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Factors associated with pre-treatment hyperferritinemia in patients with chronic hepatitis C virus infection

Yu-Ping Chang[1], Chiuan-Bo Huang[1], Jia-Horng Kao[1,2,3,4], Tung-Hung Su[1,2], Shang-Chin Huang[2,5], Tai-Chung Tseng[1,2,4], Pei-Jer Chen[1,2,3], Chun-Jen Liu[1,2,3]✉ & Chen-Hua Liu[1,2,6]✉

Pre-treatment host and viral factors may affect serum ferritin levels in patients with hepatitis C virus (HCV) infection. We delineated pre-treatment factors associated with hyperferritinemia in these patients. 1682 eligible patients underwent pre-treatment assessment for serum ferritin and various host/viral factors. Univariate and multivariate logistic regression analyses were conducted to evaluate factors associated with hyperferritinemia. Multivariate logistic regression analyses revealed that age > 50 years (adjusted odds ratio [OR]: 1.38 (95% confidence interval [CI] 1.09–1.74), p = 0.008), fibrosis stage ≥ F3 (adjusted OR: 1.36 (95% CI 1.04–1.77), p = 0.02), fibrosis index based on four parameters (FIB-4) > 3.25 (adjusted OR: 1.46 (95% CI 1.11–1.92), p = 0.01), presence of metabolic dysfunction-associated steatotic liver disease (MASLD) (adjusted OR: 1.43 (95% CI 1.21–1.76), p = 0.001), and alanine transaminase (ALT) > 2 folds upper limit of normal (ULN) (adjusted OR: 2.87 (95% CI 2.20–3.75), p < 0.001) were associated hyperferritinemia. The $\log_{10}$ value of HBV or HCV viral load was not associated with the $\log_{10}$ value of ferritin level (Spearman's rank correlation coefficient: − 0.025, p = 0.81 and 0.002, p = 0.92). In conclusion, host factors, rather than viral factors, are associated with hyperferritinemia in patients with HCV.

Keywords Hepatitis C virus, Hepatitis B virus, Hyperferritinemia, Metabolic dysfunction-associated steatotic liver disease

Globally, an estimated 58 million people have chronic hepatitis C virus (HCV) infection, with about 1.5 million new infections occurring annually[1]. The complications of HCV, including cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC), significantly contribute to the global health and economic burdens. In 2019, an estimated 290,000 deaths were attributed to HCV, primarily as a result of these complications[1].

Ferritin is essential for iron storage and homeostasis, protecting cells from iron toxicity through ferroxidase activity on its heavy chain[2]. Serum ferritin has been extensively studied for decades as a noninvasive and affordable laboratory test[3,4]. Elevated serum ferritin levels, which are observed with a prevalence of 5.9–19.0% in healthy individuals, vary among different ethnic groups[5,6]. This condition is often overlooked by general practitioners despite its wide range of etiologies, including inflammatory and infectious diseases, chronic liver disorders like cirrhosis, non-alcoholic fatty liver disease (NAFLD), and viral hepatitis, metabolic syndrome (MS), malignancies and immune-mediated syndromes such as adult-onset Still's disease, hemophagocytic lymphohistiocytosis, hemochromatosis[7]. Hyperferritinemia, once considered an indirect sign of increased iron stores, is now understood to result from complex mechanisms, including hepatocyte damage leading to ferritin release and acute

[1]Department of Internal Medicine, National Taiwan University Hospital, 7 Chung-Shan South Road, Taipei 10002, Taiwan. [2]Hepatitis Research Center, National Taiwan University Hospital, Taipei, Taiwan. [3]Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan. [4]Department of Medical Research, National Taiwan University Hospital, Taipei, Taiwan. [5]Department of Internal Medicine, National Taiwan University Hospital Bei-Hu Branch, Taipei, Taiwan. [6]Department of Internal Medicine, National Taiwan University Hospital, Yun-Lin Branch, Douliou, Taiwan. ✉email: cjliu@ntu.edu.tw; jacque_liu@mail2000.com.tw

phase reactions that stimulate ferritin synthesis and secretion[4]. While the physiological significance of ferritin is not yet fully understood, research regarding ferritin as a biomarker in relation to inflammatory diseases, cancer, neurodegeneration, and infections is currently emerging[8].

Serum levels of ferritin were first described to be linked to chronic HCV infection in 1990s[9]. Approximately 30–40% of individuals with HCV exhibit increased levels of serum iron, transferrin saturation, and ferritin[10]. However, it is not fully understood whether the hyperferritinemia is caused by the virus itself, other host factors, or the interaction between the virus and the host. Serum ferritin has been suggested as a potential predictor of treatment response and disease progression in HCV infections. Elevated ferritin levels in patients with chronic HCV infection are highly correlated with advanced hepatic fibrosis, necroinflammation, and steatosis, as well as an increased risk of developing HCC[11,12]. Furthermore, elevated ferritin levels can impact antiviral responses and on-treatment viral kinetics to interferon (IFN)-based treatments[13,14].

Before utilizing serum ferritin as a marker for chronic implications in HCV, it is essential to understand potential factors contributing to its elevation. Our study aims to identify factors contributing to pre-treatment hyperferritinemia in HCV, enabling a more precise interpretation of serum ferritin and enhancing the assessment and management of the disease.

Materials and methods

Patients

Between January 2015 and December 2023, we prospective recruited participants aged ≥ 18 years who had been diagnosed as chronic HCV infection, defined as detectable HCV antibody (anti-HCV) (Abbott HCV EIA 2.0, Abbott Laboratories, Abbott Park, Illinois, USA) and quantifiable serum HCV ribonucleic acid (RNA) (Cobas TaqMan HCV Test v2.0, Roche Diagnostics, Mannheim, Germany, lower limit of quantification (LLOQ): 15 IU/mL) for ≥ 6 months at the National Taiwan University Hospital (NTUH) and NYUH Yun-Lin Branch. Participants with decompensated cirrhosis, a history of HCC, end-stage kidney disease (ESKD), human immunodeficiency virus (HIV) co-infection, iron deficiency anemia, increased alcohol consumption which was defined as ≥ 140 g/week for females and ≥ 210 g/week for males, hemoglobinopathies which required blood transfusion including thalassemia major, sickle cell disease, systemic or organ-specific autoimmune diseases, acute bacterial infection, or those who had undergone organ transplantation were excluded from the study. All methods followed relevant guidelines and regulations. Experimental protocols were approved by the 7th Core Lab of the National Taiwan University Hospital and the 1st Common Laboratory of the National Taiwan University Hospital, Yun-Lin Branch. Informed consent was obtained from all subjects and/or their legal guardians.

Study design

Participant's baseline demographics, including age, sex, history of type 2 diabetes (T2D), hypertension (HTN) and hyperlipidemia, body mass index (BMI) with a cut-off of ≥ 23 kg/m^2 to denote overweight/obesity, platelet counts, international normalized ratio (INR), serum albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) with an upper limit of normal (ULN) of 30 U/L for males and 19 U/L for females, estimated glomerular filtration rate (eGFR) calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, fasting glucose, glycosylated hemoglobin (HbA1c), triglyceride, high-density lipoprotein-cholesterol (HDL-C), ferritin, hepatitis B virus (HBV) surface antigen (HBsAg) (Abbott Architect HBsAg qualitative assay, Abbott Laboratories, Abbott Park, Illinois, USA), HBV deoxyribonucleic acid (DNA) (Cobas AmpliPrep/Cobas TaqMan HBV DNA test, v.2.0, Roche Diagnostics, Mannheim, Germany, LLOQ): 20 IU/mL), HCV RNA, and HCV genotype (Roche Cobas HCV GT, Roche Diagnostics, Mannheim, Germany or Abbott RealTime HCV GT II, Abbott Laboratories, Abbott Park, Illinois, USA) were assessed[8,15–18].

Liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) were determined using vibration-controlled transient elastography (VCTE) (FibroScan, Echosens, Paris, France) equipped with an M or XL probe. The cut-off values of LSM for a hepatic fibrosis stages of F0-1, F2, F3, and F4 are ≤ 7.0 kPa, 7.1–9.4 kPa, 9.5–12.4 kPa, and ≥ 12.5 kPa, respectively[19]. Metabolic dysfunction-associated steatotic liver disease (MASLD) was characterized by the presence of steatotic liver disease (SLD) with a CAP cut-off value of ≥ 248 dB/m, in addition to at least one out of five cardiometabolic risk factors[20,21]. The fibrosis index based on four parameters (FIB-4) was calculated for all participants[22]. Hyperferritinemia was defined as a serum ferritin level exceeding 300 ng/mL for males and 200 ng/mL for females[2].

Statistical analysis

All statistical analyses were conducted using the Statistical Package for Social Science (SPSS version 26.0 (IBM)). Continuous variables were present as median (interquartile range [IQR]), while categorical data were present as numbers (percentage). Univariate logistic regression analysis was utilized to explore the relationship between hyperferritinemia and host/viral factors of interest, including age, sex, HBV coinfection, HCV viral load, HCV genotype, hepatic fibrosis stage, FIB-4 score, MASLD, ALT quotient, and eGFR level in the entire population. The results were reported as crude odds ratios (ORs) with 95% confidence intervals (CIs). Among participants with MASLD, we further analyzed this association with the aforementioned factors, as well as with T2D and BMI. Multivariate logistic regression analysis was performed for factors with a p-value less than 0.10 in the univariate analysis to identify independent variables associated with hyperferritinemia, and these were reported as adjusted ORs with 95% CIs. Spearman's rank correlation was used to evaluate the correlation between the $\log_{10}$ value of HBV viral load or $\log_{10}$ value of HCV viral load and the $\log_{10}$ serum ferritin level, ALT level and FIB-4 score. Among participants with HBV coinfection, those with a viral load below the LLOQ had their $\log_{10}$ value of HBV viral load set as 0. All statistical analyses were two-tailed, and statistical significance was defined as a p-value less than 0.05.

Results

Patient characteristics

Of 2343 participants diagnosed with chronic HCV infection, 661 were excluded from the study because of decompensated cirrhosis (n = 21), a history of HCC (n = 75), organ transplantation (n = 35), ESKD (n = 240), HIV coinfection (n = 253), increased alcohol consumption (n = 34), and iron deficiency anemia (n = 3). The remaining 1682 eligible participants were included in the study (Fig. 1).

The median age was 56 years (IQR: 48–63 years) and 864 (51.4%) participants were males. The median $\log_{10}$ HCV viral load was 6.07 IU/mL and 991 (58.9%) participants were infected with HCV genotype 1. One hundred forty-four (8.6%) participants were coinfected with HBV. The median $\log_{10}$ HBV viral load was 0.00 IU/mL (IQR: 0.00–2.73 IU/mL). With regard to LSM, 699 (42.4%), 416 (25.3%), 199 (12.1%), and 333 (20.2%) participants had a fibrosis stage of F0-F1, F2, F3 and F4. MASLD was present in 635 (38.6%) participants, of whom T2D, overweight/obesity, and lean/normal weight were present in 221, 407 and 7 participants. Furthermore, 1294 (76.9%), 1505 (89.5%), and 782 (46.5%) participants had ALT level > 2 folds ULN, an eGFR > 60 mL/min/1.73 m^2, and hyperferritinemia (Table 1).

Pre-treatment factors associated with hyperferritinemia in the entire population

In the univariate logistic regression analysis, age > 50 year (crude OR: 1.73; 95% CI 1.40–2.14, p < 0.001), fibrosis stage ≥ F3 (crude OR: 2.22; 95% CI 1.80–2.74, p < 0.001), FIB-4 score > 3.25 (crude OR: 2.39; 95% CI 1.93–2.95, p < 0.001), presence of MASLD (crude OR: 1.51; 95% CI 1.23–1.84, p < 0.001), ALT > 2 folds ULN (crude OR: 3.55; 95% CI 2.75–4.59, p < 0.001), and eGFR < 60 mL/min/1.73 m^2 (crude OR: 1.38; 95% CI 1.01–1.89, p = 0.04) were significantly associated with hyperferritinemia, while sex, HBV coinfection, HCV RNA at a cut-off value of 2,000,000 IU/mL, and HCV genotype were not associated with hyperferritinemia (Table 2).

Multivariate logistic regression analysis identified age > 50 year (adjusted OR: 1.38; 95% CI 1.09–1.74, p = 0.008), fibrosis stage ≥ F3 (adjusted OR: 1.36; 95% CI 1.04–1.77, p = 0.02), FIB-4 score > 3.25 (adjusted OR: 1.46; 95% CI 1.11–1.92, p = 0.01), presence of MASLD (adjusted OR: 1.43; 95% CI 1.12–1.76, p = 0.001), ALT > 2 folds ULN (adjusted OR: 2.87; 95% CI 2.20–3.75, p < 0.001) were independent factors associated with hyperferritinemia (Table 2).

Pre-treatment factors associated with hyperferritinemia in participants with MASLD

Univariate logistic regression analysis revealed that age > 50 years (crude OR: 1.68; 95% CI 1.20–2.35, p = 0.003), fibrosis stage ≥ F3 (crude OR: 1.90; 95% CI 1.38–2.63, p < 0.001), FIB-4 score > 3.25 (crude OR: 2.29; 95% CI 1.62–3.23, p < 0.001), and ALT > 2 folds ULN (crude OR: 3.12; 95% CI 2.07–4.71, p < 0.001) were significantly associated with hyperferritinemia among participants with concurrent MASLD. In contrast, T2D, BMI at a cut-off of 23 kg/m^2, sex, HBV coinfection, HCV RNA at a cut-off value of 2,000,000 IU/mL, HCV genotype, and eGFR at a cut-off value of 60 mL/min/1.73 m^2 were not associated with hyperferritinemia (Table 3).

Multivariate logistic regression analysis identified FIB-4 score > 3.25 (adjusted OR: 1.62; 95% CI 1.05–2.50, p = 0.03) and ALT > 2 folds ULN (adjusted OR: 2.55; 95% CI 1.67–3.90, p < 0.001) were independent factors associated with hyperferritinemia (Table 3).

Correlation of serum HBV DNA with ferritin, ALT and FIB-4

Among the 144 participants with HBV coinfection, 74 (53.5%) had an HBV DNA level < LLOQ and 40 (28.8%) had an HBV DNA level between LLOQ and 2000 IU/mL. After $\log_{10}$ transforming serum HBV DNA and ferritin

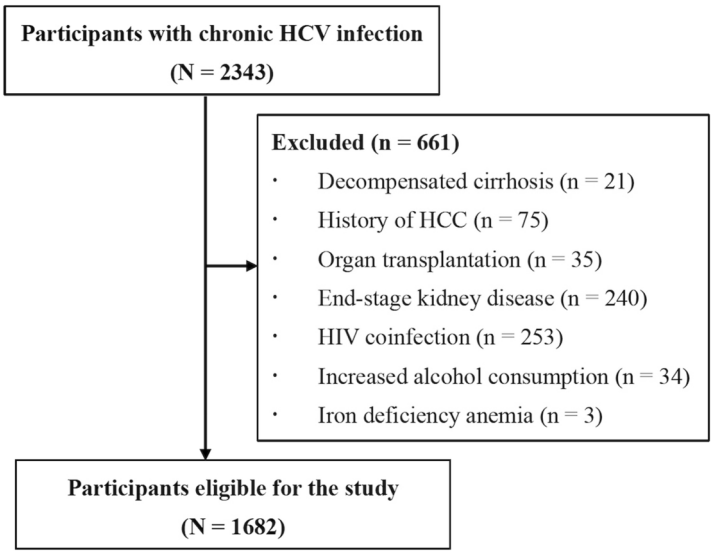

Figure 1. Study flow. *HCV* hepatitis C virus, *HCC* hepatocellular carcinoma, *HIV* human immunodeficiency virus.

Characteristics[a]	Participants (N = 1682)
Age (years)	56 (48–63)
Age > 50 years, n (%)	1161 (69.0)
Male, n (%)	864 (51.4)
HBV coinfection, n (%)	144 (8.6)
HCV RNA, log_{10}, IU/mL	6.07 (5.37–6.56)
HCV RNA > 2,000,000 IU/mL, n (%)	638 (37.9)
HCV genotype 1, (%)[b]	991 (58.9)
HBV DNA, log_{10} IU/mL[c]	0.00 (0.00–2.73)
LSM (kPa)[d]	7.5 (6.1–11.1)
Fibrosis stage (METAVIR), n (%)[e]	
F0–F1	699 (42.4)
F2	416 (25.3)
F3	199 (12.1)
F4	333 (20.2)
FIB-4	2.23 (1.42–3.73)
FIB-4 > 3.25, n (%)	515 (30.6)
CAP (dB/m)[d]	238 (214–268)
MASLD, n (%)	635 (38.6)
T2D	221 (13.4)
BMI ≥ 23 kg/m^2	407 (24.7)
BMI (kg/m^2)	25.3 (23.0–27.6)
BMI ≥ 23 kg/m^2, n (%)	1263 (76.9)
Platelet count, 10^9/L	174 (134–213)
INR	1.00 (0.96–1.04)
Albumin (g/dL)	4.3 (4.1–4.5)
Total bilirubin (mg/dL)	0.9 (0.7–1.1)
AST (U/L)	63 (40–103)
ALT (U/L)	86 (51–146)
ALT > 2 folds ULN, n (%)[f]	1294 (76.9)
eGFR, mL/min/1.73 m^2[g]	79 (68–94)
eGFR ≥ 60 mL/min/1.73 m^2[g]	1505 (89.5)
Ferritin (ng/mL)	231 (131–400)
Hyperferritinemia, n (%)[h]	782 (46.5)

Table 1. Baseline characteristics. *HBV* hepatitis B virus, *HCV* hepatitis C virus, *RNA* ribonucleic acid, *IU* international unit, *DNA* deoxyribonucleic acid, *LSM* liver stiffness measurement, *kPa* kilo Pascal, *FIB-4* fibrosis index based on four parameters, *CAP* controlled attenuation parameter, *dB* decibel, *MASLD* metabolic dysfunction-associated steatotic liver disease, *T2D* type 2 diabetes; BMI, body mass index, *INR* international normalized ratio, *AST* aspartate transaminase, *ALT* alanine transaminase, *ULN* upper limit of normal, *eGFR* estimated glomerular filtration rate. [a]Data are shown in median (interquartile range, IQR) unless otherwise indicated. [b]Participants with HCV genotype 1a, genotype 1b, genotype 1a plus 1b, or unsubtypable genotype 1 were regarded as HCV genotype 1 infection. [c]Data was set as 0 for serum HBV DNA level < lower limit of quantification (LLOQ)21. [d]Assessed by vibration-controlled transient elastography (VETE). Thirty-five participants without valid or reliable LSM or CAP were excluded from the analysis. [e]The cutoff values of LSM for a hepatic fibrosis stage of F0-1, F2, F3, and F4 are ≤ 7.0 kPa, 7.1–9.4 kPa, 9.5–12.4 kPa, and ≥ 12.5 kPa, respectively. [f]The ULN of ALT are 30 U/L for males and 19 U/L for females, respectively. [g]Calculated by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. [h]Serum ferritin levels are > 300 ng/mL for males and > 200 ng/mL for females, respectively.

levels, the Spearman's rank correlation coefficient (*r*) between log_{10} HBV DNA level and log_{10} ferritin level was − 0.025 (p = 0.81) (Fig. 2A). Regarding hepatic necroinflammation, the Spearman's rank correlation coefficients (*r*) were − 0.099 (p = 0.24) between log_{10} HBV DNA level and ALT level (Fig. 3A). Moreover, the Spearman's rank correlation coefficients (*r*) were − 0.140 (p = 0.09) between log_{10} HBV DNA level and FIB-4 score (Fig. 3C).

Correlation of serum HCV DNA with ferritin, ALT and FIB-4

The Spearman's rank correlation coefficient (*r*) between log_{10} HCV RNA level and log_{10} ferritin level was 0.002 (p = 0.92) (Fig. 2B). Regarding hepatic necroinflammation, the Spearman's rank correlation coefficients (*r*) were − 0.025 (p = 0.31) between log_{10} HCV RNA level and ALT level (Fig. 3B). Moreover, the Spearman's rank correlation coefficients (*r*) were − 0.043 (p = 0.08) between log_{10} HCV RNA level and FIB-4 score (Fig. 3D).

Parameter	Univariate analysis				Multivariate analysis[a]	
	Hyperferritinemia					
	No (n = 900)[b]	Yes (n = 782)[b]	Crude OR (95% CI)[c]	p value	Adjusted OR (95% CI)	p value
Age (years)						
> 50	573 (63.7)	588 (75.2)	1.73 (1.40–2.14)	< 0.001	1.38 (1.09–1.74)	0.008
≤ 50	327 (36.3)	194 (24.8)	Ref		Ref	
Sex						
Male	472 (49.1)	392 (50.1)	0.91 (0.75–1.10)	0.34	–	–
Female	428 (50.9)	390 (49.9)	Ref			
HBV coinfection						
Yes	73 (8.1)	71 (9.1)	1.13 (0.80–1.59)	0.48	–	–
No	827 (91.9)	711 (90.9)	Ref			
HCV RNA						
> 2,000,000 IU/mL	337 (37.4)	301 (38.5)	1.05 (0.86–1.27)	0.66	–	–
≤ 2,000,000 IU/mL	563 (62.6)	481 (61.5)	Ref			
HCV genotype 1						
Yes	514 (57.1)	477 (61.0)	1.17 (0.97–1.43)	0.11	–	–
No	386 (42.9)	305 (39.0)	Ref			
Fibrosis stage[a]						
≥ F3	212 (24.2)	320 (41.5)	2.22 (1.80–2.74)	< 0.001	1.36 (1.04–1.77)	0.02
< F3	664 (75.8)	451 (58.5)	Ref		Ref	
FIB-4						
> 3.25	199 (22.1)	316 40.4)	2.39 (1.93–2.95)	< 0.001	1.46 (1.11–1.92)	0.01
≤ 3.25	701 (77.9)	466 (59.6)	Ref		Ref	
MASLD[a]						
Present	298 (34.0)	337 (43.7)	1.51 (1.23–1.84)	< 0.001	1.43 (1.12–1.76)	0.001
Absent	578 (66.0)	434 (56.3)	Ref		Ref	
ALT, ULN						
> 2 folds	606 (67.3)	688 (88.0)	3.55 (2.75–4.59)	< 0.001	2.87 (2.20–3.75)	< 0.001
≤ 2 folds	294 (32.7)	94 (12.0)	Ref		Ref	
eGFR (mL/min/1.73 m^2)						
< 60	82 (19.1)	95 (12.1)	1.38 (1.01–1.89)	0.04	–	–
≥ 60	818 (90.9)	687 (87.9)	Ref			

Table 2. Univariate and multivariate regression analysis for factors associated with hyperferritinemia. *OR* odds ration, *CI* confidence interval, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *RNA* ribonucleic acid, *IU* international unit, *FIB-4* fibrosis index based on four parameters, *MASLD* metabolic dysfunction-associated steatotic liver disease, *ALT* alanine transaminase, *ULN* upper limit of normal, *eGFR* estimated glomerular filtration rate. [a]Thirty-five participants without valid or reliable LSM or CAP were excluded from the univariate and multivariate analyses. [b]Values in the corresponding columns are shown as numbers (percentages) unless otherwise indicated. [c]Factors with a p value of < 0.10 in univariate analysis entered multivariate analysis.

Discussion

Our study, encompassing a sample size of 1682 patients, represents the most extensive population analyzed to date in examining the factors and their correlation with pre-treatment ferritin levels in patients with HCV. To ensure the robustness of our study and minimize confounding variables, we diligently excluded patients with decompensated cirrhosis, a history of HCC, ESKD, HIV coinfection, organ transplantation, heightened alcohol consumption, and iron deficiency anemia that enhanced the reliability and validity.

We demonstrated that individuals aged > 50 years and those with ALT levels exceeding > 2 folds ULN were more likely to exhibit hyperferritinemia, consistent with previous researches[9,23,24]. Conversely, sex and eGFR at a cut-off of 60 mL/min/1.73 m^2 were not found to be correlated with hyperferritinemia. The apparent discrepancy in the influence of eGFR on ferritin dynamics compared to earlier reports may be attributed to our exclusion of participants with ESKD and the inclusion of a limited number of participants with an eGFR below 60 mL/min/1.73 m^2, who were susceptible to hyperferritinemia[25].

Patients with HCV are at risk of developing insulin resistance, which can lead to MS[26–28]. The prevalence of NAFLD among individuals with HCV is estimated to be as high as 55%, with a range spanning from 40 to 86%, influenced by the distribution of MS and HCV genotypes[29]. According to the diagnostic criteria of MASLD, an updated nomenclature aimed at encompassing a broader spectrum of etiologies for steatotic liver disease, our population exhibits a prevalence rate of MASLD of 38.6%[20]. Within MASLD alone, there is a notable correlation between hyperferritinemia and the severity of liver fibrosis[30,31]. While no studies have specifically examined

Parameter	Univariate analysis				Multivariate analysis	
	Hyperferritinemia					
	No (n = 298)[b]	Yes (n = 337)[b]	Crude OR (95% CI) [c]	p value	Adjusted OR (95% CI)	p value
T2D						
Present	96 (32.2)	125 (37.1)	1.24 (0.89–1.72)	0.20	–	–
Absent	202 (67.8)	212 (62.9)	Ref			
BMI						
≥ 23 kg/m^2	292 (98.0)	324 (96.1)	0.51 (0.19–1.37)	0.18	–	–
< 23 kg/m^2	6 (2.0)	13 (3.9)	Ref			
Age (years)						
> 50	113 (37.9)	90 (26.7)	1.68 (1.20–2.35)	0.003	1.24 (0.87–1.79)	0.24
≤ 50	185 (62.1)	247 (73.3)	Ref		Ref	
Sex						
Male	172 (57.7)	178 (52.8)	0.82 (0.60–1.12)	0.22	–	–
Female	126 (42.3)	159 (47.2)	Ref			
HBV coinfection						
Yes	27 (19.1)	38 (11.3)	1.28 (0.76–2.15)	0.36	–	–
No	271 (90.9)	299 (88.7)	Ref			
HCV RNA						
> 2,000,000 IU/mL	124 (41.6)	122 (36.2)	0.80 (0.58–1.10)	0.16	–	–
≤ 2,000,000 IU/mL	174 (58.4)	215 (63.8)	Ref			
HCV genotype 1						
Yes	169 (56.7)	202 (59.9)	1.14 (0.83–1.57)	0.41	–	–
No	129 (43.3)	135 (40.1)	Ref			
Fibrosis stage						
≥ F3	96 (32.2)	160 (47.5)	1.90 (1.38–2.63)	< 0.001	1.20 (0.81–1.79)	0.37
< F3	202 (67.8)	177 (52.5)	Ref		Ref	
FIB-4						
> 3.25	70 (23.5)	139 (41.2)	2.29 (1.62–3.23)	< 0.001	1.62 (1.05–2.50)	0.03
≤ 3.25	228 (76.5)	198 (58.8)	Ref		Ref	
ALT, ULN						
> 2 folds	208 (69.8)	296 (87.8)	3.12 (2.07–4.71)	< 0.001	2.55 (1.67–3.90)	< 0.001
≤ 2 folds	90 (30.2)	41 (12.2)	Ref		Ref	
eGFR (mL/min/1.73 m^2)						
< 60	39 (13.1)	45 (13.4)	1.02 (0.65–1.62)	0.92	–	–
≥ 60	259 (86.9)	292 (86.6)	Ref			

Table 3. Univariate and multivariate regression analysis for factors associated with hyperferritinemia in participants with MASLD. *OR* odds ration, *CI* confidence interval, *MASLD* metabolic dysfunction-associated steatotic liver disease, *T2D* type 2 diabetes, *BMI* body mass index, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *RNA* ribonucleic acid, *IU* international unit, *FIB-4* fibrosis index based on four parameters, *ALT* alanine transaminase, *ULN* upper limit of normal, *eGFR* estimated glomerular filtration rate. [a]Values in the corresponding columns are shown as numbers (percentages) unless otherwise indicated. [b]Factors with a p value of < 0.10 in univariate analysis entered multivariate analysis.

the prevalence rate of hyperferritinemia in individuals with concurrent HCV and MASLD, it can be inferred that this population likely exhibits greater rates compared to HCV or MASLD alone. The prevalence rate of hyperferritinemia among our patients reached 53%, significantly higher than the reported prevalence rates of approximately 30% in subjects with MASLD alone[32].

Chronic HCV infection induces hepatocyte injury, accompanied by the accumulation of extracellular matrix (ECM) proteins, ultimately resulting in accelerating hepatic fibrotic. The process of fibrotic change is dynamic, with later stages indicating increased severity[33,34]. While the stage of hepatic fibrosis and hyperferritinemia indicate the severity of hepatocyte damage, previous studies have provided limited evidence linking the severity of hepatic fibrosis to hyperferritinemia. Our study is the first to demonstrate a positive correlation between hyperferritinemia and the severity of hepatic fibrosis, utilizing two noninvasive indices, the FIB-4 scores and LSMs, as assessment tools. Univariate analysis revealed either higher FIB-4 scores (> 3.25) or LSMs (≥ 9.5 kPa) were associated with hyperferritinemia in both the entire population and those with MASLD. Due to the high collinearity between the FIB-4 score and LSM, and a relatively smaller sample size compared to the entire population, LSM was excluded as an independent risk factor in the multivariate model for association with hyperferritinemia among participants with MASLD. We also demonstrated that within the context of MASLD,

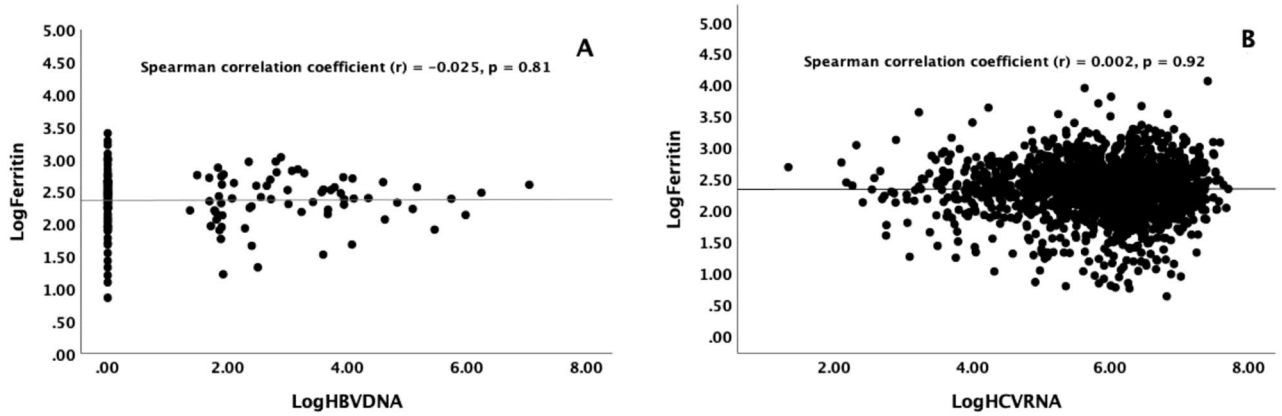

Figure 2. Correlation of serum HBV DNA/HCV RNA and ferritin levels. The Spearman's rank correlation coefficients (*r*) were −0.025 (p = 0.81) between $\log_{10}$ HBV DNA level and $\log_{10}$ ferritin level (**A**), and 0.002 (p = 0.92) between $\log_{10}$ HCV RNA level and $\log_{10}$ ferritin level (**B**).

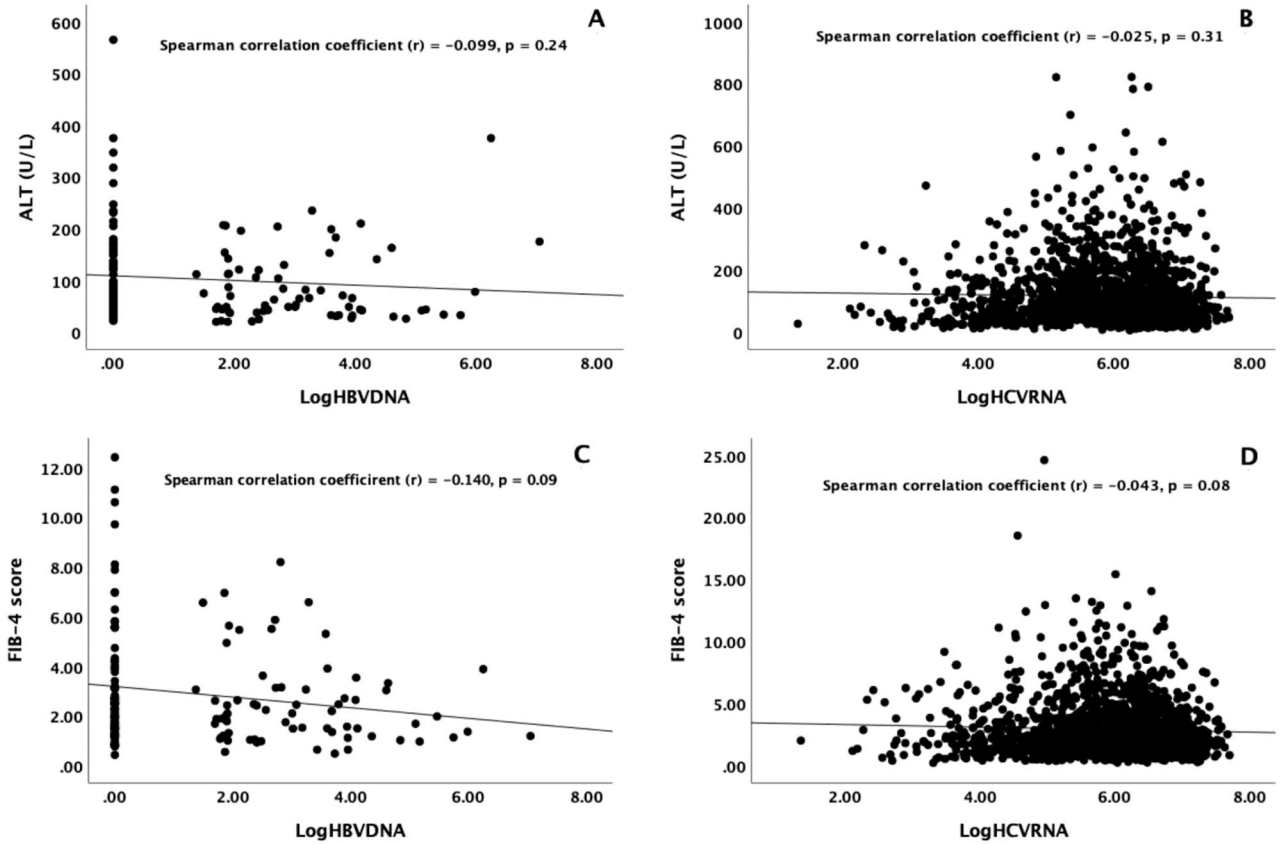

Figure 3. Correlation of serum HBV DNA/HCV RNA and ALT/FIB-4 levels. The Spearman's rank correlation coefficients (*r*) were −0.099 (p = 0.24) between $\log_{10}$ HBV DNA level and ALT level (**A**), and −0.025 (p = 0.31) between $\log_{10}$ HCV RNA level and ALT level (**B**). The Spearman's rank correlation coefficients (*r*) were −0.140 (p = 0.09) between $\log_{10}$ HBV DNA level and FIB-4 score (**C**), and −0.043 (p = 0.08) between $\log_{10}$ HCV RNA level and FIB-4 score (**D**).

T2D or obesity did not increase the risk of hyperferritinemia. This implies that the presence of MS, regardless of the potential contributing factors, may lead to systemic inflammation, thus resulting in hyperferritinemia[20,35,36].

Current evidence indicates that neither the HCV vial load nor genotype correlates with ALT levels or histological presentation, including necroinflammation or fibrosis. This is evident from our observations, showing no correlation between either ALT level or FIB-4 score with HCV viral load[37]. However, to date, no studies have investigated the correlation of hyperferritinemia with HCV viral load or genotype. This study is the first to demonstrate a lack of significant correlation between HCV viral load/genotype and hyperferritinemia.

With regard to HBV infection, prior studies hypothesized that active HBV replication may result in elevated serum levels of iron and ferritin[38]. However, our participants with HBV coinfection, characterized by an almost negligible median $\log_{10}$ HBV viral load of 0 and more than half of them without detectable HBV DNA, demonstrated that neither HBsAg nor the viral load were associated with hyperferritinemia. Additionally, the median $\log_{10}$ HBV viral load did not show a significant association with ALT levels or FIB-4 scores. While our observation fails to confirm the proposed hypothesis, the lack of association of hyperferritinemia with HBV DNA due to the suppression of HBV load from active HCV replication may not be extrapolated to individuals with HBV alone, where the HBV viral load tends to be higher than that in HBV/HCV-coinfected subjects[39,40].

Although the risk of hyperferritinemia is consistently increased among individuals with chronic HCV infection, it remains unclear if there is a dosing effect of viral load on serum ferritin levels. In contrast to the low HBV load in our coinfected participants, most of whom had active HCV replication, as evidenced by a high median viral load. However, the HCV load did not exhibit a significant association with serum ferritin levels, ALT levels, or FIB-4 scores. Based on the similar trends observed in the association of HBV or HCV load with serum ferritin levels, we deduce that rather than direct virological effects, the serum level of ferritin is augmented indirectly through immune responses to HBV or HCV.

Despite enrolling a sizable number of participants and meticulously excluding potential confounders to mitigate the imprecise risk estimates, several limitations are present in the current study. Firstly, this study was conducted exclusively in East Asians, and external validation should be conducted to confirm our findings in other ethnic populations. Secondly, we did not conduct HFE C282Y and H63D testing to exclude participants with HFE hemochromatosis. However, the presence of C282Y homozygotes and C282Y/H63D heterozygote in Asians is rare, rendering the genetic testing unnecessary[5]. Thirdly, we did not analyze the serum iron and transferrin saturation because only a minority of subjects with chronic viral hepatitis exhibit these derangements[2].

In conclusion, our study reveals that hyperferritinemia in patients with chronic HCV infection is primarily influenced by host factors such as age, severity of hepatic fibrosis, MASLD, and ALT levels, rather than viral factors. While viral factors may not directly predispose to hyperferritinemia, vigilant management of HBV and HCV using effective antiviral therapies remains essential to indirectly mitigate hyperferritinemia by reducing viral-induced hepatic necroinflammation and fibrosis.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

Conceptualization: Chun-Jen Liu, Chen-Hua Liu. Data curation: Chen-Hua Liu. Formal analysis: Yu-Ping Chang, Chen-Hua Liu. Investigation: Yu-Ping Chang, Chiuan-Bo Huang, Jia-Horng Kao, Tung-Hung Su, Shang-Chin Huang, Tai-Chung Tseng, Pei-Jer Chen, Chun-Jen Liu, Chen-Hua Liu. Methodology: Yu-Ping Chang, Chun-Jen Liu, Chen-Hua Liu. Project administration: Yu-Ping Chang, Chun-Jen Liu, Chen-Hua Liu. Resources: Yu-Ping Chang, Chiuan-Bo Huang, Jia-Horng Kao, Tung-Hung Su, Shang-Chin Huang, Tai-Chung Tseng, Pei-Jer Chen, Chun-Jen Liu, Chen-Hua Liu. Software: Yu-Ping Chang, Chen-Hua Liu. Supervision: Chen-Hua Liu. Validation: Yu-Ping Chang, Chen-Hua Liu. Visualization: Yu-Ping Chang, Chen-Hua Liu. Writing – original draft: Yu-Ping Chang, Chun-Jen Liu, Chen-Hua Liu. Writing – review & editing: Yu-Ping Chang, Chiuan-Bo Huang, Jia-Horng Kao, Tung-Hung Su, Shang-Chin Huang, Tai-Chung Tseng, Pei-Jer Chen, Chun-Jen Liu, Chen-Hua Liu.

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Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to C.-J.L. or C.-H.L.