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Original Article

Hepatitis E virus coinfection with hepatotropic viruses in Egyptian children

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Background and Purpose: Major hepatotropic viruses continue to be important causes of acute viral hepatitis in developing countries. This work was carried out to detect the seroprevalence of hepatitis E virus (HEV) markers in children with acute viral hepatitis due to hepatotropic viruses (A, B and C) and non-A, non-B, non-C acute hepatitis, and to ascertain the influence of HEV superinfection in individuals infected with hepatitis viruses (A, B and C).

Methods: We studied prospectively 162 children with sporadic acute hepatitis who reported to our hospital. Thirteen healthy controls were also included in the study. Laboratory investigations were performed, including complete liver function tests. Complete serological profiles for hepatitis viruses A, B, C and E were evaluated.

Results: HEV immunoglobulin G was detected with highest percentage among patients with hepatitis B (56.7%), followed by patients with hepatitis C virus (52.0%), hepatitis A virus (34.1%) and combined hepatitis B and C viruses (30.0%). The detection rate among patients with non-A, non-B, non-C hepatitis was 7.1%. HEV immunoglobulin M was found in 4.5% of hepatitis A virus patients and in 3.3% of hepatitis B patients. The prevalence of HEV immunoglobulin G and immunoglobulin M correlated with the levels of hepatic aspartate aminotransferase and alanine aminotransferase in patients with dual markers of infection with hepatitis E and other viruses compared to patients with acute hepatitis due to A and C viruses.

Conclusions: HEV serological markers are common among children with acute viral hepatitis, especially from hepatitis C and B viruses. There may be increased sensitivity to HEV coinfection in association with hepatitis B and C infections. Dual infection with HEV and other hepatotropic viruses was associated with greater elevation of aspartate and alanine aminotransferases.

Key words: Hepatitis A virus; Hepatitis B virus; Hepatitis C virus; Hepatitis E virus; Prevalence

Introduction

Hepatitis E, the major form of enterically transmitted non-A, non-B hepatitis, is caused by hepatitis E virus (HEV). HEV is transmitted primarily by the fecal-oral route [1]. HEV genome is a 7.2-kb, positive-sense, single-stranded RNA. It has three open reading frames (ORFs): ORF1 encodes non-structural proteins, ORF2 encodes the capsid protein, and ORF3 encodes a cytoskeleton-associated phosphoprotein [2,3]. At least four major genotypes of HEV have been reported worldwide: genotype 1 (found primarily in Asian countries), genotype 2 (isolated from a single outbreak in Mexico), genotype 3 (identified in swine and humans in the United States and many other countries), and genotype 4 (identified in humans, swine and other animals in Asia) [4].

HEV infection can be diagnosed by either detection of viral particles in stool using electron microscopy or detection of anti-HEV antibodies in serum. Similar to hepatitis A virus (HAV), HEV occurs in high concentrations in stool in the weeks immediately prior to the onset of symptoms. Viral shedding in the stool usually continues about 2 weeks after the onset of jaundice, although in a few persons viral shedding has persisted as long as 4 weeks. Antibodies to HEV

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are detectable in nearly all infected patients upon presentation of their illness [5].

Enzyme immunoassays based on recombinant proteins of HEV have been used for most seroprevalence studies. The recombinant proteins contain immunodominant epitopes encoded by ORF2 and ORF3 of the HEV genome from different strains [6].

This work was carried out to detect the seroprevalence of HEV markers in children with acute hepatitis due to viruses (A, B and C) and in non-A, non-B, non-C acute hepatitis, and to ascertain the influence of HEV coinfection in individuals infected with hepatitis viruses (A, B and C).

Methods

This study included 162 children with acute hepatitis with duration less than one month. Patients were attending Paediatric Mansoura University Hospital. Acute hepatitis was defined as an acute injury to the liver, manifested by release of liver cytoplasmic enzymes (particularly aspartate aminotransferase [AST] and alanine aminotransferase [ALT]). In a variable percentage of cases, the increase in enzymes was accompanied by symptoms such as fever, loss of appetite and abnormal bilirubin metabolism with jaundice, dark urine and pale stools. They all had two-fold or more increase of ALT and AST levels at acute onset. These were 44 patients with hepatitis A, as indicated by positive immunoglobulin M (IgM) for HAV, 30 patients with acute hepatitis B as indicated by positive anti-hepatitis B core antigen (HBc) IgM, 50 patients with hepatitis C as defined by positive viremia for hepatitis C virus (HCV) RNA by reverse transcriptase-polymerase chain reaction (RT-PCR), 10 patients with combined hepatitis B and C and 28 patients classified as non-A, non-B, non-C acute hepatitis (without evidence of virological etiology), and after exclusion of other causes of acute hepatitis such as assays for autoantibodies, ceruloplasmin, alpha1-antitrypsin, serum ferritin, and transferrin saturation, and studies were made of other metabolic disorders or systemic diseases such as morbid obesity and diabetes with deficient glycemic control. Thirteen healthy controls attending outpatient clinics for vaccination were also included in the study. Informed written consent was obtained from parents of all participants and the Ethics Committee of the Mansoura University Hospital approved the study.

The studied subjects were 90 males and 72 females with mean age ± standard deviation (SD) 7.0 ± 2.0 years. Each subject included in the study was subjected to full medical history and thorough clinical examination. Laboratory investigations were performed, including liver function tests by Synchron autoanalyzer (Beckman Coulter, Fullerton, CA, USA).

Complete serological profiles for hepatitis viruses A, B, C and E were evaluated. Immunoassay was carried out (Equipar, Saronno VA, Italy) for hepatitis A IgM, hepatitis B surface antigen and hepatitis B core IgM (acute hepatitis B infection was confirmed by positive HBc IgM), immunoglobulin G (IgG) for hepatitis C and RT-PCR for HCV RNA, and IgM and IgG for hepatitis E. Patients were diagnosed as acute viral hepatitis when serological markers were positive in addition to detection of HCV RNA in cases of acute viral hepatitis C infection.

IgG and IgM anti-HEV enzyme-linked immunosorbent assay

All serum samples from patients were tested with IgG and IgM anti-HEV enzyme-linked immunosorbent assay (ELISA) kits (Genelabs Diagnostics, Singapore), both at the start of acute hepatitis and after two weeks, for determination of seroconversion for IgM and increasing antibody titer for IgG. Fusion proteins M 3-2, B 6-1-4, and M 4-2, corresponding to the immunodominant epitopes found in ORF2 and ORF3, were used to coat the solid phase of the ELISA to detect IgG and IgM anti-HEV. The ELISA was performed according to the protocols provided by the manufacturer.

Statistical analysis

Values are given as mean ± SD or as the number of subjects and proportions. One-way analysis of variance test and independent samples Student's *t* test were used for group comparisons of normally distributed variables, and the Kruskal-Wallis test and Mann-Whitney *U* test were used for comparisons of variables with skewed distribution.

Results

162 children with acute hepatitis were included in the study, in addition to thirteen healthy children. The studied subjects were 90 males and 72 females with mean (± SD) age of 7.0 ± 2.0 years. There was history of previous blood transfusion in 30 patients. The clinical data of the studied subjects are summarized in Table 1.

Viral study of four hepatotropic viruses (A, B, C, E) was carried out. HEV IgG was detected with

Table 1. Clinical data of the studied subjects

Variable	No. (%)
Age (years; mean ± SD)	
Patients	7.0 ± 2.0
Controls	7.5 ± 1.5
Gender	
Patients (n = 162)	
Male	90 (55.6)
Female	72 (44.4)
Controls (n = 13)	
Male	7 (54.8)
Female	6 (46.2)
History of blood transfusion	30 (18.5)
Prothrombin time (sec; mean ± SD)	
Patients	13.0 ± 0.5
Controls	12.0 ± 0.5
Clinical outcome	
Complete recovery	146 (90.1)

Abbreviation: SD = standard deviation

highest percentage among patients with hepatitis B (56.7%), followed by patients with hepatitis C (52.0%), HAV (34.1%) and combined hepatitis B and C viruses (30.0%). The detection rate among patients with non-A, non-B, non-C hepatitis was 7.1%. HEV IgM was found in 4.5% of HAV patients and in 3.3% of hepatitis B patients (Table 2). None of the healthy controls had positive HEV IgM or IgG (data not shown).

The prevalence of HEV IgG and IgM was correlated to the increased levels of hepatic AST and ALT in patients with evidence of dual markers of infections with hepatitis E and other viruses compared to patients with active hepatitis A and C. However, in patients with positive HEV IgM or IgG there was no statistically significant difference in AST and ALT levels compared to patients with acute hepatitis of unknown etiology (non-A, non-B, non-C), patients with hepatitis B, and patients with combined B and C viral markers (Table 3).

Discussion

Recent studies reported very high levels of anti-HEV prevalence among healthy adults and pregnant females in rural areas in Egypt (67.7% and 84.3%, respectively) [7,8]. Their authors hypothesize that both zoonotic and anthroponotic transmission of a virulent (possibly genotype-3) HEV is occurring extensively in these rural villages and that the rate of positive antibodies increases with age.

In a previous study in children with unexplained acute hepatitis, we reported positive status for IgM and IgG for HEV in 17.2% and 12.5%, respectively [9].

In the present study, none of the healthy controls had positive HEV IgM or IgG. In contrast, previous studies have shown that in most disease-endemic areas, the rate of anti-HEV detection has ranged from 5% of children younger than 10 years of age to as high as 60% of children younger than 5 years of age [10]. This can be explained by varying epidemiologic conditions in different geographic areas in Egypt, as rural areas had higher prevalence of antibodies than urban areas [6], or differences in diagnostic technique between studies.

In the present work, we explored the seroprevalence of HEV antibodies among other hepatotropic virus infections and the influences of this association on biochemical markers of liver disease in children with acute viral hepatitis.

The surprising finding of this study was the unusually high prevalence of IgG anti-HEV antibodies among hepatitis B patients (56.7%) and HCV patients (52.0%) and those with combined hepatitis B and C viruses (30.0%). Similar findings were reported in 1994 from southern Italy [11] and in 1995 from Greece [12]. The authors noted a striking association between HEV and HCV infection, and stated that the anti-HEV prevalence was 27.0% and 10.7% among anti-HCV positive

Table 2. Distribution of hepatitis E virus (HEV) immunoglobulin M (IgM) and HEV immunoglobulin G (IgG)-positive cases among other positive hepatotropic viral markers

Viral hepatitis	HEV IgM-positive No. (%)	HEV IgG-positive No. (%)
HAV IgM-positive (n = 44)	2 (4.5)	15 (34.1)
HBV-positive (n = 30)	1 (3.3)	17 (56.7)
HCV (n = 50)	0 (0.0)	26 (52.0)
Non-A, non-B, non-C hepatitis (n = 28)	0 (0.0)	2 (7.1)
Combined hepatitis B and C (n = 10)	0 (0.0)	3 (30.0)
Total (n = 162)	3 (1.9)	63 (38.8)

Abbreviations: HAV = hepatitis A virus; HBV = hepatitis B virus; HCV = hepatitis C virus

Table 3. Comparison of biochemical liver indices between hepatitis E virus (HEV) immunoglobulin M (IgM)- and HEV immunoglobulin G (IgG)-positive patients and other groups of patients

	HEV IgM-positive (n = 3)	HEV IgG-positive (n = 63)	HAV (n = 27)	HBV (n = 12)	HCV (n = 24)	Non-A, non-B, non-C hepatitis (n = 26)	Combined HBV and HCV infection (n = 7)
Albumin (g/dL; mean ± SD)	5.5 ± 0.3	4.9 ± 0.4	4.8 ± 0.3	4.8 ± 0.6	5 ± 0.2	4.7 ± 0.7	4.8 ± 0.6
p^a			>0.05	<0.05	>0.05	<0.01	>0.05
p^b			>0.05	>0.05	>0.05	<0.005	>0.05
Total bilirubin (mg/dL; mean ± SD)	3.6 ± 3.8	4.4 ± 3.8	2.3 ± 0.8	7.6 ± 5.4	2.3 ± 0.8	7.8 ± 7.2	7.3 ± 5.9
p^a			>0.05	<0.001	>0.05	<0.05	>0.05
p^b			<0.0001	<0.05	<0.001	<0.05	>0.05
AST (IU/L; mean ± SD)	183 ± 55.1	125 ± 114.4	77.8 ± 31.7	113.6 ± 57.2	60.8 ± 7.5	142.3 ± 97.9	146.9 ± 114.8
p^a			<0.01	>0.05	<0.001	>0.05	>0.05
p^b			<0.001	>0.05	<0.001	>0.05	>0.05
ALT (IU/L; mean ± SD)	128 ± 5.5	152 ± 120.1	84.1 ± 24.6	175.6 ± 166.6	70 ± 6.7	106.8 ± 87.5	125.1 ± 97.6
p^a			<0.01	>0.05	<0.001	>0.05	>0.05
p^b			<0.01	>0.05	<0.001	>0.05	>0.05

Abbreviations: HAV = hepatitis A virus; HBV = hepatitis B virus; HCV = hepatitis C virus; SD = standard deviation; AST = aspartate aminotransferase; ALT = alanine aminotransferase

[a]Comparison between dual infection with hepatotropic viruses and positive HEV IgM cases with other viruses.

[b]Comparison between dual infection with hepatotropic viruses and positive HEV IgG and monoinfection with other hepatotropic viruses.

individuals, respectively [11,12]. Other authors noted a high rate of superinfection with HEV in hepatitis B surface antigen carriers [13,14]. Transmission of HEV occurs predominantly by the fecal-oral route. However, the parenteral route has also been implicated [15]. This hypothesis was supported by the finding that 18.5% of our patients had previous blood transfusion.

Patients with acute viral hepatitis due to hepatitis A also had high frequency of anti-HEV, probably because of similar routes of transmission for hepatitis A and E viruses [16]. This finding also supports the hypothesis that HEV is encountered early in childhood as HAV infection.

Diagnosis of HEV is primarily based on detection of specific antibody. Anti-HEV IgM antibodies indicate recent infection, but they disappear rapidly. Anti-HEV IgG antibodies can be detected in up to 96% of acute infections during the first 4 weeks, and they disappear in at least 50% of patients by 3 months after the onset of acute disease [17]. The duration of HEV antibody persistence after exposure has not been established. Recurrent acute HEV infections have been documented, suggesting that the HEV antibody does not persist on a long-term basis [18]. This could explain the low frequency of positive IgM to HEV among our patients.

In the present study, the prevalence of HEV IgG and IgM was correlated to the increased levels of hepatic AST and ALT in patients with dual markers of infection with hepatitis E and other viruses compared to patients with active hepatitis A and C alone. Mixed infection (coinfection or superinfection) with HEV plus other hepatitis viruses might have significance in these situations. Infection with HAV has been shown to cause a severe illness in adult patients with chronic liver disease, in particular patients with chronic hepatitis C [19]. Because of its similarities with HAV, HEV could also be expected to cause a superinfection in hepatitis C-infected patients.

In contrast to our findings, Kumar et al [20] reported that coinfection with multiple viruses is observed in one-quarter of patients with sporadic acute viral hepatitis in childhood. Such infection does not produce a more severe disease. Higher than reported levels of AST and ALT in our patients may be due to increased immune-mediated viral clearance or due to increased cytopathic effects of dual infection with hepatitis A and hepatitis E viruses. However, no such increase in ALT was observed in patients with HBV. This can be explained by the low number of patients in this group.

We conclude from this study that HEV serological markers are common among children with various forms of acute viral hepatitis, especially B and C viruses. Hepatitis B and C may be associated with increased sensitivity to HEV coinfection. Dual infection with HEV and other hepatotropic viruses is associated with greater elevations of AST and ALT.

References

1. Purcell RH, Emerson SU. Hepatitis E virus. In: Knipe DM, Howley PM, Griffin DE, Martin MA, Lamb RA, Roizman B, et al, eds. Fields virology. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins Co.; 2001:3051-61.
2. Zafrullah M, Ozdener MH, Panda SK, Jameel S. The ORF3 protein of hepatitis E virus is a phosphoprotein that associates with the cytoskeleton. J Virol. 1997;71:9045-53.
3. Tyagi S, Jameel S, Lal SK. Self-association and mapping of the interaction domain of hepatitis E virus ORF3 protein. J Virol. 2001;75:2493-8.
4. Jothikumar N, Cromeans TL, Robertson BH, Meng XJ, Hill VR. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. J Virol Methods. 2006;131:65-71.
5. Barnett BJ, Sehulster L. Hepatitis E: Could it happen here? Austin, TX, USA: Disease Prevention News, Texas Department of Health; 1996;56:1-2. Available from: http://www.dshs.state.tx.us/idcu/health/dpn/issues/dpn56n11.pdf
6. Yarbough PO, Tam AW, Fry KE, Krawczynski K, McCaustland KA, Bradley DW, et al. Hepatitis E virus: identification of type-common epitopes. J Virol. 1991;65:5790-7.
7. Stoszek SK, Engle RE, Abdel-Hamid M, Mikhail N, Abdel-Aziz F, Medhat A, et al. Hepatitis E antibody seroconversion without disease in highly endemic rural Egyptian communities. Trans R Soc Trop Med Hyg. 2006;100:89-94.
8. Stoszek SK, Abdel-Hamid M, Saleh DA, El Kafrawy S, Narooz S, Hawash Y, et al. High prevalence of hepatitis E antibodies in pregnant Egyptian women. Trans R Soc Trop Med Hyg. 2006;100:95-101.
9. El-Sayed Zaki M, El-Deen Zaghloul MH, El Sayed O. Acute sporadic hepatitis E in children: diagnostic relevance of specific immunoglobulin M and immunoglobulin G compared with nested reverse transcriptase PCR. FEMS Immunol Med Microbiol. 2006;48:16-20.
10. Aggarwal R, Shahi H, Naik S, Surinder K. Yachha SK, Naik SR. Evidence in favour of high infection rate with hepatitis E virus among young children in India. J Hepatol. 1997;26:1425-6.
11. Pisanti FA, Coppola A, Galli C. Association between hepatitis C and hepatitis E viruses in southern Italy. Lancet. 1994;344:746-7.
12. Psichogiou MA, Tassopoulos NC, Papatheodoridis GV, Tzala E, Klarmann R, Witteler H, et al. Hepatitis E virus infection in a cohort of patients with acute non-A, non-B hepatitis. J Hepatol. 1995;23:668-73.
13. Sheikh A, Sugitnai M, Kinukawa N, Moriyama M, Arakawa Y, Komiyama K, et al. Hepatitis E virus infection in fulminant hepatitis patients and an apparently healthy population in Bangladesh. Am J Trop Med Hyg. 2002;66:721-4.
14. Coursaget P, Buisson Y, N'Gawara MN, Van Cuyck-Grande H, Roue R. Role of hepatitis E virus in sporadic cases of acute and fulminant hepatitis in an endemic area (Chad). Am J Trop Med Hyg. 1998;58:330-4.
15. Irshad M. Hepatitis E virus: a global view of its seroepidemiology and transmission pattern. Trop Gastroenterol. 1997;18:45-9.
16. Parana R, Cotrim HP, Cortey-Boennec ML, Trepo C, Lyra L. Prevalence of hepatitis E virus IgG antibodies in patients from a referral unit of liver diseases in Salvador, Bahia, Brazil. Am J Trop Med Hyg. 1997;57:60-1.
17. Purcell RH, Emerson SU. Hepatitis E virus. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and practice of infectious diseases. Philadelphia, PA: Churchill Livingstone; 2000;345-57.
18. 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;31:818-22.
19. 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. N Engl J Med. 2006;338:286-90.
20. Kumar A, Yachha SK, Poddar U, Singh U, Aggarwal R. Does co-infection with multiple viruses adversely influence the course and outcome of sporadic acute viral hepatitis in children? J Gastroenterol Hepatol. 2006;21:1533-7.