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Hepatitis E virus infection in patients infected with human immunodeficiency virus in an endemic area in Iran

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Abstract

Some studies have suggested that hepatitis E virus is more frequent in human immunodeficiency virus (HIV) patients and can progress to chronic infection. We aimed to determine the prevalence of hepatitis E virus antibodies and RNA in a series of 100 HIV-infected patients in Tehran, Iran, with comparison to 52 healthy HIV, hepatitis B and C-negative blood donors as controls. HIV-infected patients were also tested for hepatitis E virus-RNA. Among the HIV-infected patients, 10% had antibodies to hepatitis E virus – a finding not significantly different from the uninfected controls (11.5%). No HIV-infected patients had hepatitis E virus IgM antibodies nor did any have detectable hepatitis E virus-RNA. We found no associations between anti-hepatitis E virus IgG-seropositivity and age, sex, route of HIV acquisition, aminotransferases levels, CD4, antiretroviral therapy, hepatitis B virus and hepatitis C virus co-infection. Hepatitis E virus is relatively prevalent in our HIV-infected patients, although without evidence of chronic infection and no more common than among HIV-negative controls or the general population. For the present, we do not recommend routine screening for hepatitis E virus infection in HIV-infected patients in our moderately endemic region.

Keywords

Hepatitis E virus, human immunodeficiency virus, chronic hepatitis E virus, Iran, HIV, AIDS, co-infection, hepatitis

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Introduction

Hepatitis E virus (HEV) is a single-stranded, non-enveloped RNA virus and belongs to the genus *Hepevirus* of the family *Hepeviridae*.[1] HEV is considered a typically self-limiting viral infection, which is endemic in the developing countries but can cause hepatic failure in specific high-risk groups such as pregnant women, in whom the mortality rate can reach 20%.[2] The usual transmission route of HEV is faecal-oral; but it can also be transmitted parenterally by blood transfusion[3,4] and vertically from mother to child, particularly in endemic areas.[5] Zoonotic transmission is considered to be a major cause of HEV

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infection in Europe, the United States, and Japan.[6] Other modes of transmission such as haemodialysis remain controversial.[7] HEV is generally considered non-endemic in industrialized countries with seroprevalence ranging from 1% to 20%.[2] It is more likely to be endemic in regions with poor sanitation and hygiene, such as parts of Asia, Africa, and Mediterranean, where prevalence of HEV can be as high as 50%.[8,9]

In recent years, several studies have demonstrated cases of chronic HEV infection (persistence of HEV-RNA in plasma over a 6-month period) and cirrhosis in immunocompromised patients, including organ transplant recipients,[10] patients with lymphoma and haematological malignancies,[11,12] and human immunodeficiency virus (HIV)-infected patients.[13] Some studies have suggested that patients with HIV may acquire HEV infection more frequently than individuals without HIV.[14,15] Although, other studies have not shown differences in HEV prevalence between HIV-infected and non-infected individuals.[16,17] For example, the prevalence of HEV infection in HIV patients has ranged from 0% to 11.3%[18–22] by means of serology and polymerase chain reaction (PCR).

However, studies regarding co-infection of HIV and HEV are limited and it is not clear whether HIV-infected patients are at greater risk of acquiring HEV infection due to shared modes of transmission or increased vulnerability due to immune suppression. Moreover, whether co-infected patients are more likely to develop chronic infection and severe liver damage is also not established. The possibility has considerable significance as both HIV and HEV infection are now highly endemic in many parts of the world. The present study aimed to determine the prevalence of HEV antibodies in HIV-infected patients with comparison to HIV-uninfected controls and to identify recent and active infection in an HIV-infected patient case series in Iran – a country with moderately high prevalence of both infections.

Materials and methods

Study population and patient information

From September to November 2012, a consecutive sample of 100 HIV-infected patients from the Iranian Research Center for HIV/AIDS was recruited. As a proxy for the general population, 52 healthy blood donors screened negative for HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV) were consecutively enrolled from Iranian Blood Transfusion Organization in approximate sex and age frequencies as the HIV patients. A structured face-to-face questionnaire that gathered epidemiological, clinical, and laboratory data was completed by clinicians.

Laboratory methods

All participants provided samples tested for hepatitis B surface antigen (HBsAg; Hepanosticka Biomerieux, Boxtel, The Netherlands), hepatitis B surface antibody (anti-HBs; Enzygnost, Dade Behring Marburg GmbH, Germany), hepatitis B core antibody (anti-HBc; Enzygnost, Dade Behring Marburg GmbH, Germany), and hepatitis C antibody (anti-HCV; Biorad, Segrate, Italy). A recombinant immunoblot assay (RIBA Innogenetics, Ghent, Belgium) was used to confirm anti-HCV reactivity.

Several assays were used to detect HEV. For all participants, hepatitis E antibody (anti-HEV IgM and IgG (was detected by enzyme-linked immunosorbent assay (ELISA; Dia.Pro Diagnostic BioProbes, Milan, Italy). This assay uses HEV-specific synthetic antigens derived from an open reading frame (ORF) 2 and ORF3 of all four HEV subtypes. Positive and negative controls were included in the ELISA microplates assayed. According to the manufacturer's guidelines, anti-HEV sensitivity and specificity were 100%. Among the HIV-positive participants, cell culture and reverse transcriptase-polymerase chain reaction (RT-PCR) were also done to detect HEV viral nucleic acid in the following manner. An A549 cell line was grown in mixed medium as described previously by Huang et al.[23] Cells were infected with HEV that was purified from stool sample of a patient confirmed for infection by RT-PCR. A supernatant of infected cells was used as the positive control in our PCR assays. RNA was extracted with Trizol reagent (Gibco BRL) according to manufacturer's instructions.[24] Two sets of sense and antisense synthetic oligonucleotide primers for the HEV ET1.1 region were used for detection of the HEV genome. The primers used in this study were F1, 5′-GCT ATT AGT GAG GAG TGT GG-3′(positions 4459 to 4478); R1, 5′-CAG GGC CCC AAT TCT TCT-3′ (positions 4876 to 4859); F2, 5′-GCG TGG ATC TTG CAG GCC-3′ (positions 4522 to 4539); and R2, 5′-TTC AAC TTC AAG CCA CAG CC-3′ (positions 4760 to 4741). Standard RT-PCR was performed as described by Meng et al.[24]

HIV serostatus was verified by ELISA (MP Biomedicals, Illkirch, France) with positives confirmed by Western blot (Diaplus, San Francisco, USA). For HIV-infected patients, $CD4^+$ count was determined by flow cytometry in cells/mm^3. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured by standard clinical assays (Pars Azmon Co., Iran) with auto analyzer (BT3000).

The upper limits of normal ALT and AST levels were considered as 45 and 35 U/l, respectively.

Statistical analysis

Chi-square and logistic regression analysis were used to test differences in the prevalence of HEV between cases and controls and to identify risk factors for HEV infection. In the study, however, no significant differences in prevalence or correlates for infection were noted. Data are presented as absolute counts, proportions [95% binomial exact confidence intervals (CI)], medians [interquartile range (IQR)], and means [standard deviation (SD)] where indicated.

The study was reviewed and approved by the Ethics Committee of the Iranian Society for Support of Patients with Infectious Diseases; informed consent was obtained from all patients prior to their enrollment.

Results

The 100 HIV-infected patients had a median age of 38 years (IQR: 34–43), with 71% men and 29% women. Median CD4 count was 320 cells/mm^3 (IQR: 186–440) and 36% had CD4 <250 cells/mm^3, and 80% had achieved an AIDS diagnosis. The reported routes of HIV transmission (multiple routes possible) were intravenous drug use (IDU) alone (45%), heterosexual contact alone (7%), infected blood and blood products transfusion alone (2%), heterosexual contact and IDU (17%), heterosexual contact and infected blood (3%), tattooing and IDU (6%), tattooing, IDU, and heterosexual contact (2%), IDU, heterosexual, and homosexual contact (1%), and in 17% of patients the route of HIV acquisition was not identified. Antiretroviral therapy (ART) was used in 81% of patients with a mean duration of 25 months (±3).

Prevalence of markers for viral hepatitis in the HIV-positive participants was 53% for anti-HCV, 4% for HBsAg, 50% for anti-HBs, and 31% for anti-HBc. The median of ALT and AST levels were 33.5 (IQR: 17–57) and 29.5 U/l (IQR: 18–48), respectively. Ten HIV patients were positive for anti-HEV IgG antibody for a prevalence of 10% (95% CI 5–18). None were positive for anti-HEV IgM and in no HIV patients was HEV RNA detected. Three of the HEV seropositive cases were also infected with HCV, therefore the prevalence of HEV/HCV co-infection was 3% among HIV-positive patients. Two HEV-positive cases had abnormally elevated ALT, one of them was co-infected with HCV and another had isolated anti-HBc (i.e., HBsAg and anti-HBs negative, anti-HBc positive). The ALT value was twice upper limit of normal in one case, which was co-infected with HCV. Two of the HEV-seropositive cases had CD4 count less than 250 cells/mm^3.

Among the HIV-positive patients, we found no associations between anti-HEV IgG seropositivity and age, sex, possible route of HIV acquisition, aminotransferases levels, CD4, ART duration, and HBV and HCV co-infections (Table 1).

The 52 healthy blood donors were verified as negative for HIV, HBV, and HCV infection. Anti-HEV was detected in six donors for a prevalence of 12% (95% CI 4–23). This estimate was not significantly different from the HIV-positive patients ($p = 0.769$). No blood donor controls were positive for anti-HEV IgM.

Discussion

The present study showed that the seroprevalence of HEV was moderately high (10%) in HIV patients, but similar to an age- and sex-matched series of HIV/HBV/HCV-negative controls (12%) recruited from healthy blood donors. None of our HIV-positive patients had evidence of HEV viraemia nor was anti-HEV IgM detected suggesting none had recent or active infection. We found no relationship between HEV seropositivity and possible modes of HIV transmission or stage of disease, ART use, or co-infection with HBV or HCV. The seroprevalence of HEV in our HIV-infected series of patients is similar with previous studies of general populations in Iran. For example, Mohebbi et al.[25] reported HEV seroprevalence of 9.3% in a population-based study in Tehran – a figure close to the 9.6% prevalence, a separate population-based study conducted by Taremi et al.[26] Our findings of no difference in anti-HEV seroprevalence between HIV-infected patients and controls also concur with that of Keane et al.[27] and Crum-Cianflone et al.[28] who reported that HIV-infected individuals did not appear to acquire HEV infection more frequently than their HIV-uninfected counterparts. Therefore, our data weigh against an increased acquisition or vulnerability to HEV among HIV-infected persons.

We note that our findings contrast other studies; namely, studies from Russia and Belarus found significantly higher prevalence of anti-HEV in HIV patients (11%) compared to the general population (<2%) and even higher prevalence at 40% in AIDS patients.[15] A study in Argentina also showed higher anti-HEV prevalence in HIV-infected patients (6.6%) compared to healthy controls (1.8%),[14] as did a study by Pischke et al. (5% vs. 1%, respectively).[19,29] Given the low general or control population prevalence in the settings, it may be possible that an association is not evident or detected in areas with moderate-to-high prevalence of HEV. It may also be possible that divergent results are

Table 1. Comparison of HIV-positive patients with and without anti-hepatitis E virus antibodies (IgG), Tehran, Iran, 2012.

Characteristics	Anti-HEV positive patients ($n = 10$)	Anti-HEV negative patients ($n = 90$)
Age in years		
Median (IQR)	36 (30–47)	39 (34–42)
Mean ± SD	38.11 ± 2.89	38.73 ± 0.78
Gender (M/F ratio)	5/5	66/24
HBsAg+	0 (0%)	4 (44.4%)
Anti-HBs+	5 (50%)	45 (50%)
Anti-HBc+	5 (50%)	26 (28.9%)
Anti-HCV+	3 (30%)	50 (55.6%)
CD4 (cells/mm^3)		
Median (IQR)	387 (235–469.5)	322 (159–441)
Mean ± SD	362.33 ± 40.42	326.2 ± 20.69
AST level (U/l)		
Median (IQR)	17.5 (13–37.75)	31 (18.75–48.25)
Mean ± SD	27.4 ± 7.55	37.51 ± 2.79
ALT level (U/l)		
Median (IQR)	14.5 (11.25–40.75)	34.5 (18–58.25)
Mean ± SD	26.1 ± 7.30	43.85 ± 3.46
Abnormal AST (>35U/l)	2 (20%)	42 (46.7%)
Abnormal ALT (>45U/l)	2 (20%)	33 (36.7%)
On antiretroviral therapy	8 (80%)	73 (81.1%)

Data are indicated as median (IQR), mean ± standard deviation and number (%); HIV: human immunodeficiency virus; anti-HEV: hepatitis E antibody; HBsAg: hepatitis B surface antigen; anti-HBs: hepatitis B surface antibody; anti-HBc: hepatitis B core antibody; anti-HCV: hepatitis C antibody; ALT: alanine aminotransferase; AST: aspartate aminotransferase; SD: standard deviation; IQR: interquartile range.
All p values were not significant (>0.05).

due to differences in the accuracy of different diagnostic assays.[30]

In addition to the question of whether HIV-infected patients are more likely to acquire HEV infection due to shared modes of transmission or increased vulnerability due to immune suppression, there is also concern that co-infection may result in more chronic hepatitis. Some reports have found cases of chronic HEV infection in the immunosuppressed patients, including HIV-infected individuals.[13,21,31–38] However, other studies have not corroborated a relevant role of HIV infection in chronic HEV infection.[20,27] In our study, we did not find HEV-RNA in any HIV patients, consistent with studies by Madejon et al.,[16] Renou et al.[20] and Pischke et al.[19] Therefore, our data also do not support chronic HEV infection as common among HIV patients even in a moderately highly endemic area.

In conclusion, our study revealed that HEV infection is relatively prevalent in the HIV-infected population of Tehran, but no more so than expected in healthy uninfected controls or from epidemiological studies of the general population. We also found no evidence for chronic HEV infection. These findings suggested that, for the present, systematic or routine evaluation of HEV infection in HIV-infected individuals in a country with moderate endemicity such as Iran is not required.

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Conflict of interest

The authors declare no conflict of interest.

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