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Original Article

Sero-Epidemiology of Hepatitis E Infections in patients with chronic Hepatitis C virus infection in Jahrom, Southern Iran

Fazel H[1], Shadmand E[2], Baharlou R[3], Shokouh M R[2], Hashemi S M A[1], Farahmand M[1], Ahmadi Vasmehjani A[2], Sadeghi K[1*]

1. Virology Department, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran
2. Department of Microbiology and Immunology, Jahrom University of Medical Sciences, Jahrom, IR Iran
3. Department of Immunology, School of medicine, Iran University of Medical Sciences, Tehran, Iran

Abstract

Background and Aims: Co-infection of hepatitis E virus (HEV) in chronic hepatitis C virus (HCV) infected patients can develop and cause an increase in liver disease and hepatic complications in the world. The purpose of this study was to assess the prevalence of HEV infection in patients suffering from chronic HCV infection.

Materials and Methods: Cross-sectional study testing anti-HEV antibodies in serum samples belonged to 53 chronic HCV infected patients were evaluated. Demographic and clinical data such as liver function tests and enzymes level were prospectively collected on each patient with chronic HCV infection.

Results: There were 5 HEV infected patients IgG positive among the 53 chronic HCV infected patients. These patients had an alanine aminotransferase (ALT) level twice the upper level about of the 48 HEV negative patients ($p<0.0001$).

Conclusions: Prevalence of HEV sero-positivity patients with HCV co-infection patients was 9.4%. Our results suggest that HEV screening should be implemented in HCV-infected patients with cancer.

Keywords: Hepatitis E, Sero-epidemiology, Hepatitis C

Introduction

Hepatitis E virus (HEV) belongs to the family of Hepeviridae and genus Hepevirus. HEV is a nonenveloped, spherical particle, single-stranded RNA genome, positive sense with 3 open reading frames (ORFs). Entry of the hepatitis E virus into the host is via the oral route. The virus replication is in the cytoplasm of hepatocytes and virus is released into the bile and blood. HEV is a major cause of epidemic hepatitis and acute, sporadic hepatitis in developing nations (1). Hepatitis E usually is self-limiting disease with possible mortality in the extent of 1% and clinical symptoms are similar to other viral hepatitis infections with nausea, asthenia, fever, malaise, arthralgia, abdominal pain and elevation of liver transaminases, and jaundice is which appear in approximately 65% of the symptomatic cases (2, 3). The mortality rate in pregnant women is approximately up to 25% (4). The virus can cause severe water-borne outbreaks with mortality rates ranging from 0.2 to 4% (2).

Hepatitis C virus (HCV) infection is a major global health problem with approximately 3% of the world's population being infected. The incidence and consequences of HEV superinfection among patients with chronic HCV infection or other chronic liver disease is unclear. Decompensation of preexisting cirrhosis as well as increased morbidity and mortality has been reported for HEV1 superinfection among patients with chronic hepatitis C (5). Progression of chronic hepatitis to fulminant hepatitis and cirrhosis due to HCV

* **Corresponding author:** Kaveh Sadeghi
kaveh.sadeghi91@yahoo.com
Virology Department, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran - - Tehran University of Medical Sciences, Tehran, IR Iran

infection may be caused by HEV coinfections or other hepatitis agents (6, 7). Severe response of the immune system to hepatitis viruses and direct cytopathic effect of HEV can cause liver fibrosis progression and cirrhosis (8). In recent study, HEV infection with rapidly progressive cirrhosis has been demonstrated in HCV, HBV, and HIV coinfection (7, 9) but there are limited information on the prevalence of anti-HEV IgG in patients with chronic HCV infection in Iran.

The purpose of this study was to determine the prevalence HEV co-infection with chronic HCV infection, and its effect on the severe complications of HCV infection.

Methods

Patients and Study Design. In this study, 53 anti-HCV positive patients referred to the Honari clinic (affiliated with the Jahrom University of Medical Sciences, Jahrom, Iran) from august 2015 to May 2016 were registered. Written consent forms, compiled by the Ethics Committee of Jahrom University of Medical Sciences and signed by the patients. The questionnaire, containing the demographic characteristics (age, sex, marriage, and etc) and

Table 1: Clinical, biochemical, and Immunologic features of chronic HCV patients

Variablesa	N (%)
Gender	
Male	34 (64.2)
Female	19 (35.8)
Age ± SD	Year
20-29	12 (22.6)
30-39	35 (66)
40-49	4 (7.5)
>50	2 (3.8)
HCV genotypes	
3a	37 (69.8)
1a	16 (30.2)
HCV treatment	
Yes	29 (54.7)
No	24 (45.3)
HCV load (IU/mL)	(14.86 ± 8.29) ×105
ALT ± SD (IU/L)	52.35 ± 26.38
AST ± SD (IU/L)	38.05 ± 9.24
Education	
Illiterate	9 (17)
Primary & secondary	31 (58.5)
High school	2 (3.8)
College	11 (20.8)
Marriage	
Married	43 (81.1)
Single	10 (18.9)
Resident	
Urban	48 (90.6)
Rural	5 (9.4)

clinical data such as viral load and HCV genotypes were completed for each patient. Inclusion criteria to the study were chronic HCV infection accompanied by the presence of HCV RNA in serum for more than 6 months. Samples negative for HCV RNA in blood were excluded from the study. Samples were stored at -70°C.

Laboratory tests. Anti-HCV detection was performed by a third generation enzyme immunoassay (ELISA), for HEV IgG and IgM antibodies (Diagnostic BioprobesSrl, Milano, Italy) in serum according to the manufacturer. Viral RNA extracted from the serum of patients with High Pure RNA Isolation kit (Roche, Germany). Quantitative reverse transcription polymerase chain reaction (QRT-PCR) for HCV was done using TaqMan technology according to Rosch HCV RT-PCR assay. Probe signals were detected in real-time (7500 Real time PCR system; Applied Bio Systems). Genotyping was performed similar to the report of previous study (10). Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT), liver enzymes were detected from serums.

Table 2: Characteristics of chronic HCV patients according to HEV IgG status.

	Anti-HEV IgG		
Status	**Positive (%)**	**Negative (%)**	**pa**
N	5 (9.4)	48 (90.6)	
Age			NS
20-29	0 (0)	12 (25)	
30-39	5 (100)	30 (62.5)	
40-49	0 (0)	4 (8.3)	
>50	0 (0)	2 (4.2)	
Gender			NS
Male	3 (60)	31 (64.6)	
Female	2 (40)	17 (35.4)	NS
ALT ± SD (IU/L)	91.40 ± 28.88	48.29 ± 22.81	<0.0001b
AST ± SD (IU/L)	39.60 ± 10.2	37.89 ±9.24	NSb
HCV treatment			0.033
Yes	5 (100)	24 (50)	
No	0 (0)	24 (50)	
HCV load (IU/mL)	(25.58 ± 5.59) × 105	(13.75 ± 7.74) × 105	0.002 b
HCV genotype			NS
3a	5 (100)	32 (66.7)	
1a	0 (0)	16 (30.2)	
Education			NS
Illiterate	0 (0)	9 (18.8)	
Primary & secondary	4 (80)	27 (56.3)	
High school	0 (0)	2 (4.2)	
College	1 (20)	10 (20.8)	
Marriage			NS
Married	3 (60)	40 (83.3)	
Single	2 (40)	8 (16.7)	
Resident			0.014
Urban	3 (60)	45 (93.8)	
Rural	2 (40)	3 (60)	

Statistical Methods. The outcomes for all tests were analyzed using the SPSS version 18.1. To

determine categorical parameters the Chi-square test or Fisher's exact test were used. Statistical significance was defined at P values of < 0.05.

Results

From the total of 53 patients diagnosed with HCV infection, 34 were Male (64.2%) and 19 Female (35.8%). The majority of HCV-infected patients were among 30-39 years old, 35 patients (66%). The HCV genotype 3a infection was 37 (69.8%) and was higher than 1a genotype 16 (30.2%). The average HCV viral load was (14.86 ± 8.29) ×105 IU/mL. The demographic and laboratory characteristics for the study patients are shown in Table 1. All of chronic HCV patients were negative for Anti-HEV IgM but Anti-HEV IgG was present in only in 5 patients (9.4%) positive.

All these 5 patients were in range of 30–39 years of age and 3 of them were Male (60%) and 2 Female (40%). These patients had ALT level higher than twice the upper about of 48 patient negative that this difference was significant ($p<0.0001$). HCV genotype of these 5 patients with anti-HEV IgG positive was 3a genotype. Whereas all of them had received anti-HCV drugs but HCV viral load in anti-HEV IgG positive with 3a genotype was higher than anti-HEV IgG negative patients that this difference were significant ($p=0.002$) (Table 2).

Discussion

Generally, HCV infections in patients can result in cirrhosis or HCC, and HEV infection (except in pregnant women) is a self-limiting disease but it can cause liver disease progression in immunosuppressed patients (11). HCV co-infection with other hepatitis viruses causes severe damage in liver. Previously investigators have reported the presence of infection by hepatitis viruses in different regions of Iran, but, there aren't enough data about HCV co-infection with HEV in Iran.

Previous studies have shown relatively high anti-HEV sero-prevalence in patients with HCV infection: for example Bayram reported 54%(12) and Ludi Koning reported 37%(13) IgG-HEV positive and Zaki reported 52%(14). The current study showed prevalence of IgG-HEV in HCV infected patients about 9.4%. The importance of HEV super infection in patients with HCV is not fully clear; however HEV co-infection with HCV has been implicated in deterioration of damage to the liver and with decompensation of existing cirrhosis and increased morbidity and mortality(15). This study is the first report from Iran and there is no previous data comparable to our study.

Presence of HEV antibodies have been related with liver cirrhosis in HCV patients with cancer. In this study the level of ALT enzyme in HEV co-infection with HCV patients was significantly higher than HCV infected patients, thus these results suggest that HEV could be an additional factor associated with accelerated fibrosis and cirrhosis in HCV infected population. In agreement with our data, a, reported a positive seroprevalence of HEV antibodies in HCV infected patients with cirrhosis, particularly those who developed cirrhosis (16).

HCV viral load in co-infection of HCV and HEV patients was significantly higher than HCV infected patients. This finding suggest that HEV can cause increase replication of HCV by two events: 1- HEV can decrease host immunity(17), thus provides conditions for rise of HCV viral load. 2- HEV can lyse liver cells increasingly (high level of ALT) and cause the release of HCV particles and genome. When HCV viral load is high we predict liver fibrosis, thus it can be stated that HEV co-infected whit HCV may cause further damage the liver and progression to liver cancer.

This examination proved that the most infected patients belonged to low levels of literacy section and urban residents, which some previous studies (18). Thus, the absence of enough information about endemic HEV, the low level of public awareness of the disease and transmission of HEV through fecal-oral were the main reasons for high prevalence among the population. Generally, this study

was indicated prevalence of HCV co-infection with HEV was considerable.

The main limitation of this study was the lack of HEV RNA detection in the serum of HEV sero-positive patients, to confirm HEV infection. Quantitative qualitative tests for HEV RNA are not commercially available in the Iran.

In conclusion, HEV IgG sero-positivity is present in 9.4% of HCV-infected patients and seems to be associated with the presence of cirrhosis. The exact pathogenic role of documented chronic HEV infection has yet to be determined in HCV infected patients. If a synergistic effect of HEV infection on such patients is confirmed, we must resolve both of viruses with special drug regimens and prevent liver disease progression in suitable candidates. Our results suggest that HEV screening should be accomplish in HCV-infected patients with cancer and determine kind of genotypes.

Conflict of Interest

The authors declare that there is no conflict of interests.

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