibrosis progression in HBV-related diseases [3,4,27,39,40]. Thus, the decrease in hepcidin levels with increasing fibrosis stage observed in the present cohort emphasises the correlation between serum hepcidin and progression of chronic HBV infection and provides further support for the role of hepcidin as a potential biomarker for HBV-related disease surveillance [3].

In the present study, serum albumin levels, which were significantly lower in the patients with CHB than in controls, were negatively correlated with the stage of fibrosis and positively correlated with hepcidin levels. These findings are notable given that a reduction in albumin is considered not only an indicator of liver damage but also an independent predictor of hepcidin levels by reflecting the degree of liver dysfunction [3,41].

Nonetheless, it should be noted that the potential mechanisms underlying low levels of hepcidin in liver diseases remain inconclusive [4], although the aetiology and not the severity of cirrhosis is suggested to have an impact on hepcidin response [42]. Indeed, the levels of hepcidin are consistently and significantly lower in HCV and alcoholic-related cirrhosis than in HBV-related cirrhosis, indicating the likelihood of a disease-specific hepcidin response [17,21,36].

The observed increase in prothrombin time and decrease in platelet count with increasing fibrosis stage and decreasing hepcidin level in the present study support the previously reported associations among serum markers of iron metabolism and liver function, prothrombin time and platelet count [43,44]. Overall, these

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Table 4
Correlations of hepatic fibrosis, HAI and serum hepcidin levels.

	Fibrosis		HAI		Hepcidin	
	r	P	r	P	r	P
Fibrosis	–	–	0.744	**<0.001**	−0.175	**0.051**
HAI	0.744	**<0.001**	–	–	−0.116	0.206
Hepcidin	−0.175	**0.051**	−0.116	0.206	–	–
HBVDNA	0.586	**<0.001**	0.572	**<0.001**	−0.209	**0.020**
Age	0.358	**<0.001**	0.292	**0.001**	−0.022	0.788
AST	0.591	**<0.001**	0.643	**<0.001**	−0.320	**<0.001**
ALT	0.479	**<0.001**	0.566	**<0.001**	−0.295	**<0.001**
ALP	0.455	**<0.001**	0.387	**<0.001**	−0.264	**0.001**
GGT	0.489	**<0.001**	0.418	**<0.001**	−0.104	0.207
TBil	0.216	**0.015**	0.141	0.122	−0.053	0.519
Albumin	−0.386	**<0.001**	−0.389	**<0.001**	0.189	**0.022**
Ferritin	0.407	**<0.001**	0.415	**<0.001**	−0.244	**0.006**
TS	0.219	**0.026**	0.290	**0.003**	−0.064	0.477
CRP	0.157	0.107	0.146	0.140	0.094	0.292
ESR	0.227	**0.017**	0.165	0.089	−0.083	0.346
PT	0.351	**<0.001**	0.309	**0.001**	−0.475	**<0.001**
PLT	−0.352	**<0.001**	−0.335	**<0.001**	0.280	**0.001**

HAI: histological activity index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gammaglutamyl transferase; PT: prothrombin time; TS: transferrin saturation; TBil: total bilirubin.
Spearman's rho test, r: correlation coefficient.
Bold values indicates statistically significant.

findings implicate a correlation among parameters of liver damage, liver function and iron metabolism in patients with CLD [11,37,43,44].

Recent studies have also suggest the potential therapeutic approach of restoring hepcidin levels to ameliorate liver fibrosis by reducing iron overload and acting as a paracrine signal to suppress hepatic stellate cell activation [4,14,45,46].

While the strengths of the current study include the analysis of liver histology specimens and the inclusion of healthy controls for comparisons, several limitations should also be considered. First, due to its cross-sectional design, a cause-and-effect relationship could not be established. Second, given the relatively small sample size, the present study findings may not be generalisable to the entire CHB patient population. Third, albeit a non-significant tendency was observed for lower hepcidin levels in patients with stage 0–1 fibrosis compared to those with more advanced-stage fibrosis, a relatively small sample size might have precluded the observation of a statistical significance for a correlation between fibrosis stage and serum hepcidin levels. Finally, despite the evaluation of autoimmune markers, lack of data on genetic screening tests to exclude metabolic diseases such as hemochromatosis is a limitation which would otherwise extend the knowledge obtained from the current study.

In conclusion, in this cross-sectional study we found that decreased hepcidin levels and increased ferritin and transferrin saturation levels were correlated with the severity of fibrosis and HAI. However, further comprehensive studies with larger, homogeneous cohorts are necessary to address correlations among the parameters of iron overload, liver enzymes and liver function and to explore the potential utility of hepcidin in monitoring fibrosis progression among patients with HBV-related liver diseases.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability: The data used to support the findings of this study are available from the corresponding author upon request.

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