Impact of HBV infection on clinical outcomes in patients with metabolic dysfunction-associated fatty liver disease

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Background & Aims: The new name and diagnostic criteria of metabolic-associated fatty liver disease (MAFLD) was proposed in 2020. Although chronic HBV infection has protective effects on lipid profiles and hepatic steatosis, the impact of chronic HBV infection on clinical outcomes of MAFLD requires further investigation.

Methods: The participants from a Taiwan bio-bank cohort were included. MAFLD is defined as the presence of hepatic steatosis plus any of the following three conditions: overweight/obesity, type 2 diabetes mellitus, and metabolic dysfunction. The patients with positive glycated haemoglobin were considered as having chronic HBV infection. Atherosclerosis was determined as having carotid plaques on duplex ultrasound. Advanced liver fibrosis was defined as Fibrosis-4 >2.67. Based on the status of MAFLD and HBV infection, the participants were distributed into four groups: 'dual aetiology', 'MAFLD alone', 'HBV alone', and 'healthy controls'.

Results: A total of 20,460 participants (age 55.51 ± 10.37; males 32.67%) were included for final analysis. The prevalence of MAFLD and chronic HBV infections were 38.8% and 10.3%, respectively. According to univariate analysis, 'HBV alone' group had lower levels of glycated haemoglobin, lipid profiles, and intima media thickness than healthy controls. The 'dual aetiology' group had lower levels of triglycerides, cholesterol, γ-glutamyl transferase, intima media thickness, and percentage of carotid plaques than 'MAFLD alone' group. Using binary logistic regression, chronic HBV infection increased the overall risk of advanced liver fibrosis; and had a lower probability of carotid plaques in MAFLD patients, but not in those without MAFLD.

Conclusions: The large population-based study revealed chronic HBV infection increases the overall risk of liver fibrosis, but protects from atherosclerosis in patients with MAFLD.

Impact and implications: Patients with metabolic-associated fatty liver disease can also be coinfected with chronic HBV. Concomitant HBV infection increases the overall risk of liver fibrosis, but protects from atherosclerosis in patients with MAFLD.

Introduction

Metabolic-associated fatty liver disease (MAFLD) is associated with metabolic syndrome and considered as the liver manifestation of metabolic syndrome.[1,2] The prevalence of MAFLD increases in parallel with the endemics of obesity and diabetes mellitus (DM). As nearly 1 billion people are affected by MAFLD worldwide, it produces a huge clinical and economic burden globally. Patients with MAFLD have an overall increase in mortality compared with the general population with the three main causes of death being cardiovascular disease, non-liver cancers, and liver-related diseases.[3] However, an international survey revealed a major deficiency in patient referrals and patterns of practice including diagnosis, staging, and indication of liver biopsy.[4] Chronic HBV infection induces liver-related complications and several extrahepatic manifestations including systemic vasculitides, glomerulonephritis, haematological malignancies, neurological diseases, and cutaneous manifestations.[5] Because HBV infection is classified as other aetiologies of chronic liver diseases in the exclusive criteria of non-alcoholic fatty liver disease (NAFLD), patients with HBV infection with hepatic steatosis are excluded from the diagnosis of NAFLD. The new disease name and diagnostic criteria of MAFLD was proposed in 2020 to replace the original term NAFLD.[6,7] If patients with chronic hepatitis B (CHB) show hepatic steatosis in imaging or histology and also have metabolic dysfunction, MAFLD can be diagnosed simultaneously. Thus, patients with 'concomitant HBV and MAFLD' are a new disease group and their natural history and clinical outcomes deserve further investigation.
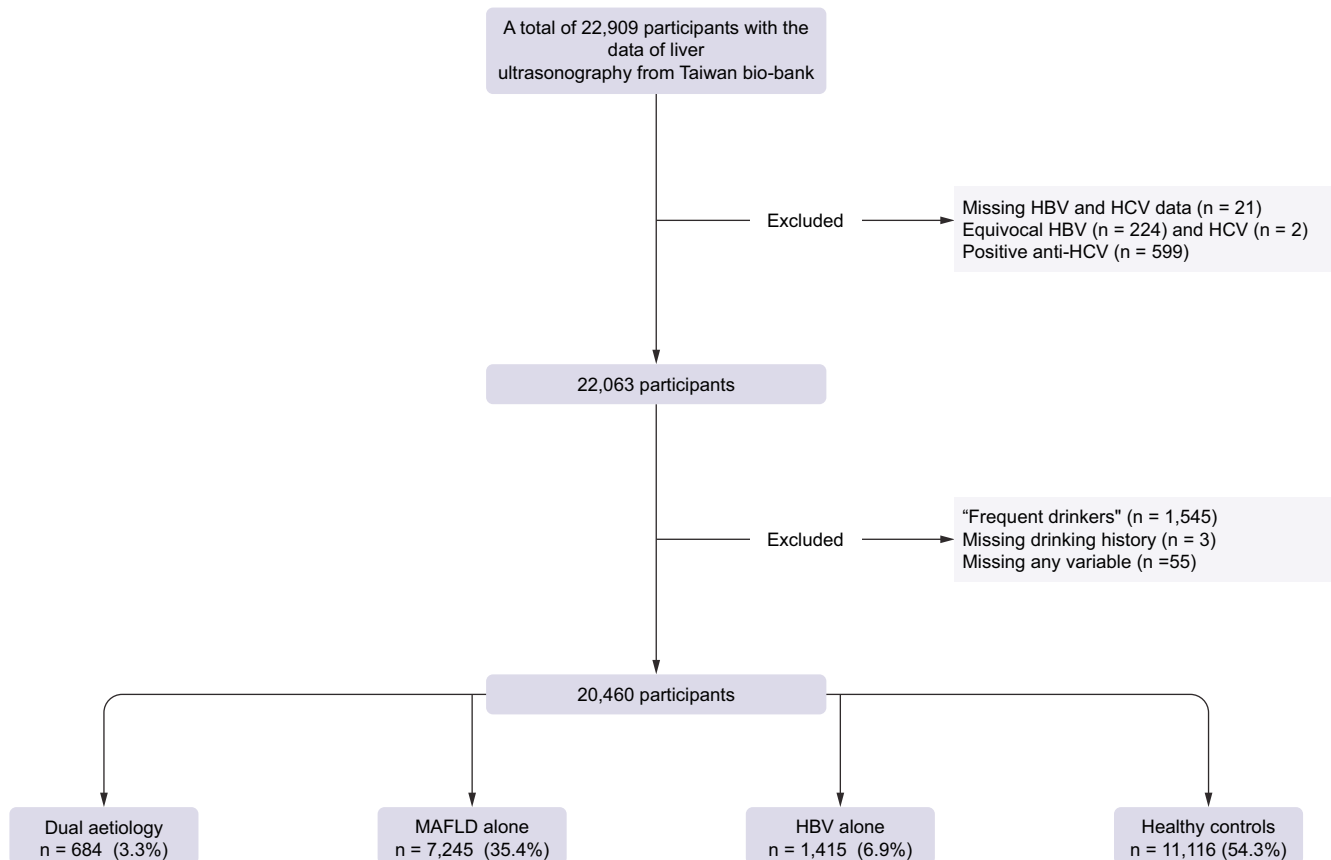
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A total of 22,909 participants with the
              data of liver
 ultrasonography from Taiwan bio-bank

                    │
   Excluded ────────►  Missing HBV and HCV data (n = 21)
                       Equivocal HBV (n = 224) and HCV (n = 2)
                       Positive anti-HCV (n = 599)
                    ▼
          22,063 participants

                    │
   Excluded ────────►  "Frequent drinkers" (n = 1,545)
                       Missing drinking history (n = 3)
                       Missing any variable (n = 55)
                    ▼
          20,460 participants
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Dual aetiology	MAFLD alone	HBV alone	Healthy controls
n = 684 (3.3%)	n = 7,245 (35.4%)	n = 1,415 (6.9%)	n = 11,116 (54.3%)

Fig. 1. Flowchart of the study design. MAFLD, metabolic-associated fatty liver disease.

The liver plays an essential role in glucose and lipid metabolism. Patients with CHB infection had lower levels of lipid profiles compared with those without.[8,9] Additionally, CHB infections were reported to protect from the development of hepatic steatosis.[10,11] However, several published studies showed an inverse association between hepatic steatosis and HBV replication.[12–14] Moreover, the risk of hepatocellular carcinoma was reduced in patients with CHB with hepatic steatosis.[15,16] However, the relationship between HBV infection and atherosclerosis remains inconclusive. Carotid duplex ultrasound can measure the intima media thickness (IMT) of carotid arteries and identify carotid plaques to assess the degree of atherosclerosis in clinical practice. Also, the degree of atherosclerosis reflects the risk of atherosclerotic cardiovascular disease (ASCVD). With the advantage of the Taiwan bio-bank cohort, a representative sample of the Taiwanese general population with carotid duplex ultrasound data, a study was conducted to investigate the impact of co-existing CHB infection on the risk of advanced liver fibrosis and atherosclerosis in patients with MAFLD.

Patients and methods

Patients and study design
The data were collected from the Taiwan bio-bank, a general population-based research database in Taiwan. The participants were enrolled through 43 recruitment stations since 2008. Until June 30, 2022, the number of participants increased to around 172,000. The methodologies of data collection from all participants are standardised procedures and have been described in previous studies.[17,18] Briefly, after obtaining informed consent, a formal questionnaire including lifestyle factors and medical comorbidities was performed by an experienced nurse. Demographic, clinical, and laboratory data were collected. All participants were invited to receive follow up at an interval of 2–4 years. At the first follow up, they received additional examinations including abdominal ultrasound, bone density measurement, and carotid duplex ultrasounds. The liver ultrasonography was performed by three hepatologists with specialist qualifications. The images were stored, but the data were based on local readings. The grade of hepatic steatosis was reported in 95.4% (mild, 58.1%; moderate, 30.4%; severe, 6.9%) of patients with MAFLD.

In the present study, participants with abdominal ultrasound data were recruited. The report of serum HBsAg being '±' was considered equivocal HBV infection. Those with equivocal HBV infection, missing data including serum HBsAg, anti-hepatitis C virus antibody (anti-HCV), record of alcohol consumption, or any required laboratory tests were excluded. Participants who reported to have persistent alcohol consumption for the past 3 months were considered as 'frequent drinkers'. The alcohol consumption was categorised into three groups: teetotalers/social drinkers, ex-drinkers, or frequent drinkers. To avoid the confounding effects of alcohol and chronic HCV infection, 'frequent drinkers' or those who were positive for anti-HCV antibody were excluded (Fig. 1). The diagnosis of MAFLD was based on the evidence of hepatic steatosis on liver

ultrasonography plus any of the following three criteria: overweight/obesity (BMI >23 kg/m^2), DM, and at least two metabolic risk abnormalities in participants who were non-diabetic and lean/normal weight. DM is defined as having a history of DM or serum glycated haemoglobin (HbA1c) >6.5%. Positive HBsAg defined CHB infection. The fatty liver index (FLI) was used to predict the grade of hepatic steatosis.[19] Fibrosis-4 (FIB-4) was calculated based on the formula: FIB-4 = age (years) × AST (U/L)/ [PLT (10^9/L) × ALT$^{1/2}$ (U/L)],[20] where AST is aspartate aminotransferase, PLT is platelets, and ALT is alanine aminotransferase. Atherosclerosis is a condition in which fatty deposits, called plaques, build up within the arterial walls. IMT is a measurement of the thickness of the innermost layer and middle layer of the arterial wall. The increased IMT is associated with a higher risk of developing atherosclerosis. Carotid plaque is a manifestation of atherosclerosis, and is used as a marker for the presence and severity of atherosclerosis in arteries. The study assessed the clinical outcomes of MAFLD, including the risks of advanced liver fibrosis and atherosclerosis. Advanced liver fibrosis was defined as a FIB-4 score of greater than 2.67. The presence of carotid plaques on duplex ultrasound was used to diagnose atherosclerosis, and this served as a surrogate marker for the risk of ASCVD.[21]

Ethical considerations

This study was performed in accordance with the principles of the 1975 Declaration of Helsinki and approved with waived informed consent by the Research Ethics Committee of Taipei Tzu Chi Hospital; Buddhist Tzu Chi Medical Foundation (approval numbers: 10-XD-055 and 11-X-074) and the Ethics and Governance Council of the TWB (approval numbers: TWBR11102-03).

Statistical analyses

The data were expressed as mean ± standard deviation for continuous variables and n (%) for categorical variables. Statistical analysis was performed using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). The data were analysed using the X^2 test and Student t test. A value of p <0.05 was considered statistically significant. The study participants were divided into four groups based on their HBV infection and MAFLD status: 'dual aetiology', 'MAFLD alone', 'HBV alone', or 'healthy controls'. The study assessed two important clinical outcomes: the risks of advanced liver fibrosis and atherosclerosis. The exposure of interest was CHB infection. As the population we were most interested in was patients with MAFLD, the clinical characteristics and outcomes were compared between the 'dual aetiology' and 'MAFLD alone' groups. As the age and/or sex of the participants were unmatched between the two groups, propensity score (PS) matching was used to assess the impact of HBV infection on the risks of liver fibrosis and ASCVD in patients with MAFLD. We utilised PS matching to enhance comparability between the two groups by balancing the known confounders. The individual PSs were estimated using a multivariable logistic regression model incorporating two covariates: age and sex. To perform the PS matching, patients with MAFLD alone were matched to patients with dual aetiology in a 1:1 nearest neighbour approach. The PS matching procedure was conducted using SAS software version 9.4 (SAS Institute, Inc., Cary, NC, USA) without replacement in this study. After PS matching, the covariates were well balanced, as evidenced by a standardised mean difference of 0.00 for age and sex. The factors associated with advanced liver fibrosis or the presence of carotid plaques were analysed using binary logistic

regression. The potential confounders included age, sex, DM, hypertension, and hyperlipidaemia for the risk of ASCVD, and age, sex, γ-glutamyl transferase (GGT), and FLI for the risk of advanced liver fibrosis. Because the impact of HBV on atherosclerosis as well as the impact of MAFLD on the clinical outcomes of patients infected with CHB were unresolved clinical questions, they were analysed and answered at the same time.

Results

A total of 22,909 participants with liver ultrasonography data were enrolled from the Taiwan bio-bank database. After exclusions, a total of 20,460 participants (age 55.51 ± 10.37; males 32.67%) were included in the final analysis. The prevalence of MAFLD and CHB infection was 38.8% and 10.3%, respectively. The percentages of 'dual aetiology', 'MAFLD alone', 'HBV alone', and 'healthy controls' were 3.3%, 35.4%, 6.9%, and 54.3%, respectively (Fig. 1).

Comparison of clinical characteristics and metabolic profiles between the 'HBV alone' group and 'healthy controls'

Compared with 'healthy controls', the 'HBV alone' group were younger, had a higher percentage of males, but lower frequencies of hypertension and dyslipidaemia histories. Furthermore, several metabolic factors including HbA1c, triglyceride (TG), total cholesterol, LDL, and HDL were lower in the 'HBV alone' group than healthy controls (Table S1).

Liver and ASCVD risks between the 'HBV alone' group and 'healthy controls'

The 'HBV alone' group had higher levels of ALT, AST, and FIB-4 index, but thinner IMTs than healthy controls (Table S1). Binary logistic regression found age, CHB infection, GGT, and FLI were

Table 1. Comparison between the 'Dual aetiology' and the 'MAFLD alone' groups (n = 7,929).

	MAFLD alone	Dual aetiology	
	n = 7,245	n = 684	*p* value
Age, years	56.40 ± 10.08	53.87 ± 9.69	<0.0001
Male, n (%)	2,916 (40.25)	319 (46.64)	0.0012
Diabetes, n (%)	887 (12.24)	75 (10.96)	0.3278
Hypertension, n (%)	1,818 (25.09)	138 (20.18)	0.0043
Dyslipidaemia, n (%)	1,220 (16.84)	81 (11.84)	0.0007
BMI, kg/m^2	26.47 ± 3.60	26.75 ± 3.66	0.0540
Glucose, mg/dl	103.49 ± 27.04	102.75 ± 27.11	0.4955
HbA1c, %	6.17 ± 1.03	6.13 ± 1.09	0.3429
TG, mg/dl	159.14 ± 122.09	143.94 ± 102.90	0.0003
CHO, mg/dl	199.80 ± 37.54	195.78 ± 38.92	0.0077
HDL, mg/dl	48.90 ± 11.07	48.57 ± 11.21	0.4473
LDL, mg/dl	125.24 ± 33.54	123.58 ± 33.63	0.2164
Uric acid, mg/dl	5.87 ± 1.40	5.90 ± 1.33	0.6222
AST, U/L	27.03 ± 14.74	29.04 ± 13.89	0.0004
ALT, U/L	30.28 ± 26.65	34.43 ± 26.99	<0.0001
GGT, U/L	28.78 ± 28.82	25.23 ± 17.44	<0.0001
Fatty liver index	42.44 ± 24.44	40.88 ± 23.90	0.1090
FIB-4	1.31 ± 0.67	1.35 ± 0.68	0.1111
NFS	-1.76 ± 1.28	-1.73 ± 1.21	0.5114
Creatinine, mg/dl	0.75 ± 0.24	0.77 ± 0.53	0.2299
eGFR, ml/min/1.732	103.48 ± 24.78	104.64 ± 23.72	0.2392
IMT, mm	0.64 ± 0.15	0.62 ± 0.13	0.0003
Carotid plaque, n (%)	2,556 (35.28)	184 (26.90)	<0.0001

Level of significance: p <0.05 (X^2 and Student t tests).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHO, cholesterol; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4; GGT, γ-glutamyl transferase; HbA1c, glycated haemoglobin; HTN, hypertension; IMT, intima media thickness; NFS, NAFLD fibrosis score; TG, triglycerides.

Table 2. Comparison between the 'dual aetiology' and the 'MAFLD alone' group using propensity score matching for age and sex.

	MAFLD alone	Dual aetiology	
	n = 684	n = 684	*p* value
Age, years	53.87 ± 9.69	53.87 ± 9.69	0.9978
Male, n (%)	319 (46.64)	319 (46.64)	>0.999
Diabetes, n (%)	81 (11.84)	75 (10.96)	0.6098
Hypertension, n (%)	157 (22.95)	138 (20.18)	0.2116
Hyperlipidaemia, n (%)	101 (14.77)	81 (11.84)	0.1113
BMI, kg/m^2	26.70 ± 3.94	26.75 ± 3.66	0.7963
Glucose, mg/dl	102.17 ± 22.84	102.75 ± 27.11	0.6647
HbA1c, %	6.10 ± 0.88	6.13 ± 1.09	0.5257
TG, mg/dl	161.57 ± 102.53	143.94 ± 102.90	0.0015
CHO, mg/dl	198.50 ± 35.90	195.78 ± 38.92	0.1790
HDL, mg/dl	48.37 ± 10.96	48.57 ± 11.21	0.7365
LDL, mg/dl	124.45 ± 31.99	123.58 ± 33.63	0.6224
Uric acid, mg/dl	5.95 ± 1.42	5.90 ± 1.33	0.4878
AST, U/L	26.94 ± 14.56	29.04 ± 13.89	0.0065
ALT, U/L	31.29 ± 35.07	34.43 ± 26.99	0.0635
GGT, U/L	29.06 ± 25.67	25.23 ± 17.44	0.0013
Fatty liver index	43.82 ± 25.77	40.88 ± 23.90	0.0289
FIB-4	1.20 ± 0.53	1.35 ± 0.68	<0.0001
NFS	-1.91 ± 1.25	-1.73 ± 1.21	0.0070
Creatinine, mg/dl	0.76 ± 0.21	0.77 ± 0.53	0.5456
eGFR, ml/min/1.732	103.75 ± 23.93	104.64 ± 23.72	0.4920
IMT, mm	0.63 ± 0.13	0.62 ± 0.13	0.4807
Carotid plaque, n (%)	226 (33.04)	184 (26.90)	0.0132

Level of significance: *p* <0.05 (X^2 and Student *t* tests).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHO, cholesterol; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4; GGT, γ-glutamyl transferase; HbA1c, glycated haemoglobin; HTN, hypertension; IMT, intima media thickness; NFS, NAFLD fibrosis score; TG, triglycerides.

factors associated with advanced liver fibrosis. Furthermore, age, male sex, hypertension, DM, and dyslipidaemia were factors associated with the presence of carotid plaques, but not CHB infection (Tables S2 and S3).

Comparison of clinical characteristics and metabolic profiles between 'dual aetiology' and 'MAFLD alone' groups
Compared with the 'MAFLD only' group, the 'dual aetiology' group were younger, and had a higher percentage of males, but lower frequencies of hypertension and dyslipidaemia histories. Furthermore, they had lower levels of TG, and total cholesterol. The percentage of DM, BMI, fasting glucose, HbA1c, HDL, LDL, and uric acid were comparable between the two groups (Table 1).

Liver and ASCVD risks between 'dual aetiology' and 'MAFLD alone' groups
The 'dual aetiology' group had higher levels of AST, ALT, but lower levels of GGT, thinner IMTs and lower percentages of carotid plaque than patients in the 'MAFLD alone' group. However, as these two groups were unmatched in age and sex, there was no difference in the FIB-4 score and NAFLD fibrosis score (NFS)

between the two groups in Table 2. By using PS matching for age and sex, the scores of FIB-4 and NFS were significantly higher in the 'dual aetiology' group as compared with the 'MAFLD only' group (Table 2). Furthermore, binary logistic regression found age, CHB infection, GGT, and FLI were factors associated with advanced liver fibrosis (Table 3). In addition, age, male sex, DM and history of hypertension were positively associated with the presence of carotid plaques; however, HBV infection had an inverse association (Table 4).

The impact of MAFLD, DM, or obesity on the liver and ASCVD risks of patients with CHB infection
Age was comparable between the 'dual aetiology' and 'HBV alone' groups. However, the 'dual aetiology' group had a higher percentage of males than the 'HBV alone' group. The 'dual aetiology' group had a lower FIB-4 score than the 'HBV alone' group, but a higher NFS. The 'dual aetiology' group had a greater IMT than the 'HBV alone' group, but the percentages of carotid plaques were comparable between these two groups (Table 5). The results remained unchanged after using PS matching for age and sex (Table S4).

Table 3. Factors associated with biomarkers of advanced liver fibrosis in patients with MAFLD (n = 7,929).

FIB-4 > 2.67	AOR	(95% CI)	*p* value
Age, years	1.151	(1.127–1.175)	<0.0001
Male	1.017	(0.776–1.332)	0.9049
HBV	1.967	(1.288–3.003)	0.0017
GGT, U/L	1.003	(1.000–1.006)	0.0314
FLI	1.007	(1.001–1.013)	0.0195

Level of significance: *p* <0.05 (binary logistic regression).
AOR, adjusted odds ratio; FIB-4, fibrosis-4; FLI, fatty liver index; GGT, γ-glutamyl transferase; MAFLD, metabolic-associated fatty liver disease; NFS, NAFLD fibrosis score.

Table 4. Factors associated with carotid plaques in patients with MAFLD (n = 7,929).

	AOR	95% CI	*p* value
Age, years	1.100	(1.093–1.107)	<0.0001
Male	1.660	(1.493–1.846)	<0.0001
HBV	0.817	(0.674–0.991)	0.0403
DM	1.577	(1.352–1.838)	<0.0001
HTN	1.575	(1.398–1.774)	<0.0001
Dyslipidaemia	1.107	(0.965–1.271)	0.1475

Level of significance: *p* <0.05 (binary logistic regression).
AOR, adjusted odds ratio; DM, diabetes mellitus; HTN, hypertension; MAFLD, metabolic-associated fatty liver disease.

Table 5. The comparison of clinical characteristics and outcomes between those with MAFLD and those without in patients with chronic HBV infection (n = 2,099).

	HBV alone	Dual aetiology	
	n = 1,415	n = 684	p value
Age, years	54.19 ± 9.78	53.87 ± 9.69	0.4808
Male, n (%)	481 (33.99)	319 (46.64)	<0.0001
Diabetes, n (%)	59 (4.17)	75 (10.96)	<0.0001
Hypertension, n (%)	159 (11.24)	138 (20.18)	<0.0001
Dyslipidaemia, n (%)	97 (6.86)	81 (11.84)	0.0001
Alcohol, n (%)	58 (4.10)	30 (4.39)	0.7584
BMI, kg/m²	22.98 ± 3.05	26.75 ± 3.66	<0.0001
Glucose, mg/dl	93.22 ± 16.85	102.75 ± 27.11	<0.0001
HbA1c, %	5.68 ± 0.61	6.13 ± 1.09	<0.0001
TG, mg/dl	90.92 ± 49.90	143.94 ± 102.90	<0.0001
CHO, mg/dl	193.33 ± 34.83	195.78 ± 38.92	0.1627
HDL, mg/dl	58.22 ± 13.97	48.57 ± 11.21	<0.0001
LDL, mg/dl	116.72 ± 29.95	123.58 ± 33.63	<0.0001
Uric acid, mg/dl	5.05 ± 1.26	5.90 ± 1.33	<0.0001
AST, U/L	28.05 ± 17.29	29.04 ± 13.89	0.1629
ALT, U/L	26.73 ± 31.27	34.43 ± 26.99	<0.0001
GGT, U/L	18.42 ± 25.93	25.23 ± 17.44	<0.0001
Fatty liver index	15.35 ± 15.78	40.88 ± 23.90	<0.0001
FIB-4	1.60 ± 0.88	1.35 ± 0.68	<0.0001
NFS	-1.84 ± 1.17	-1.73 ± 1.21	0.0413
Creatinine, mg/dL	0.74 ± 0.61	0.77 ± 0.53	0.1539
eGFR, ml/min/1.732	107.10 ± 24.84	104.64 ± 23.72	0.0310
IMT, mm	0.58 ± 0.13	0.62 ± 0.13	<0.0001
Carotid plaque, n (%)	364 (25.72)	184 (26.90)	0.5653

Level of significance: $p < 0.05$ (X^2 and Student t tests).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHO, cholesterol; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4; GGT, γ-glutamyl transferase; HbA1c, glycated haemoglobin; HTN, hypertension; IMT, intima media thickness; NFS, NAFLD fibrosis score; TG, triglycerides.

Discussion

The study included 20,460 participants and found that 3.3% had concomitant HBV and MAFLD. Using binary logistic regression analysis, researchers found that concomitant HBV infection was linked to a higher risk of advanced liver fibrosis in patients with MAFLD. These findings suggest that chronic HBV infection can worsen liver injury in people with MAFLD. Interestingly, the study also found that chronic HBV infection was inversely associated with carotid plaques in MAFLD patients, suggesting a protective effect against ASCVD. However, this protective effect was not observed in patients without MAFLD.

Chronic HBV infection can cause liver inflammation, fibrosis, cirrhosis, or even hepatocellular carcinoma.[22] With the increasing prevalence of obesity and diabetes mellitus, CHB patients with hepatic steatosis are becoming more common in clinical practice. However, the relationship between hepatic steatosis and chronic HBV infection is complex. Hepatic steatosis has been shown to suppress HBV viral replication, whereas CHB infection appears to have a protective effect on the occurrence of hepatic steatosis. A recent meta-analysis of 98 studies involving 48,472 patients with CHB infection found no significant association between hepatic steatosis and liver fibrosis progression.[23] Our study showed contradictory results in the markers of liver fibrosis between the 'dual aetiology' and 'HBV alone' groups, suggesting that the impact of concomitant MAFLD on liver fibrosis in patients with CHB infection is inconclusive. Although hepatic steatosis itself can damage the liver, the liver injury caused by CHB infection may be reduced through the suppressive effect of hepatic steatosis on HBV replication in patients with CHB infection. Therefore, further investigation is needed to determine the impact of MAFLD on liver fibrosis in patients with CHB infection. In contrast, the 'dual aetiology' group had a higher risk of advanced liver fibrosis than the 'MAFLD alone' group. It is important to note that hepatic steatosis or NAFLD is not equal to the new nomenclature of MAFLD, which requires two essential criteria including hepatic steatosis and metabolic dysfunction.[24] Therefore, the impact of concomitant HBV infection on the progression of liver diseases in patients with MAFLD has rarely been reported. This large, population-based study is the first to investigate the relationship between CHB infection and advanced liver fibrosis in patients with MAFLD, suggesting that concomitant HBV infection has a synergistic effect on liver injury in patients with MAFLD. As a result, a more aggressive anti-HBV therapeutic strategy may be necessary for patients with concomitant HBV and MAFLD in clinical practice.

Cardiovascular disease is the primary cause of mortality in patients with MAFLD, and the severity of atherosclerosis indicates the risk of ASCVD.[25] However, the relationship between CHB infection, insulin resistance, and atherosclerosis is not well understood. Moreover, the influence of concomitant CHB infection on the risk of ASCVD in patients with MAFLD patients has been poorly studied. In our large, population-based study, binary logistic regression analysis revealed an inverse association between CHB infection and the presence of carotid plaques in MAFLD patients, indicating that CHB infection may protect against the risk of ASCVD in patients with MAFLD. The protective effect of CHB infection may be attributable to its lipid-lowering effect. However, in patients with HBV without MAFLD, a population without metabolic dysfunction and at low risk of ASCVD, HBV infection did not have a protective effect on the risk of ASCVD. The discrepant results may be because of the modest protective effect of HBV infection on the risk of ASCVD, which was insufficient to reach statistical significance in this low-risk population.

Our study has several strengths. Firstly, it is the first large, population-based study to investigate the impact of concomitant HBV infection on the risks of liver fibrosis and ASCVD in patients with MAFLD. Secondly, we simultaneously assessed the risks of liver fibrosis and ASCVD using biomarkers of liver fibrosis and data from carotid duplex ultrasound. Thirdly, we excluded participants with positive anti-HCV antibody or those who were frequent drinkers to avoid the confounding effects of HCV and alcohol on the risks of liver fibrosis and ASCVD. However, some limitations should also be addressed. Firstly, the diagnosis of hepatic steatosis based on ultrasonography may miss minor degrees of steatosis. Additionally, the FLI has a modest accuracy of 0.84 in detecting hepatic steatosis. Secondly, liver biopsy, which is invasive and not well suited for population-based studies, was not available for histology. Thirdly, as data on high-sensitivity C reactive protein and insulin resistance were not available, the prevalence of MAFLD may have been underestimated. Fourthly, the data on HBV genotype, viral load, and anti-HBV treatment were not available in the study population. Finally, although the PS modelling can estimate the causal effect of HBV on clinical outcomes, the definite causal effect cannot be confirmed in this cross-sectional study.

To summarise, our study found that 'concomitant HBV and MAFLD' patients accounted for 3.3% of our study population. This newly identified disease group with a dual aetiology increases the risk of advanced liver fibrosis but protects from the risk of ASCVD when compared with 'MAFLD alone' patients. In clinical

practice, patients with CHB infection and MAFLD should be managed simultaneously, and similar surveillance and management as patients with 'MAFLD alone' should be provided, including lifestyle modifications, reduction of body weight, and treatment for comorbidities. Because of the synergistic effect of chronic HBV infection on liver injury of MAFLD, a more aggressive therapeutic strategy may be necessary in clinical practice.

New direct-acting antivirals for HBV, such as entry inhibitors, capsid assembly modulators, drugs targeting cccDNA or HBV RNA, and HBsAg secretion inhibitors, deserve further investigation and development.[26] Additionally, as the lipid-lowering effect of CHB infection may be reversed by successful viral suppression, monitoring of lipid profiles and adequate treatment are recommended during anti-HBV treatment.

Abbreviations
ALT, alanine aminotransferase; anti-HCV, anti-hepatitis C virus antibody; AOR, adjusted odds ratio; ASCVD, atherosclerotic cardiovascular disease; AST, aspartate aminotransferase; CHB, chronic hepatitis B; CHO, cholesterol; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis-4; FLI, fatty liver index; HbA1c, glycated haemoglobin; HTN, hypertension; IMT, intima media thickness; MAFLD, metabolic-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD fibrosis score; TG, triglyceride.

Conflicts of interest
All authors declare no conflicts of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions
Data collection: YMC. Statistical analysis: THH. Concept, design, and writing of article: CCW. Article revision: JHK.

Data availability statement
We declare the data was available and approved by the Ethics and Governance Council of the TWB (approval numbers: TWBR11102-03).

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2023.100836.

References
Author names in bold designate shared co-first authorship

[1] Asrih M, Jornayvaz FR. Metabolic syndrome and nonalcoholic fatty liver disease: is insulin resistance the link? Mol Cel Endocrinol 2015;418(Pt 1):55–65.

[2] Brea A, Puzo J. Non-alcoholic fatty liver disease and cardiovascular risk. Int J Cardiol 2013;167:1109–1117.

[3] Brunner KT, Pedley A, Massaro JM, Hoffmann U, Benjamin EJ, Long MT. Increasing liver fat is associated with progression of cardiovascular risk factors. Liver Int 2020;40:1339–1343.

[4] Ratziu V, Anstee QM, Wong VW, Schattenberg JM, Bugianesi E, Augustin S, et al. An international survey on patterns of practice in NAFLD and expectations for therapies – the POP-NEXT project. Hepatology 2022;76:1766–1777.

[5] Cacoub P, Asselah T. Hepatitis b virus infection and extra-hepatic manifestations: a systemic disease. Am J Gastroenterol 2022;117:253–263.

[6] Eslam M, Sanyal AJ, George J, International Consensus Panel. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. Gastroenterology 2020;158:1999–2014.e1.

[7] Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction associated fatty liver disease: an international expert consensus statement. J Hepatol 2020;73:202–209.

[8] Hsu CS, Liu CH, Wang CC, Tseng TC, Liu CJ, Chen CL, et al. Impact of hepatitis B virus infection on metabolic profiles and modifying factors. J Viral Hepat 2012;19:e48–e57.

[9] Su TC, Lee YT, Cheng TJ, Chien HP, Wang JD. Chronic hepatitis B virus infection and dyslipidemia. J Formos Med Assoc 2004;103:286–291.

[10] Cheng YL, Wang YJ, Kao WY, Chen PH, Huo TI, Huang YH, et al. Inverse Association between hepatitis B infection and fatty liver disease: a large scale study in populations seeking for check-up. PLoS One 2013;8:e72049.

[11] Wong VS, Wong GH, Chu WW, Chim AM, Ong A, Yeung DK, et al. Hepatitis B virus infection and fatty liver in the general population. J Hepatol 2012;56:533–540.

[12] **Liu Q, Mu M**, Chen H, Zhang G, Yang Y, Chu J, et al. Hepatocyte steatosis inhibits hepatitis B virus secretion via induction of endoplasmic reticulum stress. Mol Cel Biochem 2022;477:2481–2491.

[13] Hu D, Wang H, Wang H, Wang Y, Wan X, Yan W, et al. Nonalcoholic hepatic steatosis attenuates hepatitis B virus replication in an HBV-immunocompetent mouse model. Hepatol Int 2018;12:438–446.

[14] Huang SC, Kao JH. Metabolic dysfunction-associated fatty liver disease and chronic hepatitis B. J Formos Med Assoc 2022;121:2148–2151.

[15] Li J, Yang HI, Yeh ML, Le MH, Le AK, Yeo YH, et al. Association between fatty liver and cirrhosis, hepatocellular carcinoma, and hepatitis B surface antigen seroclearance in chronic hepatitis B. J Infect Dis 2021;224:294–302.

[16] Mak LY, Hui RW, Fung J, Liu F, Wong DK, Li B, et al. Reduced hepatic steatosis is associated with higher risk of hepatocellular carcinoma in chronic hepatitis B infection. Hepatol Int 2021;15:901–911.

[17] Fan CT, Lin JC, Lee CH. Taiwan biobank: a project aiming to aid Taiwan's transition into a biomedical island. Pharmacogenomics 2008;9:235–246.

[18] Lin JC, Fan CT, Liao CC, Chen YS. Taiwan biobank: making cross-database convergence possible in the big data era. Gigascience 2018;7:1–4.

[19] Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol 2006;6:33.

[20] Wang CC, Liu CH, Lin CL, Wang PC, Tseng TC, Lin HH, et al. Fibrosis index based on four factors better predicts advanced fibrosis or cirrhosis than aspartate aminotransferase/platelet ratio index in chronic hepatitis C patients. J Formos Med Assoc 2015;114:923–928.

[21] Kabłak-Ziembicka A, Przewłocki T. Clinical significance of carotid intima-media complex and carotid plaque assessment by ultrasound for the prediction of adverse cardiovascular events in primary and secondary care patients. J Clin Med 2021;10:4628.

[22] Lin CL, Kao JH. Perspectives and control of hepatitis B virus infection in Taiwan. J Formos Med Assoc 2015;114:901–909.

[23] **Jiang D, Chen C**, Liu X, Huang C, Yan D, Zhang X, et al. Concurrence and impact of hepatic steatosis on chronic hepatitis B patients: a systematic review and meta-analysis. Ann Transl Med 2021;9:1718.

[24] Cheng YM, Wang CC, Kao JH. Metabolic associated fatty liver disease better identifying patients at risk of liver and cardiovascular complications. Hepatol Int 2023;17:350–356.

[25] Zhu G, Hom J, Li Y, Jiang B, Rodriguez F, Fleischmann D, et al. Carotid plaque imaging and the risk of atherosclerotic cardiovascular disease. Cardiovasc Diagn Ther 2020;10:1048–1067.

[26] Asselah T, Loureiro D, Boyer N, Mansouri A. Targets and future direct-acting antiviral approaches to achieve hepatitis B virus cure. Lancet Gastroenterol Hepatol 2019;4:883–892.

Supplemental information

Impact of HBV infection on clinical outcomes in patients with metabolic dysfunction-associated fatty liver disease

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Table S1: Clinical characteristics and outcomes between the "HBV alone" group and the "healthy controls" in participants without MAFLD. Level of significance: p <0.05 (chi-square and student's t tests)

	Healthy controls N= 11116	HBV alone N= 1415	P-value
Age, year	55.21 ±10.62	54.19 ±9.78	0.0003
Male, n (%)	2969 (26.71)	481 (33.99)	<.0001
Diabetes, n (%)	537 (4.83)	59 (4.17)	0.2710
Hypertension, n (%)	1440 (12.95)	159 (11.24)	0.0682
Dyslipidemia, n (%)	1069 (9.62)	97 (6.86)	0.0008
BMI, kg/m^2	22.83 ±3.05	22.98 ±3.05	0.0743
Glucose, mg/dL	93.80 ±16.07	93.22 ±16.85	0.2176
HbA1c, %	5.73 ±0.64	5.68 ±0.61	0.0019
TG, mg/dL	94.90 ±54.41	90.92 ±49.90	0.0052
CHO, mg/dL	197.71 ±36.30	193.33 ±34.83	<.0001
HDL, mg/dL	59.16 ±13.73	58.22 ±13.97	0.0157
LDL, mg/dL	119.99 ±31.47	116.72 ±29.95	0.0001
Uric acid, mg/dL	5.01 ±1.25	5.05 ±1.26	0.2930
AST, U/L	23.67 ±9.07	28.05 ±17.29	<.0001
ALT, U/L	18.72 ±15.30	26.73 ±31.27	<.0001
GGT, U/L	19.06 ±20.66	18.42 ±25.93	0.3688
Fatty liver index	15.57 ±16.04	15.35 ±15.78	0.6329
FIB-4	1.46 ±0.73	1.60 ±0.88	<.0001
NFS	-1.88 ±1.20	-1.84 ±1.17	0.2835
Creatinine, mg/dL	0.71 ±0.33	0.74 ±0.61	0.0643
eGFR, ml/min/1.732	107.27 ±25.08	107.10 ±24.84	0.8096
IMT, mm	0.59 ±0.14	0.58 ±0.13	0.0209
Carotid plaque, n (%)	3088 (27.78)	364 (25.72)	0.1031

Abbreviation: DM, diabetes mellitus; HTN, hypertension; BMI, body mass index; HbA1c, glycated hemoglobin; TG, triglycerides; CHO, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; FIB-4, fibrosis-4; NFS, NAFLD fibrosis score; eGFR, estimated glomerular filtration rate; IMT, intima media thickness.

Table S2: Factors associated with biomarkers of advanced liver fibrosis in the participants without MAFLD (n=12531). Level of significance: p <0.05 (binary logistic regression)

FIB-4 > 2.67	AOR	(95% CI)	*P value*
Age, year	1.175	(1.158- 1.192)	<.0001
Male	1.143	(0.949- 1.377)	0.1593
HBV	2.215	(1.744- 2.813)	<.0001
GGT, U/L	1.008	(1.005- 1.011)	<.0001
FLI	0.986	(0.979- 0.992)	<.0001

Abbreviation: MAFLD, metabolic associated fatty liver disease; FIB-4, fibrosis-4; AOR, adjusted odds ratio; CI, confidence interval; GGT, γ-glutamyl transferase; FLI, fatty liver index; NFS, NAFLD fibrosis score.

Table S3: Factors associated with carotid plaques in the participants without MAFLD (n=12531). Level of significance: p <0.05 (binary logistic regression)

	AOR	95% CI	P value
Age, year	1.109	(1.103-1.116)	<.0001
Male	1.711	(1.555-1.882)	<.0001
HBV	1.003	(0.871-1.155)	0.9674
DM	1.521	(1.262-1.835)	<.0001
HTN	1.783	(1.579-2.015)	<.0001
Dyslipidemia	1.151	(1.001-1.323)	0.0480

Abbreviation: MAFLD, metabolic associated fatty liver disease; AOR, adjusted odds ratio; CI, confidence interval; HBV, hepatitis B virus; DM, diabetes mellitus; HTN, hypertension

Table S4: Comparison of clinical characteristics and outcomes between those with MAFLD and those without in the chronic HBV-infected patients using propensity score matching. Level of significance: p <0.05 (chi-square and student's t tests)

	HBV alone N= 684	Dual etiology N= 684	P-value
Age, year	53.88 ±9.65	53.87 ±9.69	0.9933
Male, n (%)	319 (46.64)	319 (46.64)	>0.999
Diabetes, n (%)	33 (4.82)	75 (10.96)	<.0001
Hypertension, n (%)	78 (11.40)	138 (20.18)	<.0001
Dyslipidemia, n (%)	48 (7.02)	81 (11.84)	0.0023
Alcohol, n (%)	27 (3.95)	30 (4.39)	0.6848
BMI, kg/m^2	22.95 ±2.88	26.75 ±3.66	<.0001
Glucose, mg/dL	93.92 ±17.50	102.75 ±27.11	<.0001
HbA1c, %	5.68 ±0.57	6.13 ±1.09	<.0001
TG, mg/dL	94.53 ±53.19	143.94 ±102.90	<.0001
CHO, mg/dL	193.52 ±34.27	195.78 ±38.92	0.2549
HDL, mg/dL	57.01 ±13.69	48.57 ±11.21	<.0001
LDL, mg/dL	118.10 ±29.78	123.58 ±33.63	0.0015
Uric acid, mg/dL	5.20 ±1.30	5.90 ±1.33	<.0001
AST, U/L	28.01 ±14.86	29.04 ±13.89	0.1897
ALT, U/L	27.30 ±28.86	34.43 ±26.99	<.0001
GGT, U/L	18.88 ±23.67	25.23 ±17.44	<.0001
Fatty liver index	15.89 ±15.81	40.88 ±23.90	<.0001
FIB-4	1.57 ±0.76	1.35 ±0.68	<.0001
NFS	-1.86 ±1.11	-1.73 ±1.21	0.0408
Creatinine, mg/dL	0.77 ±0.74	0.77 ±0.53	0.9879
eGFR, ml/min/1.732	105.46 ±21.88	104.64 ±23.72	0.5050
IMT, mm	0.58 ±0.14	0.62 ±0.13	<.0001
Carotid plaque, n (%)	176 (25.73)	184 (26.90)	0.6233

Abbreviation: DM, diabetes mellitus; HTN, hypertension; BMI, body mass index; HbA1c, glycated hemoglobin; TG, triglycerides; CHO, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; FIB-4, fibrosis-4; NFS, NAFLD fibrosis score; eGFR, estimated

glomerular filtration rate; IMT, intima media thickness.