Etiology of sporadic acute and fulminant non-A, non-B viral hepatitis in north India

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Background: Viral hepatitis is a major public health problem in India. Aim: To investigate the association of various hepatitis viruses in patients with acute liver diseases in north India. Methods: One hundred and thirteen patients with acute viral hepatitis (AVH; n=70) or fulminant hepatic failure (FHF; N=43) were evaluated for the presence of hepatitis A, B, C and E virus infection. Hepatitis C virus (HCV) testing was done using second-generation anti-HCV ELISA test and reverse transcriptase polymerase chain reaction (PCR) for the detection of HCV RNA in the serum of patients with non-A, non-B (NANB) hepatitis. Detection of IgM anti-HCV antibody was done in patients found negative for the above viruses (n=53). Results: Hepatitis A and B viruses accounted for 3.5% and 42.5% of the 113 cases, respectively. HCV infection accounted for 12% of the NANB cases with AVH and 15% with FHF. PCR was more useful than serological tests for the detection of HCV infection. HEV infection accounted for 49% of the NANB, non-C cases with AVH and 25% with FHF; pregnant women with HEV infection had a fulminant course. No etiological agent could be established in 28.3% of cases. Conclusion: HEV is the most important cause of NANB hepatitis; hepatitis B virus is still a major concern, while HCV is not an important cause of acute viral liver disease in India. [Indian J Gastroenterol 1997; 16: 43-45]

Key words: Viral hepatitis epidemiology, hepatitis viruses, fulminant hepatic failure

Non-A, non-B (NANB) viruses are the most common cause of acute hepatitis in Indian adults, accounting for up to 58% of cases with fulminant hepatic failure (FHF) and 44% with acute viral hepatitis (AVH). Of the NANB viruses, hepatitis E virus (HEV) has been established as the sole cause of epidemic hepatitis in Afro-Asian countries; fulminant hepatitis has been recorded during such epidemics. The role of hepatitis C virus (HCV) in acute sporadic liver disease and fulminant hepatitis remains unclear.

Second-generation anti-HCV assays (ELISA-2, RIBA-2) incorporate antigens derived from the structural region (core), c22, and from the NS5 region, c33, in addition to the original antigens 51-1 and C160-3 derived from the NS3/NS4 regions. These assays have reduced the "window period" in the diagnosis of HCV infection to between 11 weeks and 20 weeks. The only modality available for the diagnosis of HCV infection in the very early stages remains detection of viral RNA by polymerase chain reaction (PCR).

The diagnosis of HEV infection can be established by detection of IgM and/or IgG anti-HEV antibodies in serum by ELISA or by immune electron microscopy of stool samples for virus-like particles (VLP). Reverse transcriptase (rt) PCR can be used to detect viral RNA in serum. However, since the viremia is short lasting and disappears by the onset of symptoms, PCR may be negative.

The present study was undertaken to investigate the association of various hepatitis viruses with sporadic AVH and FHF.

Methods

One hundred and thirteen patients (71 men, 42 women; aged 13-60 years, mean 28), including 70 patients with AVH and 43 with FHF, were studied during the period May 1992 to May 1994.

AVH was defined as acute self-limited disease with serum aspartate aminotransferase (AST) elevation at least five-fold, or clinical jaundice, or both. FHF was diagnosed when, after a typical acute onset, the patient became deeply jaundiced and developed hepatic encephalopathy within 4 weeks of the onset of disease, with no past history of chronic liver disease.

Liver function tests (AST, ALT, alkaline phosphatase, bilirubin, total proteins and albumin, prothrombin time) were measured in all cases. The patients were followed up till biochemical recovery and/or death.

Viral markers testing

Tests for hepatitis A (HAV) and B (HBV) viruses were done in all cases. Acute hepatitis A was diagnosed by the detection of IgM antibody to HAV (IgM anti-HAV) in serum (Cloneon HAV Ab EIA, France). Acute hepatitis B was diagnosed by either HBsAg and/or IgM antibody to hepatitis B core antigen (IgM anti-HBc) in the initial sample (Cloneon MHBc EIA, France). Tests for HCV were done only in cases negative for HAV and HBV markers; these included detection of (a) anti-HCV using second-generation anti-HCV ELISA kit (Pinnacle Bio, USA) and (b) HCV RNA by rt-PCR. Sera of patients found negative for all the above markers were tested for IgM anti-HEV antibodies (GLD HEV IgM ELISA, Genelabs Diagnostics, Singapore).

rt-PCR for HCV

RNA was extracted and precipitated from 100 μl of serum. Copyright © 1997 by Indian Society of Gastroenterology
samples by acid guanidium phenol method (using a modification of the method of Chomczynski and Sacchi).5 To a 50 μl reaction mixture containing 10× PCR buffer (1 M KCl, 1 M MgCl2, 1 M Tris-HCl, pH 8.4), 10 mM dNTP's, BSA (50 ng/mL), 20 pmol of external primers #346(+)
CTGTGAGGAACACTGTGCTT and #317(-) GTGCTC GTAAGTCCAGCTGCTACGAGACCTCGG), and Taq DNA polymerase (50 μL) (Perkin-Elmer, Cetus, USA), 5 μL of extracted RNA and 200 units of Moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega, Madison, WI, USA) were added. The reaction mixture was then overlaid with 50 μL mineral oil.

rt-PCR was done using an automated thermal cycler (Perkin-Elmer, Cetus, USA), for reverse transcription, a temperature of 42°C for 1 hour was used. For PCR, 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1.5 min, and extension at 72°C for 2 min were used except that in the first cycle, denaturation temperature was maintained for 4 min. Five μL of the amplified product was used for the second round of nested PCR (35 cycles) with inner primers #364(+)
TTCAACGAGAAAGGTCTAG and #287(-)TACACGCAAAGCCTATCACGATCA.

The 251-base-pair PCR product was detected using electrophoresis in 3% NuSieve agarose gel containing ethidium bromide under an ultraviolet transilluminator and known molecular weight markers (digested with Hae III).

Results

Of the 113 patients, four (3.5%) (AVH=3, FHF=1) were positive for IgM anti-HAV and 48 (42.5%) had evidence of acute HBV infection (IgM anti-HBc positive 47, HBsAg only in the initial sample). Thus, 61 patients had NABN hepatitis: of these, six (AVH=4, FH=2) had anti-HAV antibody and eight (AVH=5, FHF=3) had HCV RNA. Of the remaining 33 patients, 21 (AVH=17, FHF=4) had IgM anti-HAV.

Clinical profile

Only two patients had history of blood transfusion 6 weeks preceding the onset of jaundice; of these, one had hepatitis B and the other tested negative for HBV and HCV. No patient gave history of intravenous drug abuse, acupuncture, tattooing, hemodialysis, occupational exposure, multiple sexual partners or homosexuality.

After 6 weeks of follow-up, all HCV-negative cases had normal or near-normal liver function profile whereas one HCV-positive patient continued to have elevated ALT levels (persisting beyond 6 months). This patient tested positive for both anti-HCV and HCV RNA at 6 months.

Of the 12 pregnant women, 10 belonged to the non-A, non-B, non-C group; IgM anti-HEV antibodies were detected in eight of these (AVH=4, FHF=4). Thus, higher frequency of HEV infection was noted in pregnant women (p<0.01, Fischer's exact test). Five of these eight pregnant women died while none of the non-pregnant anti-HEV-positive women died (p<0.05, Fischer's exact test).

Discussion

The role of HCV in sporadic, community-acquired AVH and FHF is unclear. Studies from India show that HEV positivity in AVH to vary from 10.4%-27%; some studies report that HCV is not an important cause of AVH in India (0%-0.02%).12 In the present study, we detected HCV in 14.5% of cases with acute NABN hepatitis. In the West, HCV appears to be the etiologic agent in at least 82% of cases with acute viral hepatitis.

Reports of the role of HCV in FHF are more conflicting. The frequency of HCV infection among FHF patients varies from 0% to 58%. Coinfection or superinfection with HCV may play an important role in the development of this fatal condition. In our study, we found HCV in 15.5% of cases with NABN fulminant hepatic failure. Since we did not test for markers of HCV and HEV infection in patients who were serologically HAV or HBV positive, we could not detect coinfection and/or superinfection by these hepatitis viruses.

Although various studies quote HCV as the cause in 85%-95% of cases with post-transfusion hepatitis, in India the majority of post-transfusion hepatitis are still likely to be due to HBV. In the present study, of two cases with post-transfusion hepatitis, one was due to HBV and the other was of undetermined etiology. The mode of transmission in the other patient was not known.

Hepatitis E virus is known to be an important cause of epidemics of viral hepatitis in the Indian subcontinent. It is also known to be an important cause of acute sporadic NABN hepatitis and of sporadic fulminant hepatitis in India. About 54% of the cases of AVH and FHF in our study were due to non-A, non-B, non-C infection; in this group, HEV was detected in 39.6%. HEV infection during pregnancy is known to follow a fulminant course with a high mortality. This is in agreement with our findings, where the mortality in HEV-positive pregnant women was higher than in HEV-positive non-pregnant women and FHF due to other hepatitis viruses.

In about 28% of cases, no etiological agent could be detected. Whether these cases are caused by unknown viruses remains to be established.

In conclusion, HEV accounts for a majority of cases of AVH in India and is an important cause of mortality in pregnant women. HBV is still a major concern, while HCV is not an important cause of acute viral liver disease in India.

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