HEPATITIS E VIRUS INFECTION IN EASTERN INDIA

JAYA BANSAL, JUNKUN HE, PATRICE O. YARBOUGH, SANDEEP SEN, NIEL T. CONSTANTINE, AND DILIP SEN
Cell Technology, Inc., Jessup, Maryland; Department of Virus Disease, Walter Reed Army Institute of Research, Washington, District of Columbia; Genelabs Technologies, Inc., Redwood City, California; Sen Medical Research Center, Patna, Bihar, India; Institute of Human Virology, University of Maryland, Baltimore, Maryland

Abstract. Most cases of enterically transmitted non-A, non-B hepatitis in India have so far been attributed to hepatitis E virus (HEV) infection. Most of the documented studies of hepatitis have focused on the incidence of this disease in northern, western, and south central India. A small seroprevalence study was conducted in the eastern city of Patna to assess the degree of HEV infection among acute sporadic hepatitis cases. Forty-two percent (24 of 57) of the cases of acute sporadic hepatitis were positive for anti-HEV antibodies. Absence of any serologic markers of hepatitis A, B, or E in 58% (33 of 57) of the cases with symptoms of acute hepatitis suggest that there may be as yet unidentified enterically transmitted viruses in this area.

Acute viral hepatitis is a major public health problem in developing nations having inadequate sanitary conditions.\(^1\) Non-A, non-B hepatitis viruses have been identified as a major cause of water-borne epidemics in India\(^2\) and as a significant cause of enterically transmitted sporadic hepatitis.\(^3\) About 70% of the sporadic cases among adults in Pune (Maharashtra, India; Figure 1) have been reported to be due to non-A, non-B hepatitis.\(^3\)

Most cases of enterically transmitted non-A, non-B hepatitis in India so far have been attributed to hepatitis E virus (HEV) infection.\(^2\),\(^3\) These infections are documented to be associated with as much as 20% mortality among pregnant women.\(^4\) In parts of India where epidemics resulting from HEV infection are common, this virus has been demonstrated to be one of the major pathogens associated with sporadic fulminant hepatitis.\(^5\) Also, enterically transmitted hepatitis A and E viruses were found to be associated with 60% of the cases of acute liver failure in a recent study involving a group of pediatric cases admitted to the All India Institute of Medical Sciences in New Delhi, India.\(^6\) In that study, possible coinfections with hepatitis A virus (HAV) and HEV have been implicated as the single largest etiologic subgroup causing acute hepatic failure and sporadic fulminant hepatitis in India.\(^6\)

Most of the documented studies of hepatitis E have focused on the incidence of this disease in the northern, western and south central parts of India (Figure 1).\(^2\),\(^3\),\(^5\),\(^8\) However, recurrent epidemics of water-borne hepatitis also occur in the eastern part of India, especially in the state of Bihar (Figure 1). None of these outbreaks has been thoroughly documented and characterized. Additionally, sporadic enterically transmitted cases are known to occur in eastern India throughout the warm weather seasons. It is not known whether these cases represent HAV, HEV, or HAV/HEV coinfection. In this report, we report the results of a seroprevalence study conducted to assess the degree of HEV infection among acute sporadic hepatitis cases in the eastern Indian city of Patna.

METHODS

At a pathology clinic in Patna (Figure 1), serum samples were collected between June 1992 and June 1994 from adult patients who presented with symptoms of acute sporadic hepatitis of less than 28-days duration. Hepatitis was defined as the onset of jaundice, dark urine, and lighter-colored feces in the absence of any history of liver disease. These symptoms were associated with at least a 2.5-fold elevation above the normal levels of two critical liver function enzymes: aspartate aminotransferase and alanine aminotransferase, and a serum bilirubin count of 2.0 \(\mu g/dL\). Patients with a history of liver disease, blood transfusion, tattoos, hospitalization, dental care, and sexually transmitted disease within six months of presentation were excluded from participation in this study.

Prior approval for participation of human volunteers was obtained from the Institutional Review Board of the University of Maryland School of Medicine (Baltimore, MD) and from the office of the Joint Secretary, Department of Health and Family Welfare, Government of Bihar (Patna, India) before the commencement of this study. Informed consent was obtained in writing from the participants in this study at the pathology clinic in Patna, India.

This study was conducted at a general pathology clinic that is one of the oldest and established clinics in the city of Patna. Patients are referred by their physicians to come to this clinic for tests. This pathology clinic serves patients from a wide cross-section of the population of Patna. Consequently, these patients come from a variety of socioeconomic backgrounds and therefore were not exposed to the same type of sanitary conditions. The patients who come to this clinic are not prescreened at other clinics.

A total of 60 adult patients who presented with symptoms of acute hepatitis and who satisfied the clinical criteria for participation in this study were screened for our seroprevalence study. Initially, the sera were screened by enzyme immunoassay for IgM anti-HAV and IgM anti-HBV core antigen (Abbott Laboratories, North Chicago, IL). Western blot tests\(^9\) for detection of antibodies reactive with HEV antigens were used to test 57 samples that were negative for HAV and HBV markers and thus were potentially cases of non-A, non-B hepatitis.

RESULTS

Sixty adult patients who presented with symptoms of acute hepatitis and satisfied the clinical criteria for participation in this study were screened for our seroprevalence study. Fifty-seven (95%) of 60 were potential cases of non-A, non-B hepatitis. Of the remaining three patients, two
(3%) were positive for anti-HAV IgM antibodies and one (2%) was positive for anti-HBV core IgM antibodies and HBV surface antigen. There were no cases of concurrent infection among the patients that were tested for the presence of antibodies reactive with hepatitis A, B and E viruses.

The results of the Western blot test revealed that 33 (58%) of 57 samples were negative for the presence of anti-HEV antibodies and 24 (42%) of 57 samples were positive for anti-HEV antibodies reactive with the baculovirus-expressed HEV open reading frame 2 (ORF 2)-encoded protein. Of these 24 anti-HEV antibody positive samples, two were only IgM, 12 were IgM and IgG, and 10 were IgG.

Additionally, a subset of 28 samples taken randomly from the original group of 57 potentially non-A, non-B hepatitis were tested for the presence of anti-HEV IgG and IgM antibodies by an ELISA (Genelabs Technologies, Inc., Redwood City, CA) (based on a three antigen assay with HEV ORF-2 and ORF-3 expressed in Escherichia coli). Twenty of 28 samples tested negative for anti-HEV antibodies. These 20 samples also had tested negative by the Western blot test. Eight of 28 samples tested positive for anti-HEV IgG (Table 1). These eight samples also had tested positive for anti-HEV IgG by the Western blot test (Table 1). Of the eight anti-HEV IgG antibody positive samples, four were also positive for IgM (as they had been in the Western blot). Additionally, two of these anti-HEV IgG-positive samples were negative for IgM (as they had been in the Western blot). Two of eight anti-HEV IgG-positive samples gave discordant results regarding IgM antibody status by the ELISA and Western blot tests (Table 1; samples 5 and 16).

**DISCUSSION**

In this study 24 of 57 potential cases of non-A, non-B hepatitis (42%) were positive for anti-HEV antibodies (two were only IgM, 12 were IgM and IgG, and 10 were IgG) in the baculovirus-expressed HEV ORF 2-encoded protein-based Western blot assay. Fourteen (25%) of 57 samples

**TABLE 1**

Comparison of anti-hepatitis E virus ELISA and Western blot test results

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>ELISA IgM</th>
<th>Blot IgM</th>
<th>ELISA IgG</th>
<th>Blot IgG</th>
</tr>
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<tbody>
<tr>
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<td>+</td>
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<tr>
<td>16</td>
<td>+</td>
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</tr>
<tr>
<td>45</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>+</td>
</tr>
</tbody>
</table>

* = negative; +/- = borderline pattern; + = positive; ++++ = strongly positive.
were anti-IgM antibody positive, indicating that these sporadic cases represent recent infection with HEV. It is possible that 12 IgM+/IgG- cases represent the transition to the anti-HEV IgG antibodies during the course of infection. However, the lower number cases with anti-HEV IgM antibodies among the acute phase hepatitis patients could be due to a rapid decrease in IgM levels.^

A random subset of 28 of these 57 samples were tested by an ELISA for the presence of anti-HEV antibodies. There was a 100% concordance between the ELISA and Western blots for samples that tested negative and for samples that tested positive for anti-HEV IgG antibodies. However, 75% concordance was observed for anti-HEV IgM-positive samples between the results from the ELISA and the Western blot in the subset of 28 samples that were tested by both these methods. Two of eight anti-HEV IgG-positive samples were discordant regarding IgM antibody status by the ELISA and Western blot tests (Table 1; samples 5 and 16). It is probable that sample 16 may have had anti-HEV IgM antibodies reactive only with the ORF-3 antigen, which was detectable only in the ELISA, but not in the Western blot. On the other hand, sample 5 may have had anti-HEV ORF-2 and ORF-3 IgM antibodies, but not enough to result in a definitely positive reaction in the ELISA. The relative lack of concordance between the two anti-IgM detection systems may be due to the differences in the sensitivity and specificity parameters of these tests. Performance characteristics of the IgM ELISA are a specificity of 97% and a sensitivity of 94%; these parameters are greater than 95% for the ELISA (Yarbough PO, unpublished data).

Clinical hepatitis A is uncommon among the adult population of western India because of seroconversion to HAV early in childhood. This was corroborated by the very low incidence of acute hepatitis A (3%) among the adult subjects in our study. Only adult subjects were included in this study. A low incidence of hepatitis A is expected among adult subjects due to the prevalence of hepatitis A among children and subsequent acquisition of immunity to hepatitis A. On the other hand, 57 (95%) of 60 patients with symptoms of acute hepatitis were negative for the markers of recent infection with either hepatitis A or hepatitis B. Therefore, by comparison, the observed prevalence rate of hepatitis A appears to be lower than that of non-A, non-B hepatitis in this seroprevalence study.

In this study, there were 33 (58%) of 57 acute hepatitis cases without any serologic marker of acute viral hepatitis A, B, and E. However, the absence of anti-HAV IgM, anti-HBV core antigen IgM, and anti-HEV antibodies in approximately 58% of the patients with symptoms of acute hepatitis suggest that there may be an as yet unidentified enterically transmitted virus endemic to this area. The exclusion of patients with exposure to potential risk factors for transmission of blood-borne hepatotropic pathogens within six months of presentation of hepatitis symptoms from this study most likely precluded the possibility of including cases of hepatitis C in this study. Earlier studies conducted in other parts of India have also provided evidence for the existence of a sixth human hepatitis agent, which would be the third agent to be spread by fecal contamination. Further studies will be needed to determine whether a virus other than HAV, HBV, or HEV exists in this part of India or if there is a variant of a known pathogen causing water-borne enterically transmitted hepatitis.

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Authors' addresses: Jaya Bansal, Cell Technology, Inc., 7798 Jessup Road, Jessup, MD 20794. JunKun He, Department of Virus Disease, Walter Reed Army Institute of Research, Washington, DC 20307-5100. Patrice O. Yarbough, Genelabs Technologies, Inc., 505 Pegasus Drive, Redwood City, CA 94063. Sandeep Sen and Dilip Sen, Sen Medical Research Center, Patna, Bihar, India. Niel T. Constantin, Institute of Human Virology, University of Maryland, Baltimore, MD 21201.

REFERENCES