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ORIGINAL ARTICLE

Hepatitis E Virus (HEV) Infection in Cirrhotic Egyptian Patients: IgG Seroprevalence and Acute Infection in Acute-on-chronic Liver Failure

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ABSTRACT

Background*: Hepatitis E virus (HEV) infection is enterically transmitted and usually present as acute self limiting infection. However, in cirrhotic patients, it is usually problematic being a possible trigger of decompensation with high mortality rates.* ***Objectives****: To determine the seroprevalence of HEV IgG and risk factors of HEV exposure in cirrhotic patients. Also, to determine the role of acute HEV infection as a possible trigger of acute-on-chronic liver failure (ACLF) in cirrhotic Egyptian patients in East Delta and to compare different clinical, laboratory and ultrasonographic features between acute HEV positive and negative ACLF patients.* ***Methods****: Two groups of cirrhotic patients were enrolled; group (1) included 216 patients with compensated cirrhosis and group (2) included 67 cirrhotic patients presenting with ACLF. For group (1) patients; HEV seroprevalence was determined by testing for anti-HEV IgG by ELISA, while acute HEV infection was detected in group (2) patients by testing for anti-HEV IgM and HEV RNA by ELISA and RT-PCR respectively. For ACLF cases, full history, clinical, laboratory and ultrasonographic findings were recorded.* ***Results:*** *The seroprevalence of anti-HEV IgG was 39.4 % in cirrhotic patients and well water use was the only significant predictor affecting HEV seropositivity. Acute HEV infection was detected in 13.4% (9/67) of cirrhotic patients with ACLF. It came third among the identified triggering factors of ACLF following bacterial infections (22.4%) and variceal bleeding (14.9%). Acute HEV positive ACLF patients showed significantly higher levels of liver enzymes than acute HEV negative cases.* ***Conclusion****: In our locality, securing a safe water supply seems to be the most important HEV preventive measure. Meanwhile, clinicians should consider testing for acute HEV infection in cirrhotic patients developing ACLF, particularly those showing marked liver enzyme elevation.*

INTRODUCTION

Hepatitis E is a worldwide health problem with an annual estimate of 20 million HEV infections resulting in 70, 000 HEV-related deaths[1]. Hepatitis E virus (HEV) genotypes 1 and 2 are enterically transmitted (mostly waterborne), They are endemic in many developing countries in tropical and subtropical Asia and Africa where water scarcity, inadequate sewage disposal and treatment and contamination of drinking and irrigation water lead to many epidemics and sporadic cases. HEV genotypes 3 and 4 are zoonotic infections and present as sporadic cases of foodborne infections in Europe and North America[2].

HEV infection is usually asymptomatic in the majority of patients.[3] However, it is the most common cause of acute viral hepatitis worldwide and certain patients may develop a more severe illness, particularly pregnant females and those with chronic liver diseases.[4,5] In the latter, HEV infection can result in severe and even fatal decompensation causing Acute-on-Chronic Liver Failure (ACLF) [6].

HEV induced ACLF in chronic liver disease patients were first reported from Pakistan in 2002[7]. Since then, many reports of HEV as an important cause of decompensation in about 21% of ACLF cases from Asia and Africa were published[8]. The Asian Pacific Association for the Study of the Liver (APASL) defined ACLF as "an acute hepatic failure presenting as jaundice and coagulopathy, complicated within 4 weeks by ascites and/or encephalopathy with high short term mortality in patients with previously diagnosed or undiagnosed chronic liver disease (including cirrhosis)"[9]. Different hepatic (e.g. hepatitis) and extrahepatic (e.g. Infections) ACLF triggers have been described with different outcomes, but no triggers were identified in 20%-45% of cases[10].

HEV infection is usually underdiagnosed due to the clinically inapparent course of many cases. Even in symptomatic cases, HEV is commonly misdiagnosed; Clinicians may not consider testing for acute HEV, which is often similar to other forms of acute hepatitis, either due to limited knowledge or unavailability of HEV diagnostic tests. However, the diagnosis of HEV is important for patient management, disease control and prevention in addition to characterization of HEV infection burden and epidemiology[11].

In Egypt, HEV is highly endemic and the seroprevalence of HEV is of the highest in the world, around 80% with a single reported outbreak in Upper Egypt[12,13]. It is also the country with the highest prevalence of hepatitis C virus (HCV) infection, [14] where 30% of around 125,000 HCV viremic individuals diagnosed annually present with compensated cirrhosis[15]. So studying HEV infection in cirrhotic patients is strongly required in Egypt.

The current study aims to determine HEV IgG seroprevalence and the risk factors of HEV exposure in cirrhotic patients. It also aims to determine the role of acute HEV infection as a possible trigger of ACLF in cirrhotic Egyptian patients in East Delta and to compare different clinical, laboratory and ultrasonographic features between acute HEV positive and negative ACLF patients.

METHODOLOGY

Study design and patients' selection:

This cross-sectional study was conducted in Medical Microbiology & Immunology and Tropical Medicine Departments, Faculty of Medicine, Zagazig University, during the period from January to July 2016. Two hundred and eighty three cirrhotic patients admitted to Tropical Medicine Department and 2 Intensive Care Units were enrolled and were categorized into two groups; Group (1) included 216 compensated cirrhotic patients showing no symptoms or signs of acute hepatitis; this group was studied to determine HEV IgG seroprevalence and risk factors of exposure in cirrhotic patients; Group (2) included 67 cirrhotic patients with ACLF diagnosed by development of acute hepatic decompensation manifested as jaundice (a serum bilirubin level of ≥ 5 mg/dL) and coagulopathy (an INR of ≥1.5) in a previously stable cirrhotic patient[9]; this group was studied to determine the role of acute HEV infection in ACLF development, as it was difficult to follow up patients with compensated cirrhosis indefinitely till they develop ACLF . Patients with malignancies, renal failure and any cause of immunosuppression, other than chronic liver disease, including immunosuppressive therapy were excluded.

Sample size for group (1) was calculated based on the previously reported HEV seroprevalence in cirrhotic Egyptian patients of 56% [16], with 95% confidence level and 5% absolute precision in a total population of around 500 cirrhotic patients investigated during the study period. While group (2) patients were all cirrhotic patients fulfilling the APASL definition of ACLF admitted in the same period.

Ethical statement

A written informed consent was obtained from each patient or the guardians of unconscious patients. Institutional approval was obtained from the Institutional Review Board, Faculty of Medicine, Zagazig University (ZU-IRB# 2135-20-5-2015).

***Data collection**:*

Full data were collected from patients' medical records including: Personal data and past history of surgeries and drug intake, clinical data of the present illness, laboratory data including liver function tests, kidney function tests, urine analysis, CBC, ESR, HCV Ab, HBsAg, HBc IgM, ANA and ASMA (If autoimmune hepatitis was suspected), bacterial culture results for suspected bacterial infections, abdominal ultrasonographic (US) findings and Child Pugh classification and score calculation[17].

HEV studies

Seroprevalence of HEV in cirrhotic patients was determined by detecting specific HEV IgG in group (1) by ELISA, while acute HEV infection was diagnosed in group (2) by detecting HEV IgM and/or HEV RNA in patients' sera by ELISA and RT-PCR respectively[18].

***Sample collection**:*

Three milliliters of blood were withdrawn from each patient under aseptic conditions. Blood samples were allowed to clot and sera were separated and stored at -20˚C for further testing.

Enzyme-linked immunosorbent assay (ELISA) (Bioneovan, China):

It was used for the detection of anti-HEV IgG antibodies in sera of group (1) patients and anti-HEV IgM antibodies in sera of group (2) patients according to the manufacturer's protocol.

Reverse transcriptase PCR (RT-PCR) assay:

It was used for detection of serum HEV RNA as described by Reyes et al. [19] Briefly; viral RNA was extracted from serum samples using (QIAamp Viral RNA Mini kit, Netherlands), Reverse transcription and amplification were performed using One-step RT-PCR kit (QIAGEN, INC., Nertherlands). Each reaction mixture contained 20 µl of the extracted RNA, 10 µl QIAGEN One-step RT-PCR Buffer, 400 µM of each dNTP, 2 µl QIAGEN One-step RT-PCR Enzyme Mix (Omniscript Reverse Transcriptase, Sensiscript Reverse Transcriptase, and HotstarTaq DNA Polymerase), 0.4 µM of each of sense and antisense primers; 5' GCT CAT TAT GGA GAG AGT GTG T 3' and 5' CAG GGC CCC CAA GTT CTT CT 3' respectively (Invitrogen, Thermo Fisher Scientific, USA). Finally, RNAase free water was added to reach a total volume of 50 µL. Cycling

conditions were as follows: 50°C for 30 min for cDNA synthesis by the action of reverse transcriptases, 95°C for 15 min to inactivate reverse transcriptase enzyme and activate HotStarTaq DNA polymerase. Amplification of cDNA was achieved through 40 cycles of 94°C for 30 sec, 55°C for 45 sec and 72°C for 60 sec. followed by 10 min at 72 °C for final extension. The amplified PCR products (381 bp) were visualized by agarose gel electrophoresis.

Data analysis:

The collected data were encoded and analyzed by using SPSS version 22 (SPSS Inc., Chicago, Illinois, USA). Continuous variables were expressed as mean and standard deviation (SD) and compared using Students t-test. Categorical variables were expressed as frequencies and percent and compared by Chi-square or Fisher's Exact as appropriate. For group (1), a multivariable logistic regression analysis was performed to predict the effect of gender, age, residence, drinking water source and co-infection with other hepatitis viruses on the likelihood of HEV exposure. Two-sided tests were used. P value < 0.05 was considered significant.

RESULTS

Eighty five out of 216 cirrhotic patients (group1) were HEV IgG positive with a seroprevalence of (39.4%). Different characteristics of HEV seropositive and seronegative patients in group (1) were compared and statistically significant differences existed between them regarding rural residence and drinking water source (Table 1).

Table 1: Characteristics of HEV IgG seronegative and seropositive cirrhotic patients (group 1).

Variable		HEV Sero –ve (n=131)		HEV Sero +ve (n=85)		t-test	P
Age : (year) Mean ± SD		54.98 ± 12.5		55.45 ± 12.33		0.27	0.79
Variable		**No.**	**%**	**No.**	**%**	χ^2	**P**
Gender	Male	82	62.60	59	69.41		
	Female	49	37.40	26	30.59	1.1	0.3
Residence	rural	82	62.60	78	91.76		
Urban		49	37.40	7	8.24	22.8	**<0.001****
Drinking water	Tap	105	80.15	13	15.29		
	Well	26	19.85	72	84.71	87.5	**<0.001****
Other viral hepatitis markers							
HBs Ag:							
negative (203)		124	94.66	79	92.94	0.27	0.61
positive (13)		7	5.34	6	7.06		
HCV Ab:							
negative (11)		6	4.58	5	5.88	2.2	0.06
positive (205)		125	95.42	80	94.12		

Notes: χ^2: chi square test t: student t- test ** highly significant

However, Multivariable logistic regression analysis showed that well water consumption was the only significant risk factor for HEV exposure (84.71%; OR 24.2 (10.2 to 57.4); p<0.001) (Table 2).

Table 2: Logistic regression analysis for predicting the likelihood of HEV seropositivity based on gender, age, residence, drinking water source, HBs Ag and HCV Ab.

Variables	β	S.E.	Odds ratio (95% CI)	P-value
Age	-0.03	0.02	0.97 (0.94 to 1.003)	0.08
Gender (0=female,1=male)	0.02	0.40	1.02 (0.46 to 2.2)	0.97
Residence (0=rural, 1=urban)	-0.28	0.55	0.76 (0.26 to 2.2)	0.61
Drinking water (0=tap, 1=well)	3.18	0.44	24.2 (10.2 to 57.4)	**<0.001****
HBsAg (0=neg,1=pos)	0.25	0.90	1.3 (0.22 to 7.6)	0.78
HCV Ab (0=neg,1=pos)	-0.28	0.94	0.76 (0.12 to 4.8)	0.77
Constant	-0.36	1.31	0.07	

β; Regression coefficients, S.E; standard error ** highly significant

Acute HEV infection as a trigger of decompensation in 67 ACLF patients was diagnosed by presence of HEV IgM and/or detectable HEV RNA by RT-PCR. 13.4% (9/67) of ACLF patients showed acute HEV infection; All were IgM positive and 4 (6%) had detectable HEV RNA in serum as well. These patients didn't show any history, clinical or laboratory findings suggestive of other possible triggers of ACLF. Ultrasonographic, clinical and laboratory data of acute HEV positive and negative ACLF patients were compared (Table 3).

Table 3: Ultrasonographic, clinical and laboratory characteristics of acute HEV +ve ACLF patients compared to acute HEV -ve ACLF patients (Group 2):

Variable		Acute HEV -ve (n=58) [a]		Acute HEV +ve (n=9) [b]		χ^2	P
		No	%	No	%		
Liver:	Average	4	6.90	0	0	Fisher's	>0.99
	Shrunken	54	93.10	9	100	Exact Test	
Spleen:	Enlarged	54	93.10	9	100	Fisher's	>0.99
	Removed	4	6.90	0	0	Exact Test	
Ascites:	No	7	12	0	0	1.2	0.27
	Yes	51	88	9	100		
HE:	No	22	37.93	6	66.67	2.6	0.10
	Yes	36	62.06	3	33.33		
Child Pugh Class:	C	58	100	9	100	-----	-----
Child Pugh Score:						**t**	**P**
Mean ± SD		12.16 ± 1.07		12 ± 2		0.37	0.71
Laboratory data (mean±SD)						**t**	**P**
T. bilirubin (mg/dL)		20.24 ± 3.76		21.2 ± 2.02		1.05	0.31
D. bilirubin (mg/dL)		13.73 ± 3.80		14.6 ± 2.82		0.66	0.51
T. Plasma proteins (g/dL)		6.52 ± 1.04		5.83 ± 0.65		1.9	0.06
Albumin (g/dL)		2.51 ± 0.51		2.43 ± 0.67		0.42	0.68
ALT (IU/L)		222.42 ± 78.0		933.33 ± 37.86		26.7	<0.001**
AST (IU/L)		456.89 ± 190.2		1286.7 ± 75.72		12.9	<0.001**
INR		2.96 ± 0.80		2.73 ± 0.67		0.82	0.42
Prothrombin time		24.1 ± 2.34		25.7 ± 3.33		1.45	0.09
Urea (mg/dl)		25.17 ± 14.36		30.83 ± 10.18		0.81	0.46
Creatinine (mg/dL)		1.17 ± 0.54		1.6 ± 0.63		0.96	0.36
Hb (g/dL)		10.78 ± 1.41		10 ± 1.15		0.91	0.38
WBCs ($x10^3/mm^3$)		9.19 ± 2.67		7.3 ± 1.82		0.90	0.41
Platelets ($x10^3/mm^3$)		80.42 ± 23.18		73.67 ± 8.39		1.39	0.19

[a] HCV Ab positive in all 58 cases
[b] HCV Ab positive in six cases and 3patients were HBsAg positive
χ^2: Chi-sqaure test t: t-test
HE: hepatic encephalopathy
** Highly significant

The triggering factors for decompensation were identified in 49/67 (73.13%) of ACLF cases. (Table 4)

Table 4 ACLF triggering factors identified in group (2) patients.

ACLF trigger	No (%) Total =67	Diagnostic criteria/ Remarks
Acute HEV infection	9 (13.43%)	All were HEV IgM +ve, 4 patients were HEV RNA +ve as well
Hepatotoxic drugs	6 (8.95%)	Antibiotics and NSAID
Bacterial infections (8 SBP, 3 UTI, 2 cellulitis and 2 LRTI)	15 (22.39%)	Clinical manifestations, leukocytosis, ascetic fluid PMN> 250 /mm^3, bacterial cultures, urine analysis, and chest X-ray
Bleeding varices	10 (14.93%)	Esophageal or fundal
Portal vein thrombosis	3 (4.48%)	Abdominal US and colored Doppler
Reactivation of HBV	3 (4.48%)	Positive HBc IgM antibody
Major surgury	3 (4.48%)	Splenectomy
None identified	18 (26.86%)	

Abbreviations: NSAID; Non-steroidal anti inflammatory drugs, SBP; spontaneous bacterial peritonitis, UTI; urinary tract infection, LRTI; lower respiratory tract infection, HBc IgM; hepatitis B core IgM

DISCUSSION

In Egypt, a number of studies reported a high seroprevalence of HEV in Egyptian patients of different age groups and clinical backgrounds, where high rates of about 80% were reported in highly endemic rural areas[20]. However, HEV infection is still not adequately recognized or reported in patients with underlying liver cirrhosis particularly those presenting with acute decompensation either because of poor awareness or unavailable serological and molecular diagnostic tests.

In this study the seroprevalence of HEV IgG in compensated cirrhotic patients was assessed in a mixed rural-urban Egyptian community in East Delta and it was found to be (39.4%). It is lower than that reported by an old Egyptian study on cirrhotic patients from another community; the latter didn't show a significant difference in HEV seroprevalence between cirrhotic (56%) and non cirrhotic patients (53%)[16].

Different seroprevalence rates were reported by other studies on patients with chronic liver diseases in various countries[21-25]. Patients' characteristics previously reported to influence HEV exposure risk were evaluated in a logistic regression model and drinking water source was the only statistically significant predictor affecting HEV seropositivity; well water consumers had 24.5 times higher odds to develop HEV seropositivity than tap water users (P<0.001). This is concordant with several African studies reporting fecal contamination of drinking water as the main source of HEV infection particularly in outbreaks[26]. Rural residence was significantly higher in seropositive patients; concordant results were reported for healthy subjects in Egypt and Gambia[13, 27].

There was no association between IgG seropositivity and gender or ages of the studied patients; previous African studies showed different rates of HEV seroprevalence in both sexes and in different ages[26]. Also, infection with other hepatitis viruses was not associated with HEV exposure in our study. AbdelHady et al., in a previous Egyptian study, reported a high association between HCV infection and HEV seropositivity, pointing to similar or overlapping routes of transmission[28]. Other studies that statistically assessed the effect of different confounders found no association between HEV seropositivity and HBsAg or HCVAb positivity in Egypt[29], or HCV infection in Egypt and Sweden[16,30]. The risk factors for HEV infection differs between different regions and communities depending on the predominant genotype, the route of transmission, the socioeconomic and hygienic standards and associated infectious diseases, as reviewed by Kim et al.[26]

In the current study acute HEV in ACLF cases was diagnosed by detection of HEV IgM and/or RNA in patients' sera. Accordingly, 9 (13.4%) ACLF patients had acute HEV infection; All were HEV IgM positive and 4 (6%) had detectable viremia as well. Different markers correspond to the stages of acute HEV infection; IgM negative/RNA positive in the window period, IgM positive/RNA positive in early seroconversion stage, IgM positive/RNA negative in post-seroconversion stage[31,32]. The short period of HEV viremia, which may precede clinical symptoms, and low level viremia at presentation, below the detection limit of conventional RT-PCR, might account for the low rate of HEV viremia detected in our study compared to that of IgM.

Zaki and Othman in a previous Egyptian study reported different rates; they reported HEV IgM seroprevalence and viremia in 5% and 13% of ACLF cases respectively. This difference could be attributed to inclusion of immunosuppressed patients in their study group namely liver transplantation and hepatocellular carcinoma patients who represented 44% of their ACLF patients[33]. In immunocompromised patients, HEV infection could be chronic where the viremia persists for more than 6 months, In addition, seroconversion to

detectable levels of anti-HEV antibodies is delayed or not present at all[34]. Such patients were excluded from our study. In India, Kumar-Acharya et al. reported higher HEV IgM seroprevalence and viremia rates of 44% and 10% respectively among patients with deteriorating chronic liver disease[35]. Lower rates were reported by other studies[36,37]. Variation in results can be attributed to differences in the degree of endemicity, standard of living and routes of transmission between different countries.

Acute HEV infection was the third common predisposing factor of decompensation in the studied ACLF patients following bacterial infection (22.4%) and variceal bleeding (14.9%). Potential triggers of ACLF differ by the areas of the world. For example, Shi et al, in China, reported HBV exacerbation as the most frequent ACLF trigger (35.8%) followed by bacterial infection (27.9%), upper gastrointestinal bleeding (8.9%) then HAV or HEV infection in (6.4%)[38]. Moreau et al, in a study on cirrhotic patients with ACLF from 12 European countries, found that bacterial infection was the commonest trigger (32.6%) followed by active alcoholism (24.5%) then gastrointestinal bleeding (13.2%) while other causes including viral hepatitis represented (8.6%) of the identified triggers[39]. This may be due to differences in the nature of the pre-existing liver disease or in cultures and habits of people in different countries; e.g. alcohol consumption.

On comparing different clinical, laboratory and radiological findings between acute HEV positive and negative ACLF patients, statistically significant higher levels of liver enzymes (ALT and AST) were found in acute HEV positive cases ($P<0.001$). This is likely because acute HEV is a direct hepatic insult while most other identified triggers, e.g., bacterial infections and GI bleeding, are extrahepatic insults[10]. Some studies showed no differences between characteristics of patients with and without HEV infection as a trigger of acute decompensation[21,25,36]. This may be attributed to difference in ACLF triggers reported by studies from different countries as discussed before.

Prompt diagnosis of HEV infection in ACLF cases is an important determinant of prognosis and may influence the patient's management and outcomes if ribavirin therapy is considered[40]. This perspective, in addition to the results of the current study, points out to the importance of testing for HEV infection in ACLF cases with marked liver enzymes elevation. Besides, until a vaccine becomes available, general HEV preventive measures including personal hygiene, water and food sanitation and adequate sewage disposal should be implemented, particularly for patients at high risk of fatal complications as a result of HEV infection.

Some limitations exist for this study. The performance of the used commercial HEV serological assays in this study and in other similar studies may differ that may limit the comparability of HEV seroprevalence rates. So, sensitivity and specificity of the used assays should be assessed. Another point is that we tested for HEV IgG only in the first group and HEV IgM in the second group as mentioned before, it could be more informative to test for both markers in both groups. Finally, the role of other less commonly reported routes of HEV transmission, e.g. zoonotic transmission and blood transfusion, was not explored and needs further investigation in cirrhotic Egyptian patients.

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Prof. Ahmad S. Sherbini passed away before the submission of the final version of this manuscript; Dr. Raghdaa A Ramadan accepts responsibility for the integrity and validity of the data collected and analyzed by him.

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