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Incidence, predictors and prognosis of genotype 4 hepatitis E related liver failure: A tertiary nested case-control study

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Abstract

Background/Aims: Hepatitis E virus (HEV) infection has been recognized an important insult of acute or acute-on-chronic liver failure (A(C)LF). This study aimed to identify the incidence, predictors and outcomes of A(C)LF in patients with hepatitis E.

Methods: All patients diagnosed of hepatitis E between 2012 and 2018 in the tertiary hospital were retrospectively and consecutively analysed. Patients with hepatitis E who developed A(C)LF were enrolled as cases (HEV-LF) and controls were randomly selected from those who did not develop liver failure with 1:3 ratio in the same cohort.

Results: Eight hundred and nine patients were diagnosed with hepatitis E, among which 80 were identified with HEV-related liver failure (HEV-LF) with HEV as the solely acute aetiology of A(C)LF. Sequencing of HEV genome showed genotype (GT) 4 strains in all available serum samples. Hepatitis E patients with cirrhosis underwent higher risk to develop liver failure, compared to non-cirrhotic patients. Hydrothorax, respiratory infections, lower γ-glutamyl transferase, higher lactate dehydrogenase and alpha-foetoprotein were found to be independent predictors of A(C)LF in patients with hepatitis E. The 28-day and 90-day mortality for HEV-LF was 12.86% and 30.36% respectively. Renal injury and lower triglyceride were independent factors associated with 28-day mortality. Lower alanine aminotransferase and higher International normalized ratio were independent predictors of 90-day mortality.

Abbreviations: ACLF, acute-on-chronic liver failure; AFLD, alcoholic fatty liver disease; AFP, alpha-foetoprotein; AH, alcoholic hepatitis; ALD, alcoholic liver disease; ALF, acute liver failure; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHB, chronic hepatitis B; CLD, chronic liver disease; GGT, γ-glutamyl transferase; GT, genotype; Hb, haemoglobin; HBsAg, hepatitis B surface antigen; HE, hepatic encephalopathy; HEV, hepatitis E virus; HEV-LF, hepatitis E related liver failure; HEV-non-LF, hepatitis E patients who did not develop liver failure; Ig, immunoglobulin; INR, international normalized ratio; LDH, lactate dehydrogenase; LPC, liver stem/progenitor cells; PT, prothrombin time; TBA, total bile acid; TBiL, total bilirubin; TC, total cholesterol; TG, triglyceride.

Wang, H. Liu and S. Liu authors contributed equally to this work.

Conclusions: Patients with GT4 hepatitis E are at high risk to develop A(C)LF. Different CLD status impacted the incidence of HEV-LF distinctively. The identified variables shall help to identify HEV patients with high risk for developing liver failure and the risk for death.

KEYWORDS
chronic liver disease, hepatitis E, liver failure, mortality, predictors

1 | INTRODUCTION

Liver failure involves rapid deterioration of liver function and accounts for high mortality. Clinical presentation usually includes loss of liver metabolic function, coagulopathy, hepatic encephalopathy (HE) and multi-organ failure.[1] Liver failure can present as acute liver failure (ALF), which mostly occurs in patients without any preexisting liver disease, and acute-on-chronic liver failure (ACLF), which caused by superimposed acute insult in patients with underlying chronic liver disease (CLD).[2] The main acute aetiologies of A(C)LF are hepatitis viral infection, alcoholic consumption and drug toxicity.[3,4] Yet, the causes of up to 20% of A(C)LF cases remain unknown.[5] In the past decade, Hepatitis E virus (HEV) infection is increasingly reported to be a common acute event for A(C)LF. Previous estimates assume that, globally, the majority of ALF cases occur as a result of hepatitis E and A infection. High incidence of HEV infection in some Asian countries may be responsible for this.[1]

HEV is the most common cause of acute viral hepatitis worldwide.[4] Although HEV infection generally causes an acute and self-limiting disease, it is also one of the leading causes of fulminant hepatitis, such as A(C)LF.[4] In developing countries, about 20%-40% of HEV patients, commonly attributed to genotype (GT) 1 HEV, may progress to ALF and this percentage is increased among pregnant women.[5] GT3 and GT4 HEV account for most asymptomatic HEV infections, but have been increasingly recognized to substantially contribute to liver failure as well, especially in patients with CLD that rapidly worsen to ACLF.[4,6] Previous reports from Europe and China indicated that 10%-15% and 6.5% of A(C)LF cases had evidence of HEV infection.[7,8] Overall, it is speculated that the involvement of HEV in A(C)LF is commonly underestimated or disregarded owing to lack of screening, use of insensitive assays and misdiagnosis.

To date, the incidence, predictors and prognosis of liver failure with HEV as an acute insult are largely unknown and urgently need to be elucidated. In this study, we have comprehensively investigated the incidence of liver failure in patients with hepatitis E and the predictors and prognosis of liver failure with hepatitis E as an acute insult in China, where GT4 HEV is predominant. Additionally, since hepatitis E patients with underlying CLD are particularly prone to liver failure, whether distinct CLDs and their status uniquely influence the incidence and outcome of liver failure were also elucidated.

Key Points

Genotype (GT) 3 and GT4 hepatitis E virus (HEV) infection is usually considered an asymptomatic or self-limiting disease. Our findings showed that patients with GT4 HEV infection are at high risk for developing liver failure. The identified variables shall help to identify HEV patients with high risk for developing liver failure and the risk for death.

2 | MATERIALS AND METHODS

2.1 | Study population

All the patients with symptoms of suspected acute viral hepatitis, defined as presenting elevated liver enzymes and/or jaundice and/or non-specific symptoms such as fatigue, itching and nausea, are routinely screened for HEV-specific immunoglobulin (Ig) M using ELISA at the 5th Medical Centre, Chinese people's Liberation Army (PLA) General Hospital. This hospital is the biggest tertiary hospital specialized in hepatology and infectious disease in China. The number of outpatient and inpatient visits with liver diseases is approximately 1.87 million and 0.1 million per year respectively. Patients admitted to this hospital are from all over the country. A case of hepatitis E was defined as positive serum anti-HEV IgM and/or detectable HEV RNA with clinical presentation of acute hepatitis. All patients diagnosed of hepatitis E between January 2012 and December 2018 were retrospectively and consecutively analysed.

2.2 | Study design

Patients with hepatitis E who developed A(C)LF during hospitalization were enrolled. In further analysis, those who have other possible acute events for ACLF, such as hepatitis B reactivation, alcoholic abuse, bacterial infection were excluded. Patients with hepatitis E as the sole acute aetiology of A(C)LF were recruited as cases (HEV-LF) and controls were randomly selected from those who did not develop liver failure with 1:3 ratio in the same cohort. The primary outcome was to assess the incidence of A(C)LF in patients with hepatitis E and to identify the predicting variables. The second outcome included short-term treatment outcomes at discharge, 28-day and 90-day mortality in HEV-LF patients, as well as the predictors of

mortality. We also extracted the case number of A(C)LF induced by other acute aetiologies during the same period to assess the proportion of hepatitis E as an aetiology of A(C)LF. Ethical approval was obtained from the Institutional Ethics Committee of the 5th Medical Centre, Chinese PLA General Hospital.

2.3 | Definition of liver failure, chronic liver diseases and treatment outcomes

Diagnosis and classifications of liver failure were defined by international normalized ratio (INR) and prothrombin activity, according to the 2012 China guidelines for liver failure.[9] In detail, diagnosis of ALF is based on the presence of stage 2 or 3 encephalopathy complicating end-stage disease manifestations, including profound coagulopathy (prothrombin activity ≤40% or INR ≥1.5), jaundice and hepatic atrophy in two weeks in patients with no CLD. ACLF is defined as acute deterioration of pre-existing CLD, usually related to a precipitating event. CLD was defined as follows:

1. Chronic hepatitis B (CHB) was diagnosed with hepatitis B surface antigen (HBsAg) positive for more than 6 months.[10] The severity of CHB was evaluated and classified into four categories according to clinical manifestation, laboratory test, B-ultrasonography and/or histology: (1) CHB carriers: The entries of liver function, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin (TBiL), total bile acid (TBA), albumin, prothrombin time (PT) and prothrombin activity, are at normal ranges. No obvious hepatitis manifestations presented. (2) Mild CHB: Patients present clinical symptoms of viral hepatitis. Liver function tests ranged as follows: (a) ALT: 40-120 U/L; (b) TBiL: 17-34.2 umol/L; (c) Albumin >35 g/L; (d) Albumin/Globulin (A/G): 1.3-1.5; (e) γ-globulin <20%; (f) Prothrombin activity: 71%-79%. B-ultrasonography showed normal liver. (3) Moderate CHB: Patients present with fatigue, decreased appetite and icteric sclera. Liver function tests ranged as follows: (a) ALT: 120-400 U/L; (b) TBiL: 34.2-85.5 umol/L; (c) Albumin: 33-34 g/L; (d) A/G: 1.0-1.2; (e) γ-globulin: 22%-25%; (f) Prothrombin activity: 61%-70%. B-ultrasonography revealed different degrees of liver injury. (4) Severe CHB: Patients present with unconsciousness and viscera haemorrhage. Liver function tests ranged as follows: (a) TBiL >171 umol/L; (b) Prothrombin activity <40%.
2. Alcoholic liver disease (ALD): The diagnosis of ALD based on drinking history, clinical manifestation, laboratory test, imaging examination and/or histology.[11] Patients with ALD normally had excessive alcohol use over five years. Excessive alcohol use was defined as drinking more than 20 g/day (or > 140g weekly) for women and 40 g/day (or > 210g weekly) for men. (1) Alcoholic fatty liver disease (AFLD): AFLD was generally an asymptomatic condition. Liver enzymes range from normal to modest elevation. The serum ALT level was usually less than the AST level. Bultrasonography revealed a "bright" liver with increased echogenicity. Liver biopsy showed abnormal retention of lipids within hepatocytes. (2) Alcoholic hepatitis (AH): Patients presented with clinical symptoms such as jaundice, malaise, fatigue, anorexia and fever. Generally, the AST/ALT ratio was greater than 2 and TBiL level was around 15 mg/dL or higher. Imaging examinations showed "pseudoparallel channel sign", which described a dilated hepatic artery and a dilated portal venous branch seen by ultrasound with Doppler flowmetry. Microvesicular and macrovesicular steatosis with inflammation were shown in liver biopsy specimens.
3. Cirrhosis was diagnosed either using histology or by the combination of clinical, biochemical tests and imaging examinations.[12] Evaluation of cirrhosis severity is based on Child-Pugh scoring.[13]

Short-term treatment outcomes of liver failure at discharge of hospital were classified as follows: (a) Recovery, clinical symptoms disappear, no jaundice, liver size and liver function recover to normal levels, and prothrombin activity or INR returns to normal level; (b) Improvement, disease manifestations relieve, encephalopathy disappears, TBiL level reduces over 1/3 percent and prothrombin activity level increases compared to that of before treatment; (c) Treatment failure, no response or clinical parameters were not improved to above mentioned levels; (d) Death before discharge from the hospital.

28-day and 90-day mortality were followed-up in patients with HEV-LF.

2.4 | HEV serological test

All serum samples were tested for the presence of anti-HEV IgM and IgG antibodies using commercially available HEV ELISA Kit (Wantai, Beijing, China) according to the manufacturer's instructions. Samples with signal-to-noise ratio (S/N ratio) N 1.0 were considered positive.

2.5 | Detection of HEV RNA and viral sequence analysis

Serum HEV RNA detection and sequence analysis were performed as before.[14] Total RNA was extracted from serum using the QIAamp Viral RNA mini-kit (Qiagen, Germany) according to the manufacturer's instructions. A 348-nucleotide fragment of the HEV open reading frame 2 (ORF2) was amplified using a nested PCR and sequenced to identify the genotype. The viral load of each sample was estimated using qPCR according to serial diluted artificial pseudovirus as standard using a diagnostic Kit for Hepatitis E Virus RNA (Jinhao, Beijing, China) according to the manufacturer's instruction.

2.6 | Statistical analysis

Continuous variables of normal distribution and partial distribution were expressed in mean ± standard deviation (SD) and median (interquartile-range [IQR]) respectively. Categorical variables were presented as counts (percentage). All variables of normal distribution were tested with Kolmogorov–Smirnov and the Shapiro–Wilk tests. Differences

between the two groups were analysed using Student t test for normally distributed continuous variables and Mann–Whitney U test for non-normally distributed continuous variables. Chi-square for categorical variables with continuous correction and/or the Fisher's exact test was used. Logistic regression was used to analyse risk factors for incidence and prognosis of HEV-LF. Data were analysed using SAS software (version 9.4; SAS Institute Inc, Cary, NC). The results were based on two-sided tests and $P < .05$ was defined as statistical significance.

3 | RESULTS

3.1 | Study population and incidence of HEV-LF

A total of 809 patients were diagnosed with hepatitis E during the 7 years, among whom 87 (10.75%) developed liver failure. There was no pregnant woman in all of the 809 subjects. Of note, an overall decline in the number of hepatitis E patients and an increase in the ratio of A(C)LF in hepatitis E patients were observed from year 2012 to 2018 ($\chi^2 = 26.246$, $P < .001$) (Figure 1A). In subsequent analysis, seven patients who had other confounding acute insults for developing liver failure were ruled out . They either had alcohol abuse within two weeks preceding onset of hospitalization or had reactivation of HBV. The remaining 80 patients were considered of HEV-related liver failure (HEV-LF), including 35 (43.75%) of ALF and 45 (56.25%) of ACLF. During the study period, we also found a total of 3899 patients with A(C)LF induced by other acute aetiologies, indicating that HEV infection accounts for liver failure with an approximate incidence of 2.01% (80/3979) (Figure 1B).

At discharge from hospital, 57 of the 80 HEV-LF patients (71.25%) recovered/improved, 22 (27.50%) failed in response to treatment and one (1.25%) died. Compared with HEV-related liver failure, the recovery/improvement rate was lower (57.31% vs 71.25%, $P = .013$) and the mortality was higher (9.87% vs 1.25%, $P = .010$) in patients with other aetiologies induced liver failure with HBV infection, alcohol consumption and drug as the most common acute insults, suggesting favourable outcomes of HEV-LF (Table S1).

Stored serum samples were available in 26 of 80 HEV-LF patients and in 83 of 240 HEV-non-LF controls. HEV RNA was detectable in 12 subjects and the viral load ranged between 5.70 and 8.80 $\log_{10}$ copies/mL (Table S2). Sequencing of detectable HEV genome showed GT4 strains (Figure 2, Figure S1).

3.2 | Clinical characteristics

Clinical characteristics were compared between 80 HEV-LF patients and 240 HEV-non-LF patients (Table 1). Ninety-seven percent HEV-LF patients were male, significantly higher than that in HEV-non-LF patients with a percentage of 80.42% ($P < .001$). Forty five of 80 (56.25%) HEV patients who developed liver failure had pre-existing CLD. Based on different CLD status, hepatitis E patients with cirrhosis underwent the highest risk to develop liver failure, compared to subjects without CLD or with non-cirrhotic CLD ($P = .016$). Further analysis revealed that the development of liver failure was correlated with severity of CLD in cirrhotic cases, other than in non-cirrhotic cases (Table 2). Intriguingly, the incidence of liver failure was comparable between hepatitis E patients without CLD and with non-cirrhotic CLD. Hepatitis E patients who developed liver failure were more likely to have experienced ingestion of unclean food and transfusion lately. Serum liver function tests and complications, including ascites, hydrothorax and respiratory infections were found to frequently present in hepatitis E patients developing liver failure. The extrahepatic manifestations were also compared between HEV-LF and HEV-non-LF patients (Table S3). The incidences of most extrahepatic manifestations were not significantly different between two groups, except that renal injury more frequently appeared in HEV-LF patients.

3.3 | Predictors of liver failure in patients with hepatitis E

In order to identify predictors of development of A(C)LF in patients with hepatitis E, we selected parameters which were neither the consequence of live failure nor influenced by liver failure to perform further multivariable analysis. We found that hydrothorax

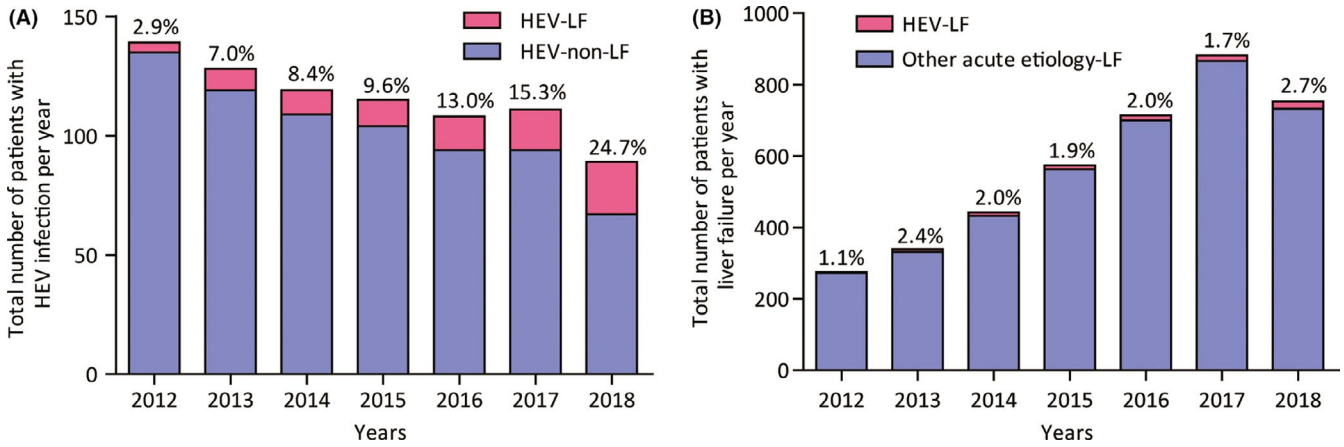

FIGURE 1 The ratio of A(C)LF in patients with hepatitis E from 2012 to 2018 (A) and the ratio of HEV induced liver failure in all A(C)LF patients from 2012 to 2018 (B). A(C)LF, acute or acute-on-chronic liver failure

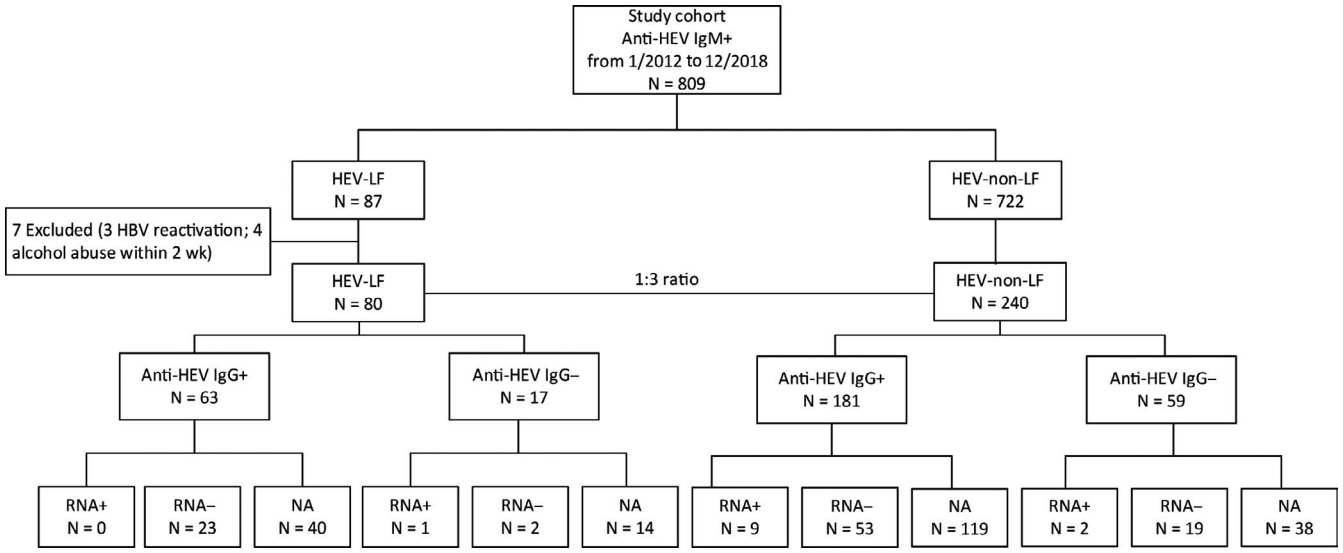

FIGURE 2 Flowchart of the cohort study. A total of 320 patients with positive anti-HEV IgM were enrolled in this study, with 240 HEV-non-LF and 80 HEV-LF patients. Results of anti-HEV IgG of the two groups are shown. Available serum samples were tested for HEV RNA and subsequently sequenced. RNA+, HEV RNA was detectable; RNA-, HEV RNA was undetectable; NA, serum samples were not available; HEV-LF, HEV-related liver failure; HEV-non-LF, hepatitis E patients who did not develop liver failure

(OR 2.417; *P* = .039), respiratory infections (OR 4.574; *P*<.001), GGT (OR 1.966; *P* = .036), lactate dehydrogenase (LDH) ≥226.5 U/L (OR 2.806; *P* = .001) and alpha-foetoprotein (AFP) ≥17.16 ng/mL (OR 2.928; *P*<0.001) at inclusion were independently associated with liver failure in patients with hepatitis E (Table 3).

3.4 | Outcomes of hepatitis E related liver failure

All of the HEV-LF patients were immunocompetent and received liver support procedures with magnesium isoglycyrrhizinate and glutathione. Among these, 17 patients also received hepatocyte growth factors injection. Electrolyte disturbance (73.75%) was the most common comorbidities followed by hypoproteinaemia (42.50%) and anaemia (42.50%) (Table 4). During follow-up, 70 and 56 patients completed the 28-day and 90-day follow -up respectively. The 28-day and 90-day mortality for HEV-LF was 12.86% (9/70) and 30.36% (17/56) respectively. All of the deaths were caused by liver failure. The courses of transaminases in deceased patients are shown in Figure S2 and S3. There were no differences in respect to short-term treatment outcome, 28-day mortality and 90-day mortality between patients receiving hepatocyte growth factors or not (Table S4).

Two of the HEV-LF patients received ribavirin treatment for flu before diagnosis of hepatitis E. Both of them improved at discharge of hospital. One patient survived at day 28 during follow-up and lack of 90-day follow-up. No follow-up data were available for the other patient.

3.5 | Predictors of death in HEV-LF patients

In Table S5, a univariate analysis of 28-day mortality was reported. No significant differences were found between survival and non-survival patients with regard to age, gender, CLD status, metabolic disorder, Child-Pugh classification and haematological examination at baseline. Non-survival patients had lower levels of total cholesterol (TC) and triglyceride (TG), and had higher levels of TBiL, creatinine, prothrombin activity and INR than survivors at 28-day. Renal injury is more frequent in non-survivors. Furthermore, based on different CLDs, the severity of any kind of CLD was not correlated with survival at 28-day (Table S6). In multivariate analyses, renal injury (OR 7.100; *P* = .017) and TG <1.77 mmol/L (OR 6.470; *P* = .043) were independent factors associated with 28-day mortality (Table 5).

Univariate analysis of 90-day mortality is also reported (Table S7). Cirrhosis was significantly related to the mortality at 90-day regardless of its severity (Table S8). HEV-LF patients presenting with ascites, shock, HE and cholecystitis were at high risk for 90-day mortality. Non-survivors had poor liver function at baseline. Levels of haemoglobin (Hb) and platelet counts were significantly lower in 90-day non-survivors than in survivors. In multivariate analyses, INR ≥1.53 (OR 8.643; *P* = .008) and ALT <388.5 U/L (OR 8.638; *P* = .011) were identified to be independent predictors of 90-day mortality (Table 5).

4 | DISCUSSION

Liver failures caused by HEV infection had been increasingly documented, but the comprehensive nature history of HEV related A(C)LF remained unexplored for long time. This study, with its largest and unique cohort of patients with GT4 hepatitis E, explored the incidence, predictors and prognosis of liver failure. Our study showed that 10.75% patients with hepatitis E developed A(C)LF with a growing tendency from year 2012 to 2018. Furthermore, the large number of hepatitis E patients in our cohort and the high incidence of HEV-LF provide statistical power of data analysis on their risk factors and outcomes. Hepatitis E patients with co-existing hydrothorax,

TABLE 1 Clinical characteristics of patients with hepatitis E who developed liver failure or not[a]

Variables	HEV-non-LF (N = 240)	HEV-LF (N = 80)	*P* value
Age	54.90 ± 12.67	55.15 ± 9.30	.850
Male gender	193 (80.42)	78 (97.50)	<.001
CLD status			
No CLD	142 (59.17)	35 (43.75)	.002[b,c]
Non-cirrhotic CLD	47 (19.58)	12 (15.00)	
Cirrhosis	51 (21.25)	33 (41.25)	
Symptoms			
Fever	43 (17.92)	31 (38.75)	<.001
Jaundice	204 (85.00)	66 (82.50)	.594
Abdominal pain	39 (16.25)	22 (27.50)	.027
Nausea/Vomit	161 (67.08)	48 (60.00)	.249
Intake of unclean food within 3 months[d]	76 (31.67)	34 (42.50)	.077
Transfusion within 3 months	12 (5.00)	10 (12.50)	.022
Metabolic disorder			
Diabetes mellitus	52 (21.67)	10 (12.50)	.072
Fatty liver	43 (17.92)	8 (10.00)	.094
Complications			
Ascites	66 (27.50)	54 (67.50)	<.001
Hydrothorax	19 (7.92)	19 (23.75)	<.001
Respiratory infections	16 (6.67)	21 (26.25)	<.001
Gastrointestinal injury[e]	29 (12.08)	14 (17.50)	.219
Cholestasis	31 (12.92)	7 (8.75)	.318
Cholecystitis	56 (23.33)	19 (23.75)	.939
Laboratory parameters			
Liver function			
TP 60-83 (g/L)	59.66 ± 7.41	55.03 ± 7.95	<.001
Albumin 35-55 (g/L)	32.75 ± 5.17	29.15 ± 4.82	<.001
Pre-albumin 160-400 (mg/L)	70.00 (46.00-106.50)	42.00 (24.00-66.00)	<.001
ALT 5-40 (U/L)	449.00 (165.50-943.00)	299.50 (125.50-1220.50)	.553
AST 5-40 (U/L)	210.00 (86.50-562.50)	181.00 (90.00-608.00)	.890
ALP 40-150 (U/L)	170.00 (139.00-227.50)	168.00 (135.00-221.00)	.816
GGT 11-50 (U/L)	153.00 (91.00-265.00)	94.00 (59.00-160.00)	<.001
TBA 0-10 (umol/L)	151.95 (60.00-235.95)	193.00 (158.00-241.00)	<.001
ChE 5000-12000 (U/L)	4325.50 (3286.50-5444.50)	3187.00 (2182.00-4400.00)	<.001
LDH 109-245 (U/L)	218.00 (180.00-283.50)	254.00 (213.00-332.00)	.001
Glucose 3.9-6.1 (mmol/L)	4.80 (4.40-5.80)	4.60 (3.90-6.30)	.111
TC 2.8-5.2 (mmol/L)	2.91 (2.38-3.82)	1.72 (1.22-2.40)	<.001
TG 0.56-1.7 (mmol/L)	2.16 (1.47-3.29)	1.76 (1.19-2.18)	<.001
Serum proteins			
HDL-c 1.16-1.42 (mmol/L)	0.47 (0.32-0.72)	0.29 (0.21-0.37)	<.001
LDL-c 2.1-3.1 (mmol/L)	2.44 (2.06-3.09)	1.91 (1.58-2.29)	<.001
Haematological examination			
Leukocytes 3.97-9.15 ($\times 10^9$/L)	5.64 (4.40-7.40)	7.63 (5.50-9.74)	<.001
Hb 131-172 (g/dL)	136.00 (124.00-148.00)	137.00 (119.50-151.00)	.781
Platelet count 85-303 ($\times 10^9$/L)	170.00 (126.00-221.00)	134.00 (96.50-172.50)	<.001

(Continues)

TABLE 1 (Continued)

Variables	HEV-non-LF (N = 240)	HEV-LF (N = 80)	*P* value
Serum tumour markers			
AFP 0-10.0 (ng/mL)	14.08 (4.29-53.80)	31.12 (7.69-125.20)	.003
CA125 0-35 (U/mL)	18.21 (11.50-34.67)	31.51 (16.88-68.37)	<.001
CA19-9 0-39 (U/mL)	47.86 (18.54-133.55)	99.86 (39.80-238.00)	<.001
CA72-4 0-8.2 (U/mL)	1.16 (0.91-2.11)	1.18 (0.93-1.92)	.768
CEA 0-3.4 (ng/mL)	2.18 (1.36-3.35)	2.77 (1.86-3.66)	.006

Abbreviations: AFP, alpha-foetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CA, carbohydrate antigen; CEA, carcino embryonic antigen; ChE, cholinesterase; CLD, chronic liver disease; GGT, -glutamyl transferase; Hb, haemoglobin; HDL-c, high density lipoprotein cholesferol; LDH, lactate dehydrogenase; LDL-c, low density lipoprotein cholesterol; TBA, total bile acid; TC, total cholesterol; TG, triglyceride; TP, total protein.

[a]Normally distributed continuous variables are expressed in mean ± standard deviation (SD), whereas other continuous variables are expressed in median (interquartile range [IQR]). Categorical variables are presented as counts (percentage).

[b]*P* = .001, Cirrhosis vs No CLD (post-hoc analysis).

[c]*P* = .016, Cirrhosis vs Non-cirrhotic CLD (post-hoc analysis).

[d]Ingestion of unclean food included undercooked pork or contaminated water.

[e]Gastrointestinal injury included gastritis, intestinal infection, ileus, duodenal ulcers.

TABLE 2 Association of severity of chronic liver diseases and the risk of developing liver failure in patients with hepatitis E

Variables	HEV-non-LF N = 98	HEV-LF N = 45	*P* value
Non-cirrhosis			
ALD			
AFLD (N = 15)	13 (43.33)	2 (28.57)	.394
AH (N = 22)	17 (56.67)	5 (71.43)	
CHB			
CHB carriers (N = 7)	5 (29.41)	2 (40.00)	.745
Mild CHB (N = 6)	6 (35.29)	0 (-)	
Moderate CHB (N = 6)	4 (23.53)	2 (40.00)	
Severe CHB (N = 3)	2 (11.77)	1 (20.00)	
Cirrhosis			
Child-Pugh A (N = 25)	22 (43.14)	3 (9.09)	.003
Child-Pugh B (N = 29)	15 (29.41)	14 (42.42)	
Child-Pugh C (N = 30)	14 (27.45)	16 (48.49)	

Abbreviations: ALD, alcohol liver disease; AFLD, alcoholic fatty liver disease; AH, alcoholic hepatitis; CHB, chronic hepatitis B.

respiratory infections, lower GGT, higher LDH and higher AFP were at high risk to develop liver failure. The mortality rate of HEV-LF ACLF is quite high: 12.86% at 28 days and 30.36% at 90 days. Renal injury and low TG are independently associated with 28-day mortality and high INR and low ALT are independently associated with 90-day mortality.

Based on the previous Asian estimates, 20%-60% of ALF cases are assumed to have evidence of GT1 HEV infection.[4] GT3 HEV infections are seldom described but increasingly contribute to a substantial proportion of acute liver injuries in western countries (10%-15%).[7,15,16] A recent small cohort study in China, representing GT4 HEV data, has indicated the potential contribution of HEV to liver failure.[8] In the current study, 2.01% A(C)LF were identified to have HEV infection as an acute insult by review of 3979 A(C)LF patients, which is lower than the ratio from another Chinese study (6.5%), as well as Asian and European studies. The pronounced role of other acute aetiologies, such as HBV reactivation, accounting for the majority of liver failures in China, could be responsible for the discrepancy. Nevertheless, we found a considerable number of patients with GT4 hepatitis E (averagely 10.8% with an increasing trend by year) developed liver failure, suggesting that despite HEV contributes to a small proportion of A(C)LF in China, patients with hepatitis E should be taken caution of developing A(C)LF.

The striking finding of this study is serum AFP as a specific marker for liver failure in HEV patients. AFP expression is induced by liver stem/progenitor cells (LPC), contributing to liver regeneration and reflecting the severity of hepatocyte loss.[17] Thus the increasing serum AFP level may indicate the hepatocellular regeneration initiated by severe liver damage.[18,19]

TABLE 3 Multivariate analysis of predictors of liver failure in patients with hepatitis E

Variables	Odds ratio (95% CI)	*P* value
Hydrothorax	2.417 (1.047-5.580)	.039
Respiratory infections	4.574 (2.005-10.438)	<.001
GGT <140 U/L	1.966 (1.046-3.692)	.036
LDH ≥226.5 U/L	2.806 (1.519-5.184)	.001
AFP ≥17.16 ng/mL	2.928 (1.575-5.443)	<.001

Note: Variables included in the analysis: CLD status, transfusion within 3 months, respiratory infections, diabetes mellitus, fatty liver, hydrothorax, GGT, LDH, AFP.
Abbreviations: AFP, alpha-fetoprotein; GGT, -glutamyl transferase; LDH, lactate dehydrogenase.

TABLE 4 Outcomes of HEV related liver failure

Variables	N = 80
Comorbidities [N (%)]	
Electrolyte disturbance	59 (73.75)
Hypoproteinaemia	34 (42.50)
Renal injury	23 (28.75)
Shock	7 (8.75)
Spontaneous bacterial peritonitis[a]	19 (23.75)
Heart dysfunction	5 (6.25)
Anaemia	34 (42.50)
Pancreatic injury	4 (5.00)
Outcomes [N (mortality)]	
Death within 28 days[b]	9 (12.86)
Death within 90 days[c]	17 (30.36)

[a]The gold standard for the diagnosis of spontaneous bacterial peritonitis is based on a polymorphonuclear leukocyte count of 250 cells/mm^3 or more, irrespective of the results of ascitic fluid culture.
[b]Data of 28-day mortality were available for 70 HEV-LF patients.
[c]Data of 90-day mortality were available for 56 HEV-LF patients.

TABLE 5 Multivariate analysis of predictors of mortality at 28-day and 90-day

	Odds ratio (95% CI)	*P* value
28-day all-cause mortality[a]		
Renal injury	7.100 (1.413-35.670)	.017
TG < 1.77 mmol/L	6.470 (1.060-39.500)	.043
90-day all-cause mortality[b]		
INR ≥ 1.53	8.643 (1.752-42.628)	.008
ALT < 388.5 U/L	8.638 (1.649-45.239)	.011

Abbreviations: ALT, alanine aminotransferase; INR, international normalized ratio; TG, triglyceride.
[a]Variables included in the analysis: renal injury, TG.
[b]Variables included in the analysis: ALT, INR, CA125.

It is not surprising to find renal injury as an independent predictor of 28-day mortality of HEV-LF, complying with prior studies.[20-24] Furthermore, Urrunaga et.al not only found that renal dysfunction complicating ALF predicted a significantly worse outcome, but also emphasized the association between mortality and the degree of renal dysfunction.[25] Accordingly, underlying kidney diseases might pre-dispose HEV-LF patients to subsequent multiorgan failure in the context of severe liver injury, which definitely led to high mortality.

Underlying CLD has been widely described to contribute to HEV-LF.[4,16,26-28] This study is the first to our knowledge to describe the magnitude of the association in terms of distinct categories and status of CLD. In patients with hepatitis E, development of ACLF is significantly associated with cirrhotic CLD, when compared to non-cirrhotic CLD or no CLD patients. Further analysis even revealed a correlation between severity of cirrhosis and risk of liver failure. In agreement, cirrhotic individuals superinfected with HEV were frequently reported to develop liver decompensation or liver failure.[28-30] However, it remains to determine the possible mechanisms: (1) HEV potentiates the damage of cirrhotic liver that leads to liver failure; (2) a direct effect of viral genome or protein on hepatocytes; (3) the immune- or inflammatory-mediated activation of cell death pathways. Intriguingly, non-cirrhotic CLD seems to have no effect on the development of ACLF, since the incidence of liver failure was comparable between hepatitis E patients without CLD and with non-cirrhotic CLD. We thus postulated that the overall status of liver, other than specific aetiology of CLD, is more pivotal for determination of risk of liver failure in patients with hepatitis E.

The mortality of HEV-related A(C)LF ranges from 0% to 67% with a median of 34% over the world.[4] Studies from India and Pakistan indicated a mortality rate of 25%- to 67% in GT1 HEV-LF patients.[29,30] One study from Europe indicated 27.27% mortality of HEV -related A(C)LF within 6-month.[16] Previous studies in China have indicated a mortality of 34% to 42% when HEV as an acute insult of A(C)LF.[8,31] In this study, 28- and 90-day mortality were 12.86% and 30.36% respectively. The overall mortality of HEV-LF is higher in China than those in Europe. This is probably explained by more severe pathogenicity of GT4 HEV to humans compared to GT3 HEV.[32] Furthermore, our study revealed significantly better short-term outcomes of A(C)LF with HEV as an acute insult than other acute aetiologies, with in-hospital mortality of 1.25% vs 9.87% respectively. This observation concurs with findings from Indian studies, which showed that the mortality of HEV-LF was 13%-45%, in contrast to 33%-70% of A(C)LF with other acute aetiologies.[33-35] Rapid and effective spontaneous clearance of HEV is likely to interpret this result.[36]

Whether CLD affects the clinical outcomes of liver failure with HEV as an acute insult remained unclear. Studies from Southeast Asia and Europe showed that HEV infection in patients with CLD have a poor prognosis with mortality rates of 25% to 70%.[27,29,30] A Chinese study reported higher mortality in HEV-LF patients with CLD (5/10, 50%) than in those without CLD (3/9, 33%), but no statistical significance.[8] This study has shown that 58.82% non-survival patients at 90 days had CLD, compared to 53.85% survival patients (*P* = .731). Although CLD is not a risk factor of mortality in the context of HEV-LF, cirrhotic liver was significantly related to the 90-day mortality regardless of its severity, suggesting that the overall status

of liver, other than specific etiology of CLD, play important role in outcomes HEV-LF. We then elucidated whether and to what extent the distinct CLDs influence the mortality of HEV-LF. Notably, the stratification analysis of the prognosis in HEV-LF patients according to different CLDs showed that there was no clear association of severity of CLD and mortality. The small sample size of CLD based HEV-LF patients is one of the potential reasons. In summary, our study is the first to highlight the unique role of different CLDs in prognosis of HEV-related A(C)LF.

In our study, only a few cases had detectable HEV RNA. But these patients with anti-HEV IgM positivity were considered proven HEV cases because anti-HEV IgG were moderately present in most of them, which is a suggestion of recent HEV infection.[6,37-41] According to the nature of HEV infection course, the immune response to HEV is accompanied with initial short-lived IgM followed by more durable IgG antibody.[42] HEV RNA does not persist for long, which can be just detected in sera and stool samples during the incubation period and early phase of disease, and then disappears from serum about 3 weeks after the onset of symptoms with recovery.[43] The time of HEV infection is not certain in these patients of our study. The delayed hospitalization after onset of acute hepatitis symptoms may be a reason that we could not capture the viral RNA. Although the virus can be shed in stool for 2 weeks longer than in serum[43], stool samples were not available in this study. Eventually, the retrospective study spanned seven years therefore some early obtained serum sample could have viral RNA degradation.

There are some limitations in our study. Firstly, the retrospective study led to missing follow-up data of HEV viral clearance, prolonged prognosis of HEV-LF and the association between them. Secondly, some patients with mild acute hepatitis E would have been missed because they did not visit the hospital. Therefore, we might have overestimated the incidence as the hospitalized patients tended to have more severe hepatitis and other complications.

In conclusion, although HEV infection is not a common cause of A(C)LF in China, patients with hepatitis E are at high risk for development of A(C)LF. Cirrhosis is the predominant CLD that is associated with the development of liver failure in HEV patients. The identified variables in patients with hepatitis E shall be important to identify risk population with high risk for developing liver failure and risk population with high risk for mortality causued by HEV-LF , who are recommended with specific intensive monitoring and effective prevention program with patient-tailored strategies.

CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest. All authors read and approved the final manuscript.

AUTHORS CONTRIBUTIONS

Y. W. contributed to the study concept and design, obtained funding, analysis and interpretation of data, literature search and writing of the manuscript; H. L. contributed to acquisition and analysis of data, prepared the tables and drafted the manuscript; C. Y. and S. W. contributed to lab experiments; Y. J. contributed to viral sequencing and analysis; A. L. and S. L. contributed to sample collection. N. K. contributed to critical revision of the manuscript; Q. P. and M. P. contributed to the study concept and critical revision of the manuscript; J. Z. contributed by providing clinical samples and supervising the study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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