ELSEVIER

Journal of Hepatology 46 (2007) 387–394

Journal of Hepatology

www.elsevier.com/locate/jhep

Hepatitis E virus (HEV) infection in patients with cirrhosis is associated with rapid decompensation and death☆

Subrat Kumar Acharya[1,*], Praveen Kumar Sharma[1], Rajbir Singh[3], Sujit Kumar Mohanty[2], Kaushal Madan[1], Jyotish Kumar Jha[2], Subrat Kumar Panda[2]

[1]*Department of Gastroenterology, All India Institute of Medical Sciences, New Delhi 110029, India*
[2]*Department of Pathology, All India Institute of Medical Sciences, New Delhi 110029, India*
[3]*Department of Biostatistics, All India Institute of Medical Sciences, New Delhi 110029, India*

***Background/Aims*: India is hyper-endemic for hepatitis E virus (HEV). HEV infection in cirrhosis may cause high mortality. Prospective study evaluating HEV infection in cirrhotics is scarce.**

***Methods*: Consecutive patients with cirrhosis and healthy controls were included. Cirrhotics were categorized to 3 groups, (Group I – rapid decompensation, Group II – chronically decompensated, Group III – cirrhotics without decompensation). Sera from cirrhotics and controls were tested for HEV-RNA (RT-PCR). HEV-RNA positivity among cirrhotics and controls was compared. Natural course and mortality rate between HEV infected and non-infected cirrhotics were assessed during a 12-month follow-up.**

***Results*: 107 cirrhotics and 200 controls were included. 30 (28%) cirrhotics and 9 (4.5%) controls had detectable HEV-RNA ($p < 0.001$). HEV- RNA positivity among Group I ($n = 42$), II ($n = 32$) and III ($n = 33$) cirrhotics was 21 (50%), 6 (19%) and 3 (10%), respectively ($p = 0.002$). 70% (21/30) with HEV infection and 27% (21/77) without it had rapid decompensation ($p = 0.001$). Mortality between HEV infected and non-infected cirrhotics at 4 weeks (43% vs. 22%, $p = 0.001$) and 12 month (70% vs. 30%, $p = 0.001$) was different. Multivariate analysis identified HEV infection, Child-Pugh's score, renal failure, and sepsis as independent factors for mortality.**

***Conclusions*: In India, cirrhotics were prone to HEV infection, which was associated with rapid decompensation and death.**

***Keywords*: HEV; Cirrhosis; Decompensation; Death**

1. Introduction

Hepatitis E virus (HEV) and hepatitis A virus (HAV) are hyper-endemic in the Indian subcontinent, China, Central Asia and Africa [1–4]. A recent editorial in New England Journal of Medicine reported, a seroprevalence of HEV among 20% of blood donors in USA, identification of Swine HEV in USA and evidence of HEV epidemic in Japan [5]. HEV infection has also been documented in Australia and European Union [2,6–8].

In hyper-endemic countries like India, sub-clinical HAV infection in childhood causes acquirement of protective anti-HAV antibody in almost all by the age of 16 years [9,10]. In contrast, the prevalence of IgG anti-HEV antibody among adults in these region varies from 17.5% to 56% [11–13]. Protective role of IgG-anti-HEV is unclear and repeated HEV infection in an individual

Received 1 June 2006; received in revised form 6 September 2006; accepted 9 September 2006; available online 3 November 2006

☆ The authors who have taken part in this study have declared that they received funding from the Indian Council of Medical Research which enabled them to carry out their study.

* Corresponding author. Tel.: +91 11 26594934; fax: +91 11 26588641.

E-mail address: subratacharya2004@yahoo.com (S. Kumar Acharya).

0168-8278/$32.00
doi:10.1016/j.jhep.2006.09.016

have been documented [14,15]. Therefore, in hyperendemic areas of HAV and HEV, while large immune population against HAV exist, the adult population remain susceptible to HEV infection, as documented during multiple epidemics of HEV in Asia and Africa [1,2].

HAV and HEV infection in healthy individuals are associated with a mortality rate of 0.04–4% [1,2]. However, in non-endemic area with non-immune population, HAV infection in patients with pre-existing chronic liver disease have been reported to cause death in 40% of such cases [16]. Therefore, HAV vaccination is recommended for patients with chronic liver disease [17]. Recently, few case series from Pakistan and India indicate that, HEV infection in pre-existing chronic liver disease may be associated with rapid decompensation and higher mortality [11,13,18]. In Indian subcontinent, 97–100% of patients with cirrhosis of liver are immune to HAV infection [9,19], whereas, adult population with cirrhosis may be prone to contract HEV infection. Prospective study, defining the frequency of HEV infection among patients with cirrhosis, and its subsequent natural course has not been evaluated. The present prospective study was conducted, to identify whether patients with cirrhosis of liver are at high risk to contract HEV infection and whether HEV infection in them alters the natural course of the disease.

2. Patients and methods

2.1. Patients

During January 2003 to December 2004, all patients with a diagnosis of liver cirrhosis and admitted to Gastroenterology ward were included in the study. The diagnosis of cirrhosis was established by conventional clinical, biochemical, imaging and endoscopic criteria. Presence of irregular liver surface with altered attenuation and porta-systemic collaterals with or without ascites on dual phase contrast enhanced tomographic scan (dual phase CECT) with endoscopic documentation of presence of esophageal varices were the conventional criteria to diagnose cirrhosis of liver. Liver biopsy was performed, if diagnosis of cirrhosis could not be obtained by conventional criteria. Liver biopsy was done, after patient's consent and if coagulation profile was normal.

Cirrhotic patients with overt sepsis, gastrointestinal bleeding, primary liver cancer, portal or hepatic vein thrombosis, surgical obstructive jaundice, hepatorenal syndrome and those consuming alcohol during previous 6 months were excluded from the study, because each of these events is known to cause decompensation and death. Age and sex matched replacement and voluntary blood donors attending blood bank during the study period were included as healthy controls.

2.2. Methods

2.2.1. Investigations

All patients were subjected to clinical examination and had routine haematological, biochemical and liver function tests (serum bilirubin, alanine amino transferase, aspartate aminotransferase, serum alkaline phosphatase, total serum protein, serum albumin and prothrombin time estimation). Each patient also had real time grey scale ultrasonography; dual phase contrast enhanced CT scan, upper gastrointestinal endoscopy and alfa-feto protein estimation.

Sera from each patient were tested for hepatitis B surface antigen (HBsAg), IgM and total antibody to hepatitis core antigen (Anti HBc IgM and Anti HBc), antibody to hepatitis 'C' virus using commercial ELISA (Xcyton, Bangalore, India). HCV RNA in each patient's sera was tested by reverse transcriptase nested polymerase chain reaction (RT-PCR) using primer's from 5′-nontranslated region of hepatitis C virus genome by method already described by us earlier [20]. Patients positive for HBsAg and/or Anti HBc were also tested for IgG and IgM antibody against hepatitis D virus using commercial ELISA (Wellcome, UK). Appropriate tests for autoimmune liver disease [21], Wilson's disease [22], haemochromatosis [23] and α-1-antitrypsin defficiency [24] were performed. Etiology of cirrhosis was determined based on results of above investigations. At the time of hospitalization, each patient's Child-Pugh's score was determined [25]. Test for HEV-RNA in the sera of each patient and control was performed by RT-PCR.

2.2.2. Reverse transcription and nested PCR for HEV [26–28]

RNA was extracted and precipitated from 100 μl of serum samples by acid–guanidinium–phenol method. The precipitated RNA was washed with 70% ethanol and vacuum dried. RNA was resuspended in 50 μl diethyl-pyrocarbonate (DEPC) treated water and used for the first round of RT-PCR after denaturation at 94 °C for 10 min. A 100 μl reaction mixture was set up that contained the RNA along with 1× PCR buffer [10 mM Tris–HCl, pH 8.8, 2.5 mM $MgCl_2$, 50 mM KCl and 0.1% gelatin (w/v)], 50 pmol external primers (Table 1), 200 μM of each dNTP, 40 U RNAsin (Promega, Madison, WI, USA), 8 U avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI, USA), and 2.5 U *Taq* DNA polymerase (Amersham, UK). The reaction mixture was overlain with 100 μl mineral oil and incubated at 42 °C for 60 min, followed by 32 cycles of amplification. Ten microliters of the amplified material was used for the second-round nested amplification with internal primers (Table 1). The primers for HEV were from the nonstructural region (ORF-1) [26]. Samples were tested in duplicate, and the tests were repeated if differing results were obtained. Only repeatedly positive reactions were considered positive. Positive and negative controls were included at the extraction step and in both rounds of amplification.

The amplified PCR products were confirmed either by Southern hybridisation [26] or liquid oligomer hybridisation.[28] with internal oligo-probes end labelled with γ-[32] P-ATP (New England Nuclear, Boston, MA). The probes were oligonucleotide sequences that were located between but did not include the primers (Table 1).

2.2.3. Study design

All cirrhotics included in the study were prospectively categorized into three distinct groups as per following criteria.

Table 1
Primers used for detection of HEV-RNA

Outer primers	(3043)	5′-CGGGATCCACACACATCTGAGCTACATTCGTGAGCT-3′
	(3044)	5′-CGGAATTCAAAGGCATCCATGGTGTTTGAGAATGAC-3′
Inner primers	(HEV-1)	5′-GGAATTCGACTCCACCCAGAATAACTT-3′
	(HEV-3)	5′-GGAATTCACAGCCGGCGATCAGGACAG-3′
Inner probe	(DET-3)	5′-ACTCCTCCATAATAGCACACTCTAGACCCAGAG-3′

Group 1: Patients with cirrhosis presenting with serum bilirubin >2.5 mg/dl with ALT >2.5 times of upper limit of normal or sudden deterioration of Child-Pugh's score by 3 or more over pre-existing score within 30 days. This group was considered as the hypothetical test group in whom, HEV infection might have been responsible for rapid deterioration (acute on chronic liver failure) [29].

Group 2: Patients with cirrhosis of liver without above features, but presenting with long-standing decompensation (⩾6 months) in the form of ascites. During the study period, these patients were hospitalized for short duration for either large volume paracentesis or for their 6 monthly-protocolised check-up for detection of early HCC.

Group 3: Patients with Child's A cirrhosis having Child-Pugh's score of ⩽6. These patients were hospitalized for short duration for liver biopsy and other investigations.

Voluntary or replacement healthy blood donors attending blood bank during the study period were included as healthy controls.

Each patient after discharge from the hospital was followed up 4 weekly for at least 12 months. During each visit, their Child-Pugh's score and renal function (blood urea and serum creatinine) were evaluated. After inclusion to the study, if any complications like variceal bleeding, hepatorenal syndrome, sepsis, encephalopathy or death occurred during the hospital stays and follows-up, they were recorded and therapy was instituted. Diagnosis of these complications was made as per accepted criteria [30–33].

Primary outcome measures was the frequency of HEV viremia among the three categories of patients with cirrhosis of liver and healthy controls. Furthermore, four-week and 12-month mortality among patients with cirrhosis with and without HEV infection was also assessed. The secondary outcome measures included complications encountered among cirrhotics with and without HEV infection during the first 4 weeks and subsequently during 12 months follow-up.The endpoint of the study was death and completion of 12 months follow-up. The censoring time for mortality was end of 12 months of follow-up.

2.2.4. Statistics

Descriptive statistics for continuous variables included mean ± SD as well as median (range), and for categorical variables frequency distribution with percentage were calculated. To compare the continuous variables among groups of patients, one-way analysis of variance (ANOVA) with post hoc analysis (Bonferroni) was used. Data with

Table 2
Comparison of demographic, clinical and biochemical parameters among three groups of patients with cirrhosis of liver

Variables	Group I	Group II	Group III	*p* value
Number of patients	42	32	33	
Age in years (mean ± SD)	44.6 ± 14.6	47.3 ± 13.5	43 ± 11.9	NS
Sex (male:female)	32:10	22:10	29:4	NS
Etiology				
HBV	18	10	14	
HCV	3	2	8	
Mixed (HBV + HCV)	7	3	4	NS
Alcohol	7	8	1	
Others	3	4	1	
Cryptogenic	4	5	5	
Presenting features[†]				
Jaundice	39 (93%)	5 (16%)	0	0.0001
Ascites	36 (86%)	19 (59%)	0	0.02
Encephalopathy	15 (36%)	17 (53%)	0	NS
Renal failure	7 (17%)	4 (12.5%)	0	NS
Disease duration in months (mean ± SD)	7.17 ± 11.4	31.35 ± 32.04[*]	34.9 ± 26.3[*]	0.02
Liver function tests				
Serum bilirubin (mg/dl)				
Mean ± SD	16.6 ± 11.5	3.3 ± 4.5[*]	1.4 ± 1[*,#]	0.0001
Median (range)	15 (5.5–28.9)	1.8 (0.9–4.7)	1.2 (0.7–1.8)	
ALT (IU/L)				
Mean ± SD	213.1 ± 202.8	55 ± 51.1[*]	52 ± 30.5[*,#]	0.0001
Median (range)	145.5 (74–291.8)	4.1 (30–60.8)	40 (31–61)	
Serum albumin (g/dl)				
Mean ± SD	2.7 ± 0.6	2.4 ± 0.8[*]	3.6 ± 0.6[*,#]	0.0001
Median (range)	2.6 (2.3–2.9)	2.3 (2–2.8)	3.5 (3.1–4.0)	
Prothrombin time prolongation over control (seconds)				
Mean ± SD	12.7 ± 10.5	7.5 ± 7.7[*]	2.8 ± 1.2[*,#]	0.0001
Median (range)	10 (6–16)	5 (2–8.8)	3 (2–3)	
Child-Pugh's score (at inclusion)	11.1 ± 2.48	9.9 ± 2.3[*]	5.7 ± 1.5[*,#]	0.0001
Serum creatinine (mg/dl)	1.2 ± 1.1	1.1 ± 0.8	1.0 ± 0.2	NS

Group I patients with cirrhosis of liver with rapid hepatic decompensation (scute on chronic liver failure).
Group II chronically decompensated cirrhosis.
Group III stable cirrhosis.
[†] Comparison between Groups I and II.
[*] $p < 0.05$, Groups I vs. II and Groups I vs. III (post-hoc analysis).
[#] $p < 0.05$, Groups II vs. III (post-hoc analysis).
HBV, hepatitis B virus; HCV, hepatitis C virus; ALT, alanine aminotransferase; and NS, not significant.

negative-skewed distribution were compared using non-parametric test such as Mann–Whitney test. χ^2 test was used for categorical variables. A Kaplan–Meier curve was plotted to depict survival among groups and log-rank test was used to compare the survival among the groups. A p-value of 0.05 was considered as statistically significant. SAS 8.0 statistical software was used for analysis.

Death was considered as dependent variable and variables like age, sex, disease duration, Child-Pugh's score, ALT levels, etiology of underlying cirrhosis, HEV-RNA positivity or negativity and complications were considered as independent variables. Among these independent variables, HEV-RNA status, etiology of cirrhosis and complications were considered as categorical variable and the remaining were continuous variable. A univariate Cox's proportional hazard regression was performed to calculate unadjusted relative risk (RR) with its 95% confidence interval (CI). Subsequently, a multivariate Cox's proportional hazard regression was performed to identify the adjusted RR and the CI of the independent variable for mortality among the patients with cirrhosis. Variables with statistical significance on univariate analysis were used for multivariate analysis.

Ethical clearance was obtained before the study was initiated and each patient's consent was obtained for his or her inclusion in the study.

3. Results

One hundred ninety-two consecutive patients with cirrhosis were included in the study. Eighty-five of them were excluded due to, gastrointestinal bleeding ($n = 48$), overt sepsis ($n = 24$), presence of hepatocellular cancer ($n = 3$), portal vein thrombosis ($n = 1$) and unconfirmed diagnosis of cirrhosis ($n = 9$).

Thirty (28%) of 107 with cirrhosis and 9 (4.5%) of 200 controls included in the study had detectable HEV-RNA in their sera ($p < 0.001$).

Out of 107 patients with cirrhosis included in the study, 42 were categorized as Group I, 32 as Group II and 33 as Group III cirrhotics (Table 2). The Child-Pugh's score and liver function tests among Group I were significantly worse than Groups II and III cirrhotics (Table 2). HEV-RNA positivity among Groups I, II and III cirrhotics were 50% ($n = 21$), 19% ($n = 6$) and 10% ($n = 3$), respectively ($p = 0.002$) (Table 3). On post hoc analysis also, HEV-RNA positivity frequency among Group I in comparison to Groups II and III patients was found to be significantly higher ($p < 0.05$) (Table 3).

Twenty-six (62%) out of 42 cirrhotics in Group I (rapid decompensation group) were asymptomatic before the present illness without history of previous liver disease and 21/26 (86%) of them had detectable HEV-RNA in their sera. Of 30 cirrhotic patients with positive HEV-RNA, 21 (70%) had rapid decompensation (gr 1), 6 (20%) were chronically decompensated (gr II) ($p < 0.001$) and remaining 3 (10%) were stable cirrhotics (gr III) ($p < 0.0001$) (Table 4). The former 21 patients with cirrhosis having HEV-RNA positivity were asymptomatic in the past, and presented with hepatic decompensation as the initial presenting features and all died

Table 3
Comparison of HEV-RNA positivity status, mortality frequency and 4 week complication frequencies among the three groups of patients with cirrhosis of liver

Variables	Group I	Group II	Group III	p value
Number of patients	42	32	33	–
HEV RNA +ve	21 (50%)	6 (19%)*	3 (10%)*	0.002
Mortality (4 weeks)	21 (50%)	6 (19%)*	0*,#	0.001
Mortality (12 months)†	27 (64.3%)	16 (50%)	1 (3%)*,#	0.0001
Child-Pugh's score				
At inclusion	11.1 ± 2.48	9.9 ± 2.3*	5.7 ± 1.5*,#	0.0001
At 4 weeks	11.5 ± 2.74	7.9 ± 3.9*	5.7 ± 1.5*,#	0.0001
At 6 months	3 ± 5	6 ± 6	5.8 ± 1.7	NS
Complications 4 weeks††				
Variceal bleed	10 (24%)	11 (34%)	0	NS
Spontaneous bacterial peritonitis	3 (7.5%)	8 (25%)	0	NS
Encephalopathy	23 (55%)	13 (41%)	0	NS
Renal failure	20 (48%)	6 (19)	0	0.02
Infection	10 (24%)	6 (19%)	0	NS
Follow-up (months)				
Mean ± SD	4 ± 4.19	5.9 ± 4.25	6 ± 4	
Median	1	5.5	12	
Range	0.5–12	0.5–12	2–12	

Group I patients with cirrhosis of liver with rapid hepatic decompensation or (acute on chronic liver failure).
Group II chronically decompensated cirrhosis.
Group III stable cirrhosis.
* $p < 0.05$, Groups I vs. II and Groups I vs. III (post-hoc analysis).
# $p < 0.05$, Groups II vs. III (post-hoc analysis).
† One patient in Group III died due to intracranial bleed at the end of 6 months follow-up.
†† Complication frequencies were compared between Groups I and II.
NS, not significant.

Table 4
Comparison of demographic, clinical, biochemical parameters as well as Complication and mortality frequencies among patients with cirrhosis of liver with and without HEV infection

Variable	Cirrhotics with HEV infection	Cirrhotics without HEV infection	*p*
Number of patients	30	77	–
Age in years (mean ± SD)	46 ± 14.6	44.5 ± 13.1	NS
Sex (male:female)	24:6	59:18	NS
Etiology			
HBV	12	30	
HCV	2	11	
HBV + HCV	6	8	NS
Alcohol	4	12	
Others	1	7	
Cryptogenic	5	9	
Liver function tests			
Serum bilirubin (mg/dl)			
Mean ± SD	13.2 ± 13	6 ± 8	0.006
Median (range)	5.8 (1.85–25.7)	1.9 (0.9–6.8)	
ALT (IU/L)			
Mean ± SD	191 ± 252	88 ± 88	0.03
Median (range)	82.5 (35–264)	56 (34–112.5)	
Serum albumin (g/dl)			
Mean ± SD	2.8 ± 0.6	2.9 ± 0.8	NS
Median (range)	2.75 (2.3–3.0)	(2.4–3.4)	
Prothrombin time prolongation over controls (s)			
Mean ± SD	10 ± 7	7 ± 9	0.001
Median (range)	8.1 (4–13.8)	3.9 (2–7.5)	
Serum creatinine (mg/dl)	1.1 ± 0.6	1.2 ± 0.9	NS
Child-Pugh's score			
At inclusion	10.5 ± 2.5	8.4 ± 3	0.001
At 4 weeks	11.2 ± 2.8	9 ± 4	0.02
At 6 months	4.3 ± 5.8	4.1 ± 5.0	NS
Categorization to Group I cirrhosis (acute on chronic liver failure)	21 (70%)	21 (27%)	0.001
Complication at 4 weeks[a]			
Variceal bleed	7 (23%)	14 (18%)	NS
Spontaneous bacterial peritonitis (SBP)	2 (7%)	9 (12%)	NS
Encephalopathy	18 (60%)	19 (25%)	0.001
Renal failure	15 (50%)	11 (14%)	0.002
Infection	8 (27%)	7 (9%)	0.04
Mortality			
At 4 weeks	13 (43%)	14 (22%)	0.001
Within 12 months	8 (27%)	9 (12%)	0.03
Total	21 (70%)	23 (30%)	0.001

HEV, hepatitis E virus; HBV, hepatitis B virus; HCV, hepatitis C virus; ALT, alanine aminotransferase; and NS, not significant.
[a] Complication frequencies between the two groups after 4 week until the end of follow-up were similar.

within 12 months of hospitalization (Table 4). Thirteen (64%) of these 21 patients died within 4 weeks of hospitalization (Table 4).

Both 4-week and 12 month mortality rate was significantly higher among Group I cirrhotic than in Groups II and III (Table 3 and Fig. 1).

To determine the natural course of HEV infection among the cirrhotics, further comparison was made between cirrhotics with HEV-RNA positivity ($n = 30$) and cirrhotics without HEV-RNA in their sera ($n = 77$) (Table 4). The liver function tests and Child-Pugh's score (at inclusion and at 4 weeks) were significantly worse among cirrhotics with HEV infection than in cirrhotics without it (Table 4). Twenty-one (70%) of 30 HEV-RNA positive cirrhotics had rapid decompensation and were categorized as Group I cirrhotics, whereas 21/77 (27%) patients without HEV infection were categorized to Group I ($p < 0.001$) (Table 4). Proportion of patients who developed serious complications like encephalopathy, renal failure, and sepsis was significantly higher among patients with HEV infection than those without HEV infection (Table 4).

In comparison to patients without HEV infection, HEV infected patients had a higher mortality rate at 4

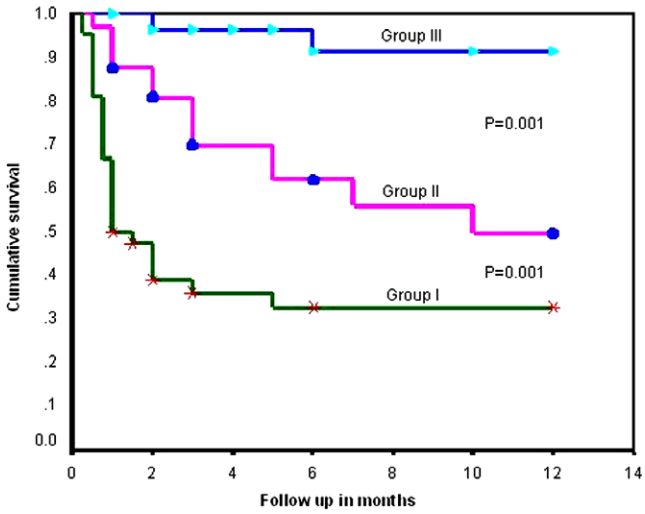

Fig. 1. Kaplan–Meier curve showing 12-month cumulative survival frequency among the three groups of cirrhotics included in the study. Group I (acute on chronic liver failure), Group II (chronically decompensated cirrhotics), Group III – (Childs 'A' stable cirrhotics). The mortality among Group I cirrhotics was significantly higher than Group II ($p = 0.001$) and Group III cirrhotics ($p = 0.001$). [This figure appears in colour on the web.]

weeks (43% vs. 22%,$p = 0.001$) as well as at 12 months (70% vs. 30%, $p = 0.001$) (Table 4). The Kaplan–Meier curve (Fig. 2) depicting 12-month cumulative survival frequency between HEV infected and noninfected cirrhotics also revealed same findings ($p = 0.003$).

Multivariate analysis revealed that higher Child-Pugh's score (RR-1.36, 95%CI 1.19–1.56), HEV-RNA positivity (RR-1.88, 95% CI 1.01–3.49), presence of renal failure (RR-5.4, CI 2.2–15.2) and sepsis (RR-3.19, CI 1.50–6.77) were independent predictors of mortality (Table 5).

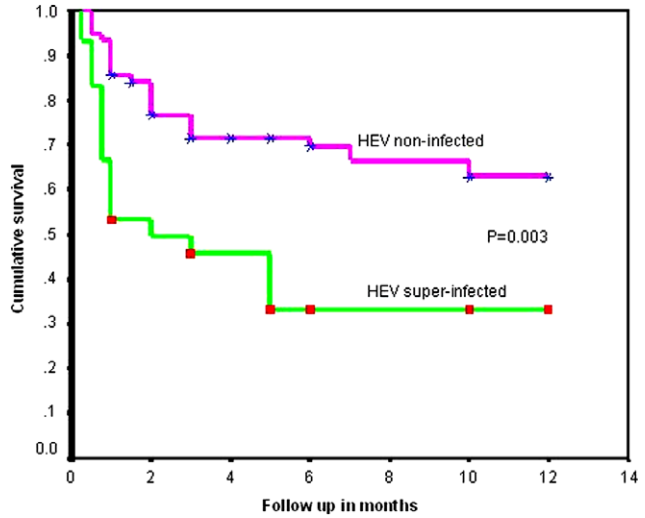

Fig. 2. Kaplan–Meier curve showing 12-month cumulative survival frequencies among patients with cirrhosis of liver with and without HEV infection. The mortality among HEV-infected cirrhotics was significantly higher than in-patients of cirrhosis without HEV infection ($p = 0.003$). [This figure appears in colour on the web.]

4. Discussion

Usually HAV or HEV infection causes mild self-limiting disease. However, HAV infection in cirrhotics has been reported to cause liver failure and high mortality [16]. More than 95% patients with chronic liver disease in India have protective antibody against HAV [19]. Therefore, HAV infection in Indian patients with chronic liver disease is infrequent. In contrast, despite hyper-endemicity of HEV in South Asia and particularly in India, prevalence of IgG anti-HEV in adult population has been reported to vary from 17.5% to 56% [11–13] and protective role of IgG anti-HEV is unclear. In four recently reported case series [11–13,18], rapid hepatic decompensation associated with increased mortality was documented among patients with pre-existing chronic liver diseases with HEV super-infection. Three of these studies were retrospective analysis of few cases of cirrhosis with rapid decompensation [11–13] and only one study was prospectively done which included small number of patients ($n = 32$) [18]. All these studies used presence of IgM anti-HEV in patients sera as evidence of acute HEV infection. Commercial ELISA that detects antibodies against Open reading frame 2 (ORF2) and ORF3 were used in these studies. In hyper-endemic area, higher sensitive tests are appropriate and the commercial anti-HEV test available are known to be more specific and less sensitive [34–38]. Furthermore, sensitivity of these tests in hyper-endemic area has not been optimally evaluated until date. Available information indicate that the sensitivity of commercial ELISA test in detecting IgM antibodies against ORF2 and ORF3 encoded peptide is less than 50% [36]. Among cirrhotics in whom immune system is compromised, IgM anti-HEV studies following HEV infection have not been performed. Therefore, the estimation of HEV-RNA may be a reliable marker of HEV infection in cirrhotics than IgM anti-HEV assessment to indicate presence of active viral infection. In the present study, HEV-RNA was used as evidence of active HEV infection.

The present study revealed that, patients with cirrhosis of liver constitute high-risk group to contract HEV infection. Until date, pregnant females were the only high-risk population to contract HEV infection [1,2]. No study had evaluated the proneness of cirrhotics to contract HEV infection. In the previously described case series [11–13] and the prospective study [18], HEV infection in cirrhosis was associated with rapid deterioration of liver function, but whether cirrhotics were prone to contract HEV infection was not evaluated.

Detection of HEV-RNA in 4.5% healthy donors may appear high, particularly to those working in non-en-

Table 5
Risk factor of mortality among patients with cirrhosis by Cox's proportional hazard model[a]

Variable	Categories	Unadjusted RR and (95% CI)	Adjusted RR and (95% CI)
Age	Continuous	1.03 (1.01–1.05)	
Disease duration	Continuous	0.99 (0.97–1.00)	
Child-Pugh's score	Continuous	1.45 (1.29–1.65)	1.36 (1.19–1.56)
ALT level at inclusion	Continuous	1.01 (1.00–1.03)	
Serum creatinine at inclusion	Continuous	1.52 (1.18–1.97)	
Sex	Male	1	
	Female	1.46 (0.78–2.85)	
HEV-RNA	Negative	1	
	Positive	3.03 (1.67–5.48)	1.88 (1.01–3.49)
Renal failure	Absent	1	
	Present	13.30 (5.98–29.58)	5.40 (2.20–15.20)
Sepsis	Absent	1	
	Present	10.23 (5.24–19.97)	3.19 (1.50–6.77)

HEV,-hepatitis E virus; ALT, alanine aminotransferase.
[a] Encephalopathy, sepsis and renal failure were complication after inclusion into the study.

demic regions for HEV. Recent reports from the Indian subcontinent have repeatedly documented presence of HEV-RNA in the sera of 1.5–4% of healthy voluntary donors [39–41]. Furthermore, post transfusional HEV infection and exposure have also been documented [35,37]. In another recent report, 244/2070 (12%) of the children residing in northern India and attending medical facilities for minor ailments were found to be IgM anti-HEV positive [42]. These facts indicate that HEV infection without overt features of liver injury (Sub-clinical infection) is frequent in India [41]. It is well known that HEV infection resolves quickly and does not cause chronic infection [1,2,5]. Therefore, short duration sub-clinical acute HEV infection without overt clinical manifestation among general population in northern India is well known and is responsible for maintenance of HEV-reservoir in human [41].

The study also documented that; superinfection of HEV in-patients with pre-existing cirrhosis of liver were associated with rapid hepatic decompensation causing high mortality. This observation was supported by the fact that, the frequency of HEV-RNA positivity among patients presenting with rapid decompensation (group I) was significantly higher ($p = 0.002$), than in cirrhotics without rapid decompensation (groups II and III) (Table 3), and the frequency of rapid decompensation (categorization to Group I) among HEV-RNA positive cirrhotics was significantly higher ($p = 0.001$) than similar events among the cirrhotics without HEV infection (Table 4). Furthermore, 26 (62%) out of 42 cirrhotics in-group I (rapid decompensation group) were asymptomatic before the present illness and 21/26 (86%) of these patients had detectable HEV-RNA in their sera. Therefore it is possible that HEV super-infection in these 21 patients had resulted in rapid decompensation from a compensated asymptomatic state of chronic liver disease. Thirteen of these latter 21 patients died within 4 weeks of inclusion into the study and all of them succumbed to their illness within 12 months of follow-up. Therefore, HEV infection in cirrhotics is not only associated with rapid hepatic decompensation, but also with higher mortality. The latter statement is further reinforced by the fact that, the mortality rate and risk of hepatic decompensation among HEV infected cirrhotics were significantly higher than in similar patients without HEV infection (Table 4) and HEV positive status was shown to be an independent risk factor for mortality (Table 5). In a report from China, HEV super-infection in patients with chronic HBV infection was reported to have worse hepatic histology than those without HEV superinfection [43].

In conclusion, the present study revealed that in India, cirrhotics constituted high-risk group to contract HEV infection and subsequently had rapid deterioration of hepatic reserve leading to high mortality.

Acknowledgement

The study was funded by a grant from the Indian Council of Medical Research.

References

[1] Aggarwal R, Krawczynski K. Hepatitis E: an overview and recent advances in clinical and laboratory research. J Gastroenterol Hepatol 2000;15:9–20.
[2] Krawczynski K, Aggarwal R, Kamili S. Hepatitis E. Inf Dis Clin North Am 2000;15:9–20.
[3] Tsega E, Krawczynski K, Hannson BG, Nordenfelt E, Negusse Y, Alemu W, et al. Outbreak of hepatitis E virus infection among military personnel in Northern Ethiopia. J Med Virol 1999;34:232–236.
[4] Mushawar IK, Dawson GJ, Bile KM, Magnus LO. Serological study of an enterically transmitted non-A, non-B hepatitis in Somalia. J Med Virol 1993;40:218–221.
[5] Emerson SU, Purcell RH. Running likes water – The omnipresence of hepatitis E. New Eng J Med 2004;351:2367–2368.
[6] Coursaget P, Depril N, Buisson Y, Molinie C, Roue R. Hepatitis types E in a French population: detection of anti-HEV by

synthetic peptide-based enzyme linked immunosorbent assay. Res Virol 1994;145:51–57.
[7] Moaven L, Van Aslen M, Crofts N, Locarinini SA. Seroepidemiology of hepatitis E in selected Australian population. J Med Virol 1995;42:326–330.
[8] Grieco A, Miele L, Gasbarrini G, Grillo R. Sporadic HEV in Italy. Gut 2001;48:580.
[9] Batra Y, Bhatkal B, Ojha B, Kaur K, Saraya A, Panda SK, et al. Vaccination against hepatitis A virus may not be required for school children in Northern India: results of a seroepidemiological survey. Bull WHO 2002;80:728–731.
[10] Acharya SK, Batra Y, Bhatkal B, Ojha B, Kaur K, Hazari S, et al. Seroepidemiology of hepatitis A virus infection among school children in Delhi and North Indian patients with chronic liver disease; Implications for HAV vaccination. J Gastroenterol Hepatol 2003;18:822–827.
[11] Hamid SS, Atiq M, Shehzad F, Yasmeen A, Nissa T, Salam A, et al. Hepatitis E virus superinfection in-patients with chronic liver disease. Hepatology 2002;36:474–478.
[12] Kaur H, Oberoi A, Chander R, Pawan G, Verma M. Epidemiology of hepatitis E and A in Ludhiana, India. Trop Gastroenterol 2002;23:76–78.
[13] Ramachandran J, Eapen CE, Kang G, Abraham P, Hubert DDJ, Kurien G, et al. Hepatitis E superinfection produces severe decompensation in patients with chronic liver disease. J Gastroenterol Hepatol 2004;19:134–138.
[14] Khuroo MS, Kamili S, Dar MY, Moecklii R, Jameel S. Hepatitis E and long term antibody status. Lancet 1993;34:1355.
[15] Ke WM, Tan D, Li JG, Izumi S, Shinji Y, Hotta H, et al. Consecutive evaluation of immunoglobulin M and G antibodies against hepatitis E virus. J Gastroenterol 1996;31:811–822.
[16] Vento S, Garofano T, Renzini C, Cainelli F, Casali F, Ghironzi G, et al. Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. New Eng J Med 1998;338:286–290.
[17] Centre for disease control and prevention. Prevention of hepatitis A through active or passive immunization: recommendation of the Advisory Committee on Immunisation Practices (ACIP). MMWR 1996; 45:1–30.
[18] Kumar A, Aggarwal R, Naik SR, Saraswat V, Ghosal UC, Naik S. Hepatitis E virus is responsible for decompensation of chronic liver disease in an endemic region. Ind J Gastroenterol 2004;23:59–62.
[19] Acharya SK, Batra Y, Saraya A, Hazari S, Dixit R, Kaur K, et al. Vaccination against hepatitis A virus (HAV) is not necessary for Indian patients with chronic liver disease. Results of a serological study. Natl Med J India 2002;15:267–268.
[20] Panigrahi AK, Nanda SK, Dixit RK, Acharya SK, Zuckerman AJ, Panda SK. Diagnosis of hepatitis C virus associated chronic liver disease in India: comparison of HCV antibody assay with a polymerase chain reaction for the 5′ non coding region. J Med Virol 1994;44:176–179.
[21] Byland DJ, Mcttutchisen J. Autoimmune liver disease. Clin Lab Med 1997;17:483–497.
[22] Abe A, Yamashita S, Noma A. Sensitive direct colorimetric assay for copper in serum. Clin Chem 1989;35:552–554.
[23] Stacy DL, Han P. Serum ferritin measurement and the degree of agreement using four techniques. Am J Clin Pathol 1992;98:511–515.
[24] Norman MR, Mowat AP, Hutchision DC. Molecular basis, clinical consequences and diagnosis of alpha-1-antitrypsin deficiency. Ann Clin Biochem 1997;34:230–246.
[25] Pugh RNH, Murray-Lyon M, Dawson JL, Pietroni MC, William R. Transaction of esophagus for bleeding esophageal varices. Br J Surg 1973;60:646–649.
[26] Jameel S, Durgapal H, Habibullah CM, Khuroo MS, Panda SK. Enteric non A, non B hepatitis: epidemics, animal transmission and hepatitis E virus detection by the polymerase chain reaction. J Med Virol 1992;37:263–270.
[27] Mohanty S, Acharya SK, Panda SK. Antibodies against hepatitis E virus (HEV) protein in acute hepatitis. J Gastroenterol hepatol 2002;36 (Suppl):A29. Abstract.
[28] Abbot MA, Poiesz BJ, Byrne BC, Kwok S, Shinky JJ, Ehrlich GD. Enzymatic gene amplification: qualitative and quantitative methods for detecting proviral DNA amplified in vibro. J Infect Dis 1988;158:1158–1169.
[29] Sen S, William R, Jalan R. The Pathophysiological basis of acute on chronic liver failure. Liver 2002;22 (Suppl 2):5–13.
[30] Fernandez J, Navasa M, Gomez J, Colmenero J, Vila S, Arroyo V, et al. Bacterial infections in cirrhosis: epidemiological changes with invasive procedure and norfloxacillin prophylaxis. Hepatology 2002;35:140–148.
[31] Navasa M, Rodes J. Bacterial infections in cirrhosis. Liver Int 2004;24:277–280.
[32] Gines P, Guevara M, Arroyo V, Rodes J. Hepatorenal syndrome. Lancet 2003;362:1819–1827.
[33] Moore KP, Wong F, Gines P, Bernardi M, Ochs A, Salerno F, et al. The management of ascites in cirrhosis: report on the consensus conference of the International ascites club. Hepatology 2003;38:258–266.
[34] Takahasi M, Kusakai S, Mizuo H, Suzuki K, Fujimura K, Masuko K, et al. Simultaneous detection of immunoglobuin A (IgA) and IgM antibodies against hepatitis E virus (HEV) is highly specific for diagnosis of acute HEV infection. J clin microbiol 2005;43:49–56.
[35] Lin CC, Wu JC, Chang TT, Chang WY, Yu ML, Tam AW, et al. Diagnostic value of immunoglobulin G (IgG) and IgM anti-hepatitis E virus (HEV) tests based on HEV-RNA in an area where hepatitis E virus is not endemic. J Clin Microbiol 2000;38:3915–3918.
[36] Li K, Zhuang H, Zhu W, Ruan B, Jiang J, Li S, et al. A preliminary study on hepatitis E virus antibody IgG and IgM for the diagnosis of acute hepatitis E. Zhonghua Nei Ke Za Zhi 1999;38:733–736.
[37] Thakral D, Panda SK. HEV biology-recent developments and future implications. Trop Gastroenterol 2004;25:1–3.
[38] Panda SK. Hepatitis E virus: recent advances. Trop Gastroenterol 2000;21:47.
[39] Arankalle VS, Chobe LP. Hepatitis E virus: Can it be transmitted parenterally?. J Viral Hepatol 1999;6:161–164.
[40] Khuroo MS, Kamili S, Yattoo GN. Hepatitis E virus infection may be transmitted through blood transfusion in an endemic area. J Gastroenterol Hepatol 2004;19:778–784.
[41] Aggarwal R. Hepatitis E: Is it a blood borne pathogen. J Gastroenterol Hepatol 2004;19:729–731.
[42] Mathur P, Arora NK, Panda SK, Kapoor SK, Jailkhani BL, Irshad M. Seroepidemiology of hepatitis E virus (HEV) in Urban and rural childrens of north India. Indian Paediatr 2001;38:461–475.
[43] Shang Q, Yu J, Xiao D, Xu C, Chen C, Zhang G. The effect of hepatitis E virus superinfection on patients with chronic hepatitis B: a clinicopathological study. Zhnghua Nei Ke Za Zhi 2002;41:656–659.