Superinfective Hepatitis E Virus Infection Aggravates Hepatocytes Injury in Chronic Hepatitis B*

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Summary: Hepatitis E virus (HEV) infection is a major cause of morbidity in endemic areas. Its consequences among chronic hepatitis B (CHB) patients have been under-reported. The aim of this study was to assess the impact of superinfective HEV infection (acute and past) on virological and clinical features of patients with CHB infection. Clinical, biochemical, virological and immunological data of 153 CHB patients including 98 with hepatitis B virus (HBV) monoinfection and 55 with HBV-HEV superinfection with both HEV and HBV infection was retrospectively investigated and analyzed in this study conducted in Wuhan, China. An overall anti-HEV IgG seroprevalence was found to be 35.9% in CHB patients. HBV-HEV superinfection patients showed significantly higher rate of complications (ascites, hepato-renal syndrome & encephalopathy) (all with P=0.04), cirrhosis (P<0.001) and acute-on-chronic liver failure (P<0.001) than HBV monoinfection patients. They also displayed elevated ALTs (P<0.001) and total serum bilirubin (P<0.001) with diminished albumin (P<0.001) and HBV viral load (P<0.001). Cytokines assay revealed increased expression of IL-6 (P=0.02), IL-10 (P=0.009) and TNF-α (P=0.003) in HBV-HEV superinfection patients compared to HBV monoinfection patients. Our study demonstrated that HEV superinfection in CHB patients was associated with progressive clinical manifestation, which is likely due to the enhanced expression of cytokines related with hepatocytes necrosis. HEV was also associated with repressed HBV replication, but the underlying mechanism requires further investigation.
Key words: hepatitis E; hepatitis B; superinfection; liver failure; replication

Hepatitis B virus (HBV) and hepatitis E virus (HEV) infections are the diseases of global significance that affect a large number of people. According to a recent Global Hepatitis Report, up to 257 million people are suffering from chronic hepatitis B (CHB) infection worldwide, the highest number (68%) being in Western-Pacific and African regions[1]. HEV infection is estimated to affect 20 million people every year worldwide, and approximately 44 000 deaths were reported in 2015[2]. Both of these infections are epidemic in China, the country that has recently changed from high to moderate prevalence of HBV[3]. Anti-HEV IgG seroprevalence is estimated to be ranging from 10%–50% in China[4].

During the chronic course of HBV infection, overlapping with other hepadnoviruses including HEV is common. Interaction between HBV and acute HEV infections has been a focus of many studies in both low and high endemic areas, with limited data in persistent seropositivity. Correlation of acute HEV coinfection with detrimental disorder of several liver markers and enhancing of early evolution into more severe complications and poor outcome among CHB patients, has been clearly established[5], but the consequences of anti-HEV IgG seropositivity that have enduring post-infection persistence as well as influences on HBV viral load and genotypes, have been rarely described.

In China where two genotypes of HBV namely genotypes B and C are common, the latter is known to be the most virulent, which frequently presents with higher viral load and lower rate of spontaneous Hepatitis B envelope antigen (HBeAg) seroconversion[6]. However, the possibility that this tendency is influenced by HBV-HEV superinfection remains to be established.

The aim of this cross-section study therefore, was to determine the impact of HEV in HBV superinfection on associated clinical, serological, virological and immunological factors in such patients.

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*This work was supported by the Innovation Team Development Plan of the Ministry of Education (No. IR14R20) and National Natural Science Foundation of China (No. 81571989).

1 MATERIALS AND METHODS

1.1 Patients

This study was conducted between November 2016 and June 2017 and it involved inpatients with naïve CHB in the Department of Infectious Diseases of Tongji Hospital in Wuhan, China. Informed consents were obtained from all enrolled patients who supplied the blood samples for this study. The patients were serially recruited and grouped into two categories of either HBV monoinfection or HBV-HEV superinfection. Patients with hepatitis A (HAV), hepatitis C (HCV), hepatitis D (HDV), alcoholic liver diseases, autoimmune hepatitis, fatty liver disease, drug induced liver injury, Japanese schistosomiasis and other non-viral hepatitis were excluded from the study.

CHB diagnosis was made when patients had positive hepatitis B surface antigen (HBsAg) at 6 months or beyond. HBV-HEV superinfection patients were named in this study if they were detected positive for HEV immunoglobulin G antibodies (anti-HEV IgG) and positive or negative for HEV immunoglobulin M antibodies (anti-HEV IgM) after CHB infection. Patients with HBsAg positive and HEV seronegative for IgG and IgM were named as HBV monoinfection patients. The diagnosis of liver cirrhosis in this study was based on clinical, biochemical, ultrasound, radiographic imaging and/or pathological confirmation of liver tissue from the study patients. The diagnosis of acute-on-chronic liver failure (ACLF) in this study was based on 2014 APASL ACLF guideline[7].

1.2 Sample Collection and Testing

Clinical and laboratory data on biochemistry, hematology and serology were obtained from the medical records. Previously, these tests were performed in the infectious disease laboratory within 1 h of samples collection. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total bilirubin, international normalization rate (INR) and prothrombin activity (PTA) were analyzed by an automatic biochemical analyzer (Abbot Diagnostic, USA).

1.3 Pathogenic Detection

HBV deoxyribonucleic acid (DNA) titer was measured by using a standard generic assay of Cobas TaqMan HBV Test assay (Roche Diagnostics, China) with the linear detection range of 20–1.7×10^8 IU/mL. HBsAg and HBeAg were measured by using HBsAg quantitative Elecsys (Roche Diagnostics, China) and AXSYM HBe2.0 (Abbott Laboratories, USA; range 0.15–200 PEIU/mL) respectively. Anti-HBs and anti-HBe levels were determined by the ARCHITECT qualitative assays (Abbott Laboratories, USA). Anti-HEV IgG, anti-HEV IgM, anti-HAV IgM and anti-HCV were measured by using a commercial ELISA kit (Beijing Wantai Company, China) according to the manufacturer's instructions.

1.4 HBV DNA Extraction and Amplification

About 200 μL of plasma that was obtained from each patient's blood within 6 h or pre-stored at –80°C was used in this process. HBV DNA was extracted using QIAmp DNA Mini kit (QIAGEN, German) according to the manufacturer's instructions. The HBV polymerase gene region was then immediately amplified with two primers: P1, forward (nt 2121-2139) TTG GCC AAA ATT CGC AGT C and P2, reverse (nt 2996 to3014) GCG TCA GCA AAC ACT TGG C. Polymerase chain reaction (PCR) was performed by Quick Taq HS DyeMix (TOYOBO, Japan) in a volume of 50 μL under the following thermocycler reaction conditions: 94°C for 2 min, 94°C for 30 s, 50°C for 40 s, 72°C for 40 s and 68°C for 10 min.

1.5 DNA Sequencing

The PCR products were analyzed by electrophoresis using 1.5% agarose gel and DNA was extracted by QIAquick Gel Extraction Kit (QIAGEN, German) according to the manufacture's manual. The primers P1 and P2 were used to determine nucleotide sequences from both strands and the purified products were used as templates in the cycle sequencing reactions. The resulting sequences were read directly with automated DNA sequencer (Applied Biosystems, USA), followed by phylogenetic analysis for detection of HBV genotypes as previously described[8].

1.6 Measurement of Cytokines

The cytokines interleukin (IL-2, IL-4, IL-6, IL-10, IL-17A), tumor necrotic factor (TNF)-α, and interferon (IFN)-γ were measured and analyzed in this study by using a commercial kit (BD Cytometric Bead Array Human Th1/Th2/Th17, USA) according to the manufacturer's instructions. In brief, 10 μL of each cytokine's capture bed was mixed in one tube and centrifuged at 200 g for 5 min. The supernatant was carefully aspirated and equal volume of serum enhancement buffer was added, followed by thorough vortex. The solution was then incubated at room temperature for 30 min. Fifty microliters of mixed capture beads were then added to 50 μL of each serum sample, followed by titration by Human Th1/Th2/Th17 PE detection reagent. The solution was incubated for 3 h at room temperature and protected from light. Each assay was washed twice in 1 mL and 300 μL of wash buffer after centrifuging at 200 g for 5 min. The samples were immediately transferred to the cytokine analyzer BD FACS Canto II (BD Bioscience, USA), and the concentrations were estimated using FCAP Array™ Software Version 3.0.

1.7 Statistical Analysis

In this study, categorical variables were described in frequencies and percentages and were compared using Fisher's exact test or Chi-square test. Continuous

variables were reported as medians with interquartile ranges (IQR) and were compared by Mann-Whitney U test. *P*-values of less than 0.05 were considered to be statistically significant. All analyses were conducted by using STATA program 13.0 (College Station, USA).

2 RESULTS

2.1 Study Enrollment

During the study period, a total of 305 patients with CHB infection according to the criteria were recruited. In the recruited patients, 152 patients were excluded due to missing data, coinfection with other kinds of hepatitis viruses or combination with other liver diseases. Finally there were 153 patients included for analysis (fig. 1). The study was approved by the Ethics Committee of Tongji Hospital (No. TJ-C20151108) and the consent was obtained from the participants.

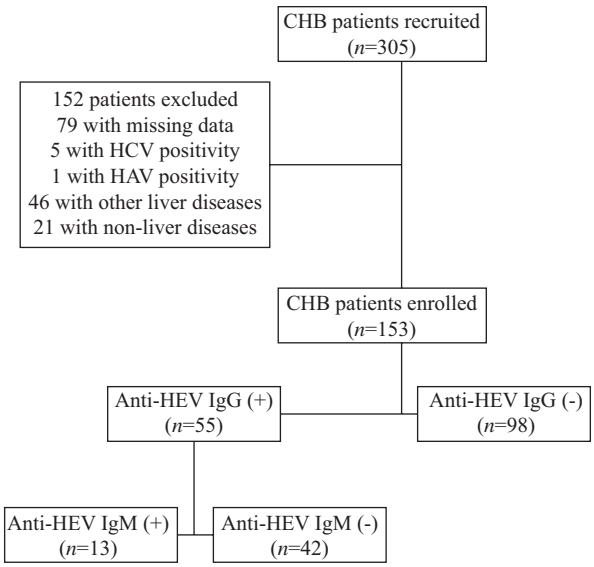

Fig. 1 Patients' enrollment flow chart

2.2 Patient Demographic and Baseline Characteristics

Among the 153 enrolled patients, male patients outnumbered females with the ratio of male to female being 1.6:1. The age range was 20–73 years and median age was 49 (IQR 37–56) years. Sixteen percent of the participants had at least one chronic complication of CHB (ascites, hepato-renal syndrome or hepatic encephalopathy) during enrollment. HBV genotype characterization could be obtained in 70/153 (45.8%) patients and HBV genotypes B and C were the only significant isolates observed. The majority of the studied patients (86.3%) had detectable HBV DNA. None of the patient was on antiviral therapy for HBV. Details of the patients' baseline characteristics are summarized in table 1.

Table 1 Baseline characteristics of all enrolled patients (*n*=153)

Variable	Number (%) or median (IQR)
Sex	
Male	94 (61.4)
Female	59 (38.6)
Age (years)	49 (37–56)
Complications	
Ascites	15 (9.8)
Hepato-renal syndrome	5 (3.3)
Hepatic encephalopathy	5 (3.3)
Liver cirrhosis	35 (22.9)
Acute-on-chronic liver failure	12 (7.8)
Hepatocellular carcinoma	3 (1.9)
HBV genotypes (*n*=70)	
A	1 (1.4)
B	42 (60.0)
C	26 (37.1)
D	1 (1.4)
HBsAb positivity	2 (1.3)
HBeAg positivity	68 (44.4)
Detectable DNA	133 (86.3)
ALT (IU/L)	55 (29–184)
AST (IU/L)	50 (27–124)
γ-glutamate (IU/mL)	45 (21–118)
Alkaline phosphatase (U/L)	84 (62.5–114.5)
Total bilirubin (umol/L)	20.4 (12.2–69)
Albumin (g/L)	42.5 (34.5–45.3)
Prothrombin activity (%)	63 (46–80)
International normalized ratio	1.4 (1.1–1.8)

2.3 Comparison between HBV Monoinfection and HBV-HEV Superinfection Patients

Table 2 shows comparison of demographic data and baseline laboratory and clinical pattern between the patients with HBV monoinfection (anti-HEV IgG$^-$ & anti-HEV IgM$^-$) and those with HBV-HEV superinfection (anti-HEV IgM$^+$ and/or anti-HEV IgG$^+$) in CHB. Both gender and sex were comparable between the two groups. Moreover, HBeAg seropositivity rate between HBV monoinfection and HBV-HEV superinfection patients was also comparable with respective rates being 46.9% *vs.* 40% (*P*=0.41). The prevalence of liver cirrhosis, ACLF and related complications (ascites, hepato-renal syndrome & encephalopathy) were significantly higher in HBV-HEV superinfection group than in those of monoinfection with HBV. Regarding the DNA quantification, viral load for hepatitis B peaked significantly among patients of monoinfection with HBV as compared with those of superinfections [7.2 (5.3–8.0) *vs.* 4.8 (3.8–6.4) $\log_{10}$IU/mL; *P*<0.001]. The mean level for several liver tests was also more deranged among the HBV-HEV superinfected patients.

On genotypic analysis, the superinfection group comprised more of the patients with HBV genotype C than HBV monoinfection group (47.2% *vs.* 5.9%, *P*=0.002). There was no significant difference in presentation of other genotypes between the two

Table 2 Comparison of baseline and hospitalization characteristics with complications (*n*=153)

Variable	Monoinfection (*n*=98), *n* (%)/median (IQR)	Superinfection (*n*=55), *n* (%)/median (IQR)	*P* value
Gender			0.07
Male	55 (56.1)	39 (70.9)	
Female	43 (43.9)	16 (29.1)	
Age (years)	47.5 (33–56)	52 (44–57)	0.06
Ascites	6 (6.1)	9 (16.4)	0.04
Hepato-renal syndrome	1 (1.0)	4 (7.3)	0.04
Hepatic encephalopathy	1 (1.0)	4 (7.3)	0.04
Liver cirrhosis	11 (11.2)	24 (43.6)	<0.001
ACLF	2 (2.0)	10 (18.2)	<0.001
HCC	2 (2.0)	1 (1.8)	0.92
Serological markers			
HBeAg seropositivity	46 (46.9)	22 (40)	0.41
HBsAg $\log_{10}$ (IU/mL)	3.2 (0–4.2)	3.7 (2.7–4.2)	0.03
Viral load $\log_{10}$ (IU/mL)	7.2 (5.3–8.0)	4.8 (3.8–6.4)	<0.001
Liver tests			
ALT (IU/L)	41 (24–108)	99 (37–408)	<0.001
AST (IU/L)	38 (23–74.2)	100 (42–255)	<0.001
ɣ-glutamate (IU/mL)	24.5 (18–59)	85 (45–132)	<0.001
Alkaline phosphatase (U/L)	72 (59–89)	106 (84–142)	<0.001
Total bilirubin (μmol/L)	15.4 (11.0–23.4)	52.1 (21.2–269.8)	<0.001
Albumin (g/L)	44.3 (41.3–46.1)	35.8 (30.4–41.9)	<0.001
Prothrombin activity (%)	74.5 (59–85)	60 (46–78)	0.13
International normalized ratio	1.2 (1.1–1.4)	1.5 (1.2–1.8)	0.11
HBV genotypes (*n*=70)			0.002
B	26 (49.1)	16 (94.1)	
C	25 (47.2)	1 (5.9)	

groups.

2.4 HEV Seroprevalence

An overall seroprevalence of anti-HEV IgG among all CHB patients in this study was 55/153 (35.9%). Anti-HEV IgM was detected in 13/153 (8.5%) patients, of whom all were IgG positive. Anti-HEV IgG seroprevalence among patients with ACLF, liver cirrhosis and HCC was found to be 83.3%, 68.5% and 33.3%, respectively while that of anti-HEV IgM was 8.3%, 14.3% and 0%, respectively.

2.5 Distribution of HEV Seropositive Patients according to Gender and Age

Among all the 55 patients with anti-HEV IgG positivity, there were no significant differences in age and gender. Their mean ages were 51.5 (40–61) years and 52 (46–56) years for females and males respectively (*P*=0.98).

2.6 Factors Associated with HBV-HEV Superinfection in Different Categories of CHB Patients

Among liver cirrhosis patients, significantly high frequency of HBV-HEV superinfection was observed compared to monoinfection subjects (68.6% *vs.* 31.4%; *P*<0.001). The same trend was also observed among patients with ACLF where 83.3% of them were HBV-HEV superinfected while 23.1% were HBV monoinfected (*P*<0.001). Furthermore, the HBV-HEV superinfection group comprised the majority of patients with suppressed HBV viral load. All of the patients with low viral load (<2000 IU/mL) were HBV-HEV superinfected (*P*<0.001). Remarkably, a strong association was observed between HBV-HEV superinfection with deranged markers for liver failure like total bilirubin (*P*=0.002), serum albumin (*P*<0.001) and PTA (*P*=0.02) (table 3).

2.7 Serum Cytokines

In this study, seven common cytokines (IL-2, IL-4, IL-6, IL-10, IL-17A, TNF- α and IFN-γ) were analyzed in the sera of 83 patients (47 with anti-HEV IgG positivity and 36 with anti-HEV IgG negativity) that were randomly selected. Fig. 2A showed a general increased trend in the percentage of cytokines secretion among anti-HEV IgG^+/IgM^+ seropositive patients compared to IgG^+/IgM^- or IgG^-/IgM^- group. TNF-α was expressed in 76.9% *vs.* 26.1% (*P*=0.003), IL-10 was expressed in 46.1% *vs.* 8.7% (*P*=0.009) and IL-6 was expressed in 100% *vs.* 65.2% (0.02) in the respective IgG^+/IgM^+ *vs.* IgG^-/IgM^- groups. There is an increased trend of cytokines secretion in HEV seropositive patients (anti-HEV IgG and anti-HEV IgM) compared to seronegative patients, but the differences did not reach significant value even after adjusting for age and sex (fig. 2A).

In quantification analysis, significantly higher concentrations of TNF-α were observed in IgG^+/IgM^+ group than in IgG^-/IgM^- group [26.2 (13.4–36.5) *vs.* 10.3 (6.1–14.6), *P*=0.04] pg/mL] (fig. 2B). There were

Table 3 Association between anti-HEV seropositivity with clinical and laboratory variables

Variable	Anti-HEV IgG$^-$	Anti-HEV IgG$^+$	Chi square (χ^2)	*P* value
Cirrhosis			20.98	<0.001
Yes	31.4 (11/35)	68.6 (24/35)		
No	73.3 (87/118)	26.3 (31/118)		
Acute on chronic liver failure			12.69	<0.001
Yes	23.1 (2/12)	83.3 (10/12)		
No	68.1 (96/141)	31.9 (45/141)		
Hepatocellular carcinoma			0.009	0.92
Yes	66.7 (2/3)	33.3 (1/3)		
No	96 (64.0)	54 (36.6)		
Viral load (IU/mL)			55.81	<0.001
<2000	0	100 (26/26)		
≥2000	77.2 (98/127)	22.8 (29/127)		
HBsAg (Ng/mL)			0.86	0.35
<3200	67.5 (54/80)	32.5 (26/80)		
≥3200	60.3 (44/73)	39.7 (29/73)		
ALT			0.20	1.61
<2 ×UNL*	68.7 (55/80)	31.3 (25/80)		
≥2 ×UNL	58.9 (43/73)	41.9 (30/73)		
Total bilirubin (μmol/dL)				
0–20.5	81.4 (57/70)	18.6 (13/70)	16.92	<0.001
20.6–85	53.9 (21/39)	46.1 (18.39)	2.37	0.12
>85	45.5 (20/44)	54.5 (24/44)	9.28	0.002
Albumin (g/L)			26.80	<0.001
<35	25.0 (8/32)	75.0 (24/32)		
≥35	74.4 (90/121)	25.6 (31/121)		
Prothrombin activity (%)			5.59	0.02
<40	25.0 (2/8)	75.0 (6/8)		
≥40	66.2 (96/145)	33.8 (4/145)		

Results are reported as % (*n*/total number). *Upper normal limit=41 IU/L

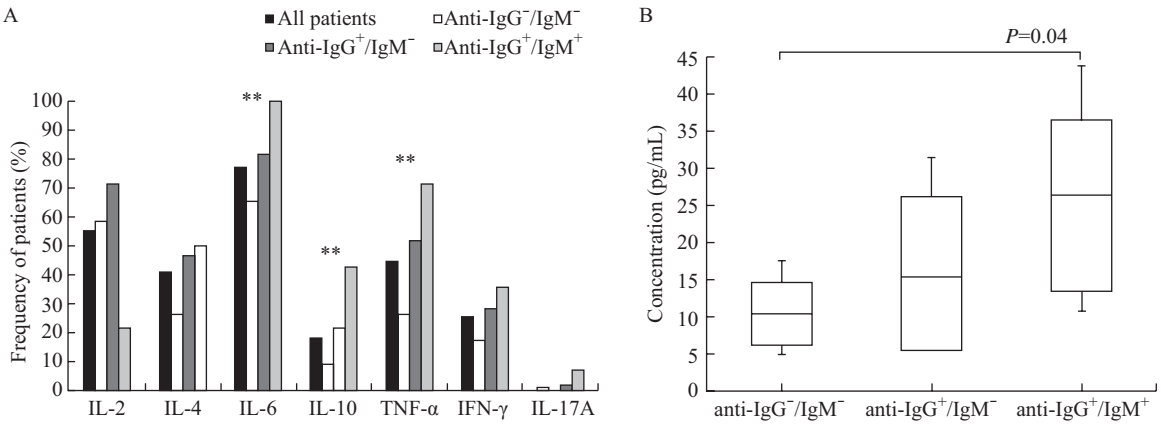

Fig. 2 Cytokines prevalence and concentration comparison between anti-HEV IgG$^+$/IgM$^+$ group, anti-HEV IgG$^+$/IgM$^-$ group and anti-HEV IgG$^-$/IgM$^-$ group

A: Cytokines secretion rates in above different groups. Minimum detection limits for concentrations (pg/mL) were 2.6 for IL-2, 4.9 for IL-4, 2.4 for IL-6, 4.5 for IL-10, 3.5 for TNF-α, 3.7 for IFN-γ and 18.9 for IL-17A. Categorical variables were compared by Fisher's exact test and expressed as percentages.

B: The mean concentration of TNF-α in above different groups was compared by using Mann-Whitney U test. Box plots illustrate medians with 25 and 75 percentiles.

**Significant *P* value between IgG$^-$/IgM$^-$ and IgG$^+$/IgM$^+$ groups

increased odds towards elevated concentration of other cytokines among IgG$^+$/IgM$^-$ and IgG$^+$/IgM$^+$ groups compared to IgG$^-$/IgM$^-$ group, but the differences were not statistically significant (*P*>0.05).

3 DISCUSSION

This study was conducted to assess the magnitude and significance of HEV among patients with CHB

in China. We have demonstrated an association of patients with liver cirrhosis, liver dysfunction with related complications and acquiring of HEV superinfection. The study has revealed that HEV-HBV superinfection is strongly associated with decreased HBV replication despite of the underlying mechanism being unclear. HEV superinfection was also correlated with aggravated liver injury through altered secretion of cytokines resulting in hepatocytes damage in CHB patients. Lower susceptibility for HEV superinfection was mostly observed in genotype C-infected HBV patients.

A great variation of prevalence of HEV seropositivity is typical, depending on the genetic disparity, geographic location, yearly season and occupation. Anti-HEV IgG seroprevalence of 9.2% and 38.1% among CHB patients have been reported in two respective counties of Shandong province recently[9]. In their study, Fu *et al*[10] reported seroprevalence of 25.1% in the south-western part of the country. Previously at the current study site, the prevalence of coinfection was reported to be 35.7% with highest incidences occurring during the first quarter of the year which is denoted as a vulnerable time for increased infection[11]. A similar rate (35.9%) has been observed in the index study which implies a stable pattern of the condition.

Peaked rates of acute HEV infection among CHB patients with chronic liver diseases have been previously reported in Egypt. These patients were also found to have exacerbated rate of hepatic decompensation and excessive risk of mortality in that study[12]. Acharya *et al*[13] reported an increased risk of decompensation (70% *vs.* 20%; $P<0.001$) and mortality (43% *vs.* 22%; $P=0.001$) among cirrhotic patients with acute HEV infection compared to those without. A Chinese study by Zhang *et al*[14] showed an existence of more advanced condition with a poorer prognosis among CHB patients coinfected with acute HEV than in the patients with monoinfection of HBV or those with HBV-HAV coinfection. Consistently, patients with liver diseases presented with an increased rate of HEV seropositivity in the index study. The current study however was not empowered to determine the prognosis of these patients. It is worth noting that an existence of HEV IgG suggests either persistence from a previous infection or a new infection. Either way, this crucial finding underscores that the chronic hepatitis B patients should be an important focus group in preventive strategies including HEV vaccination that is currently not being routinely provided[15].

The impact of HEV superinfection in HBV DNA replication has been debatable for long time due to insufficient evidence as the few available data are conflicting. In their study, Cheng *et al*[16] reported that HBV infection was dormant in acute HEV, and the profound clinical effect during coinfection was significantly triggered by HEV. Moreover, in a recent case report, HBsAg sero-clearance occurred during HEV exacerbation in a patient with CHB and chronic HEV dual infection[17]. Contrarily, a large Vietnamese survey data showed a significant increase in HBV viral load among HEV superinfected patients compared to HBV monoinfected, which suggests the HBV predominance effect[18]. Another study by Zhang *et al*[14] reported that HBV replication was neither stimulated nor suppressed in the presence of HEV. Therefore, the findings from the present study strengthen the evidence that HBV DNA replication might be suppressed by the presence of HEV, and that suppression is likely to persist to post-infection period. However, we don't know its underlying molecular mechanism now. We speculate that as an RNA virus, HEV might function as a ribozyme to inhibit the replication of HBV DNA. This complex mechanism deserves further studies.

Previous studies have correlated acute HEV superinfection with accelerated hepatocytes damage and liver failure in patients with CHB, as demonstrated by elevation of transaminases and deterioration of liver function, respectively. These parameters displayed more severe condition in HEV-HBV coinfected patients than in HBV monoinfected patients[19–21] and HEV monoinfected patients[16]. Similar observations were found in this study which also suggests that the effects also occur in non-acute phase of HEV. Coupled with above finding that HEV decreased HBV replication, a concept that hepatocytes injury is predominantly triggered by HEV and only a trivial effect from HBV in hepatocytes injury can be suggested. This interesting finding is similar to the result previously reported by Cheng *et al*[16]. These results further suggest importance of undertaking intensive immunological researches to ascertain the underlying mechanisms that both HBV and HEV viruses initiate immune mediated response as a key mode of pathogenesis.

Similar to the previous reports from China[22–24], four HBV genotypes (A–D) were identified in this study, and genotypes B and C were the predominant isolates in the central area. We also found that, HBV superinfection group mostly comprised HBV-genotype B, which might be due to small number of comparison patients in superinfection group.

To our knowledge, the present study is the first to evaluate common cytokines in the context of HBV monoinfection relative to HBV-HEV coinfection that may contribute to understanding of differences in virus-specific immune responses from the host. This is the innovation of our study. Our important sightings here are the prominent expression of IL-6 & TNF-α that are regarded as pro-inflammatory cytokines[25], and a remarkable increased secretion of most of the cytokines in acute HEV state in the setting of CHB infection. The

pattern of immune response that is induced by HBV mono-infection has been well established. In acute HBV infection, Th1 (IFN-γ, TNF-α), $CD4^+/CD8^+$ (IL-2) and Kupffer (IL-6) cells with their corresponding cytokines are normally stimulated and released to initiate the immune response[26]. Similarly, IFN-γ and TNF-α which are the hallmark of T-cell response, have also been found to increase in acute HEV mono-infected patients, and spontaneously decline after the recovery[27]. Indeed, the current study demonstrated the same increasing trend of cytokine variation and an exacerbated effect in coinfection groups, implying a synergistic effect. However, we were unable to analyze the concurrent cytokine producing cells population, thus a special precaution was necessitated in interpreting these results. Also, an overall lower rate of cytokines detection that was encountered in the present study might have substantially contributed to the lower concentration profiles. It is also possible that the limited cytokines secretion indicates that their absence is important for viral persistence or it does not play any role in particular infection. The major limitation of this study was its retrospective nature and small number of enrolled patients, therefore a prospective study with more patients is necessary to be carried out in the future.

In conclusion, our findings suggest that in CHB patients, liver dysfunction and chronic liver diseases are strongly associated with HEV superinfection. The superinfection is also correlated with severe manifestations and reduced HBV DNA replication. Thus, this study has provided an important baseline information concerning the general outcome of intersection between CHB and HEV infection which substantiates a need for comprehensive research on the underlying molecular and immunological interactions.

Acknowledgement

We extend our sincere gratitude to all staff of Clinical and Research Laboratories of Infectious Disease of Tongji Hospital.

Conflict of Interest Statement

We have no conflict of interest to declare.

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(Received Feb. 5, 2019; revised May 5, 2019)