Original Research Article

Aggravating Effects of Hepatitis E virus infection on patients with chronic liver disease in Ibn-Sina hospital Sudan

Abstract

Background: Hepatitis E virus (HEV) is one of the most common causes of acute hepatitis. Sudan is considered as hyper-endemic for HEV. HEV infection in patients with preexisting chronic liver diseases (CLDs) has been reported to result in severe clinical manifestations and poor outcomes. However data on the role of HEV infection in worsening of pre-existing CLD are limited.

Objective: To determine hepatitis E virus (HEV) infection and its effect on severity of CLD.

Methods: A descriptive cross-sectional study that consecutively enrolled 87 CLD patients in Ibn-Sina Specialized Hospital was carried out during the period from August 2020 to December 2020. Data regarding demographics, CLD causes, clinical manifestations and comorbidities were collected. The screening for anti-HEV antibodies was performed in all patients by using enzyme linked immuno-sorbent assay (ELISA)

Results. Hepatitis B virus (HBV) was the commonest etiology of chronic liver diseases being detected in 45/87(51.7%) patients. On the other hand;among all subjects; 43/87(49.4%) patients were HEV seropositive with anti-HEV Ig-G being detected in 32(36.8%) and concurrent anti-HEV Ig-M and Ig-G in 11(12.6%) patients. Jaundice (OR= 3.8, CI95%: 1.5-9.4; P. value=0.004), HCC (OR= 4.1, CI95%: 1.4-11.9; P. value=0.008), child-Pugh class-C (OR= 26, CI95%: 5.4-94.0; P. value=0.000) and child-Pugh class-B (OR= 5.3, CI95%: 1.6-17.0; P. value=0.000) were associated independently with anti-HEV positivity.

Conclusion: The frequency of hepatitis E virus (HEV) among Sudanese patients with chronic liver disease (CLD) was considerably high (mainly past infection- Ig-G).

Furthermore, HEV was associated with advanced liver failure statuses (child-Pugh class-B & C), jaundice, and hepatocellular carcinoma.

Keywords: Hepatitis E virus, Chronic liver diseases, ELISA , Ibn-Sina hospital , Sudan.

Introduction

"Chronic liver disease (CLD) in the clinical context is a disease process of the liver that involves a process of progressive destruction and regeneration of the liver parenchyma leading to cirrhosis. "Chronic liver disease" refers to disease of the liver which lasts over a period of six months. It consists of a wide range of liver pathologies which include inflammation (chronic hepatitis), liver cirrhosis, and hepatocellular carcinoma" (1)

"CLD is a significant cause of mortality due to complications i.e. cirrhosis. Findings of low hemoglobin, thrombocytopenia and hypoalbuminemia, acholia correspond well with advanced stages of chronic liver disease. The complications of cirrhosis include portal hypertension, ascites, hepatic encephalopathy, and hepatorenal syndrome" (2,3,4).

"HEV is a small, enveloped, single-stranded RNA virus of the Hepeviridae family. According to its epidemiological characteristics and survival indicators, 4 different HEV genotypes that affect humans have been described. Genotypes 1 and 2 cause disease in humans only and are responsible for epidemics through faecal-oral and waterborne transmissions. Genotypes 3 and 4 are more likely to infect domestic and wild pigs and cause infections in humans" (5).

"HEV infection is an enterically transmitted liver disease that is typically self-limited (6,7), but it can trigger fulminant hepatitis in pregnant women where it causes 20-25% mortality" (8). "Infection with HEV can also progress and become either rapidly fatal or

chronic in immunosuppressed individuals" (9). "Liver cirrhosis can also result from HEV infection under certain circumstances" (10).

"The global disease burden of HEV has been reported to be at least 20 million cases per year leading to an estimated 3.7 million symptomatic cases with 70000 fatalities and 3000 stillbirth (11,12) however, it,s global prevalence rate is thought to be under-reported". "HEV is found worldwide, but the disease is more common in East and South Asia. Currently, infection with HEV represents the most common cause of acute viral hepatitis among adults in Southern Asia and Sub-Saharan Africa" (13). "Regions with prevalence rate of more than 25% include Central America, the Middle East and large parts of Africa and Asia. Epidemics of HEV have been reported in African countries, including Sudan, Ethiopia, Somalia, Chad, the Democratic Republic of the Congo, and Uganda" (14).

"The first reported cases of HEV infection in Sudan occurred in 1992; since then several larger outbreaks have been observed particularly in refugee camps in the Darfur region. Furthermore, all of these outbreaks have been shown to be associated with high mortality rates in pregnant women. Large outbreaks have also been reported in camps with Sudanese refugees in Chad and Ethiopia. Sudanese HEV strains causing outbreaks among displaced individuals in Darfur in 2004 were shown to belong to genotype 1. However, little is known about the genetic variability of HEV strains from different regions of Sudan" (15).

The natural history of hepatitis E in patients with CLD is not well understood, and superimposed infection may aggravate the prognosis and increase mortality, that is by causing a severe liver decompensation and occurrence of severe extra hepatic manifestations (16).

"Several studies have reported a high prevalence of HEV antibodies in patients with chronic liver disease and others have suggested that superinfection with HEV in patients

with underlying chronic liver disease can cause severe hepatic decompensation, leading to increased morbidity and mortality" (17,18,3).

Materials and Methods

Study design

This is a prospective cross-sectional Hospital-based study. The study was conducted during the period from August 2020 to December 2020 and aimed to investigate the adverse effects of HEV infection in CLD patients in Khartoum State ,Sudan

Study area

The study was conducted in Ibn-Sina Specialized Hospital for gastrointestinal and liver diseases, which is the largest tertiary center for gastroenterology and hepatology diseases in Sudan, and contains state of the art equipments for both diagnosis and intervention. The center provides service to patients from all over the country.

Study population:

All eligible patients diagnosed with CLD in Ibn-Sina Specialized Hospital during study period.

Inclusion criteria:

The inclusion criteria included adult patients (>18 years) and both sex were consecutively enrolled in the study.

Exclusion criteria:

The exclusion criteria included refusal to participate in the study.

Sample size

This study enrolled 87 CLD patients calculated by Cochrane' formula as follow:

$$n = \frac{Z^2 P(1-P)}{e^2}$$

n= sample size

z= level of confidence (1.96)

p= pervious prevalence (3.3%) (ref?)

e= the degree of accuracy desired (0.05)**.**

$n = \frac{1.96^2 0.033(1-0.033)}{0.05^2}$ = 87 patients

In the final count, there were suitable 87 patients and were all included in the study.

Data collection tools and methods:

Data collection was carried out by the principal investigator. Data was collected through structured questionnaires composed of: demographics, CLD causes and states of origin, clinical manifestations and comorbidities.

The screening for anti-HEV antibodies were performed to all patients by using IgG and IgM detecting enzyme linked immuno-sorbent assay (ELISA) (EUROIMMUN, Germany).

Study variables

1. Independent variables:
   - Socio-demographic characteristics of participants: age, gender, residence, occupation
   - Cause of CLD
   - CLD status
   - Clinical manifestations
   - Comorbidities
   - CTP classification

2. Dependent variables:
    - HEV status

Data analysis:

Data was analyzed by using the computer program Statistical Package for Social Sciences (SPSS V. 21.0). The analyzed data was presented in tables and figures designed by Microsoft Excel 2010. Chi-square test was used in bivariate analysis and logistic regression in multivariate analysis and all P. values of 0.05 or less were considered as significant.

Ethical Approval and Consent

An ethical approval was obtained from Sudan medical specialization board (SMSB). Approval acceptance by the hospital authority was also obtained. Written and verbal consent were obtained from patients. Data were used anonymously by using identity numbers instead of names in order to protect patient's identity and kept securely and in separate file. No reference to any individual participant was made in study reports. Subject identities were being known only by the study staff.

Results

In this study,out of the enrolled 87 chronic liver disease patients, 59(68%) were males and 28(32%) were females with male to female ratio of2.1:1, their mean age was 50.3±15 years and most of them (n=50; 57.5%) aged from 40-60 years (table 1).In regard to residence, most of the patients lived in central areas (n=31; 35.6%) and Khartoum state (n=29; 33.3%) and the majority of patients were workers and farmers (27.6% for each) (table 1).

With regard to Hepatitis E virus status the data showed that, 43(49.4%) were HEV seropositive, among them anti-HEV Ig-G was detected in 32(36.8%) and Anti-HEV Ig-M and Ig-G in 11(12.6%) patients (table 1).

Table (1) also illustrated that, HEV was not significantly associated with demographic characteristics (P. value= 0.448), gender (P. value= 0.438), and residence (P. value= 0.150).

The causes of CLD were not significantly correlated with HEV (P. value > 0.05) (table 1).

However, HEV was present in 53.8% of decompensated liver disease patients comparing to 36.4% of compensated liver disease patients, but the difference was not significant (P value= 0.121) (table1)

HEV was significantly higher in jaundiced patients more than in those without jaundice (74.4% vs 43.2%; P. value= 0.003). However, HEV occurrence was not significantly associated with ascites (P. value= 0.556), encephalopathy (P. value= 0.106) and coagulopathy (P. value= 0.111) (table1).

HEV antibodies were present in 73.9% of HCC patients comparing to 40.6% of non-HCC patients and the difference was highly significant (P. value= 0.006) (table 1).

The presence of comorbidities was not significantly correlated with HEV seropositivity(P. value > 0.05) (table 1).

The association between HEV and child-Pugh classifications showed that, HEV was significantly present in child-Pugh class-C (85%) and child-Pugh class-B patients (53.8%) more than in those with child-Pugh class-A (P. value= 0.000) (table 1).

In multivariate logistic regression, only jaundice (OR= 3.8, CI95%: 1.5-9.4; P. value=0.004), HCC (OR= 4.1, CI95%: 1.4-11.9; P. value=0.008), child-Pugh class-C (OR=

26, CI95%: 5.4-94.0; P. value=0.000) and child-Pugh class-B (OR= 5.3, CI95%: 1.6-17.0; P. value=0.000) were associated independently with anti-HEV positivity (table 2).

Table 1: The association between independent variables (demographic characteristics, cause of CLD, CLD status, clinical manifestation, HCC, comorbidities, and CTP classifications) and HEV seropositivity in 87 CLD patients.

			HEV		*P* value
			Positive	**Negative**	
Demographic characteristics	**Age (Yrs)**	< 40	7	12	
		40-60	26	24	0.448
		> 60	10	8	
	Gender	Male	30	29	0.438
		Female	13	15	
	Residence	Khartoum	14	15	
		Central	17	14	
		West	12	9	0.150
		East	0	2	
		North	0	4	
CLD causes	**HBV**		24	21	0.295
	HCV		2	3	0.511
	Alcoholic liver disease		5	2	0.207
	AIH		4	6	0.384
	Other		0	5	0.055
	Unknown		9	8	0.479
CLD status	Compensated		8	14	0.121
	Decompensated		35	30	
Clinical manifestation	**Ascites**		27	28	0.556
	Slight (n=25)				
	Moderate (n=21)				
	Massive (n=9)				
	Jaundice		32	19	0.003
	Encephalopathy		9	4	0.106
	Coagulopathy		17	11	0.111
HCC	Yes		17	6	0.006
	No		26	38	
Comorbidities	DM		3	10	0.068
	Hypertension		1	4	0.360

	Renal disease	0	4	0.116
	Gout	1	0	0.494
CTP classifications	Class-A	5	23	
	Class-B	21	18	0.000
	Class-C	17	3	

Table 2: Multivariate logistic regression to detect predictors of HEV in CLD patients

	OR	**CI95%**	**P. value**
Jaundice			
Yes	3.8	1.5-9.4	0.004
No (ref)	1	-	-
HCC			
Yes	4.1	1.4-11.9	0.008
No	1	-	-
CTP			
Class-A (ref)	1	-	-
Class-B	5.3	1.6-17.0	0.004
Class-C	26.0	5.4-94.0	0.000

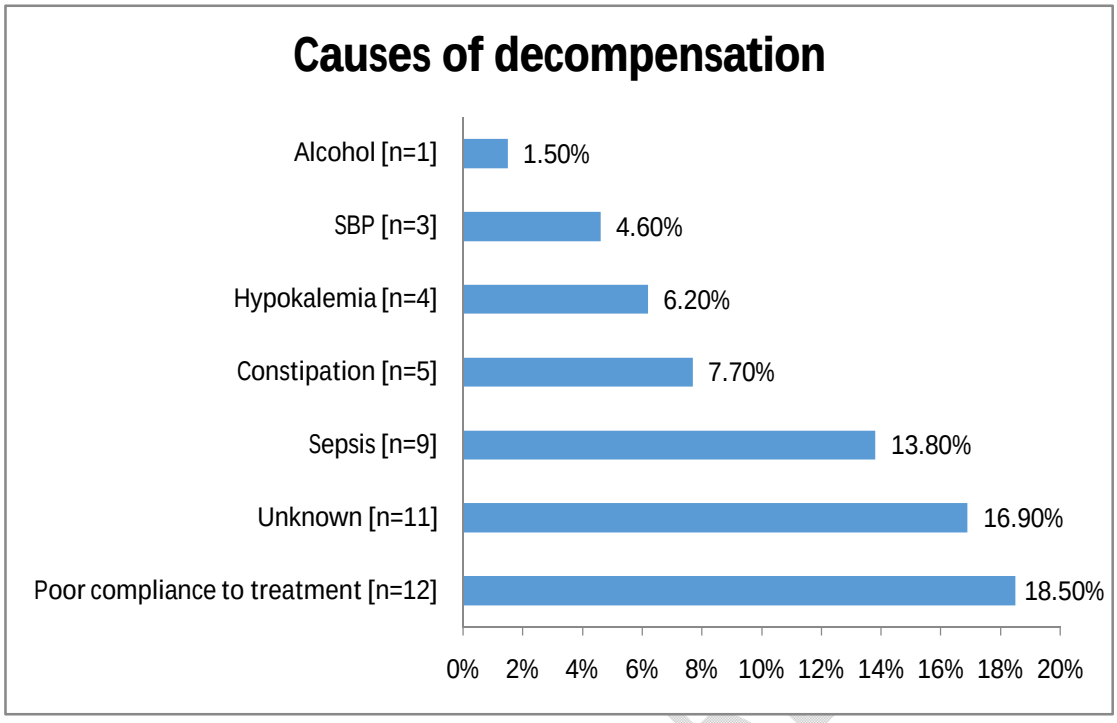

Figure 1: Causes of decompensation in LCD patients take out this figure

Discussion

In the present study we aimed to determine the frequency of hepatitis E virus (HEV) infection[both past and acute] and its effect on severity of CLD among CLD patients in Ibn-Sina Specialized Hospital, Khartoum, Sudan.

The present study showed that, the most affected patients with CLD were males (68% vs. 32%) with male to female ratio of 2.1:1, the mean age was 50.3±15 years and most of them 50(57.5%) aged from 40-60 years(table 1).Our findings indicated that 43(49.4%) of our patients were HEV seropositive whereon anti-HEV Ig-G was detected in 32(36.8%) and both anti-HEV Ig-M and Ig-G in 11(12.6%) patients, in other words most of patients (36.8%) had past HEV infection and 12.6% had acute infection with both IgG and IgM antibodies. This latter group of patients may be past the acute infection stage and starting to produce IgG antibodies in the presence of declining IgM.

Our rate of HEV in CLD was similar to that reported by Kumar et al (50%) (19)., Zhang et al (41.2%)(20) and Kondili et al (36.6%)(21).

Whereas, our findings were higher than those reported by studies of El Sayed et al (13%)(22). Garg et al (15.3) (23), Kumar et al (14.5%) (19), Jha K et al (9.6%) (24) and Museja et al (8In the United States, Kyvernitakis et al reported anti-HEV IgM was not detected in any patient (25).

and Fontana et al found that only 3 patients (0.4%) exhibited anti-HEV IgM (26), interestingly Shimakawa et al did not detect HEV in any patient in Gambia, (27). Moreover, we reported lowered rate of HEV comparing to Krishna et al (66.1%) in India (28)

These discrepancies in results might be explained by several factors such as differences in sampling, sample sizes and study populations biological and genetic characteristics , as well geographical, variations.

Our findings conformed and proved high and remarkable burden of HEV infection among CLD in our context.

Noticeably, the current study showed that, HEV was associated with advanced liver failure statuses as child-Pugh class-C (OR= 26, CI95%: 5.4-94.0; P. value=0.000) and child-Pugh class-B (OR= 5.3, CI95%: 1.6-17.0; P. value=0.000) that were significant predictors of HEV in CLD patients. These observations were in agreement with a study carried out in Vietnam by Hoan et al where an association was found between HEV infection and a higher Child-Pugh score (29) . One of the most interesting finding in the present study is that HEV was significantly associated with development of hepatocellular carcinoma (HCC) (OR= 4.1, CI95%: 1.4-11.9; P. value=0.008), this association could be explained by possible HEV superinfection of patients with chronic liver disease. This result is in accordance with a Cameroonian study conducted by Marie et al(30) in 2017 in which anti-HEV carriage in HCC patient was (41.8%) compared to non-HCC subjects (12.6%;)with CLD (P = 0.000; OR = 4.8, 95%CI: 2.3-10.6) (30) . Also in the study of Kondili et al, the presence of end stage liver disease (ESLD) (OR 4.3, CI95%1.4–12.8) was associated independently with anti-HEV

positivity, indicating that presence of anti-HEV is associated with advanced stages of chronic liver disease (21).

Conclusion

The present study concluded that, the frequency of hepatitis E virus (HEV) among Sudanese patients with chronic liver disease (CLD) was considerably high (mainly past infection with Ig-G). Furthermore, HEV was associated with advanced liver failure statuses (child-Pugh class-B & C), jaundice, and hepatocellular carcinoma. Our findings also indicate that testing for HEV as well as other hepatitis viruses in CLD patients is imperative in a country like Sudan where such viruses are endemic to allow for earlier diagnosis and appropriate therapeutic interventions.

Ethical approval: The study protocol was ethically approved by the Central Laboratory of Ministry of Higher Education and Scientific Research, Sudan. Researchers respected the respondents' autonomy and confidentiality, and written consent with signatures was obtained from respondents after informing them about the objectives and benefits of the research.

Data availability: None.

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