Clinicobacteriologic Study of Mother-to-Neonate Transmission of Hepatitis E Virus in Egypt

Maysaa El Sayed Zaki, MD,1 Amena Abd El Aal, MD,1 Ahmed Badawy, MD,2 Douaa Raafat El- Deeb, MD,1 and Nermin Youssef Abo El-Kheir, MD1

From the 1Department of Clinical Pathology and 2Department of Obstetrics and Gynecology, Mansoura Faculty of Medicine, Mansoura, Egypt.

Key Words: Gynecological; Infectious diseases; Obstetric and perinatal; Pediatrics

DOI: 10.1309/AJCPT55TDMJNPLLV

ABSTRACT

Objectives: To study the presence of hepatitis E viremia in neonates with congenital infections.

Methods: We included 29 neonates with clinical signs and symptoms suggesting congenital infections, along with their mothers. The control group comprised 29 healthy neonates and their mothers. Laboratory evaluations were performed for each sample for liver function profiles and virological studies for hepatitis viruses B, C, and E.

Results: The most common viral markers in mothers were for hepatitis C immunoglobulin G (IgG) (41%), followed by hepatitis B surface antigen (34%) and hepatitis E virus (HEV) IgG (31%). The most common presentations in neonates were respiratory distress syndrome, followed by preterm birth and signs of sepsis (both 41%) and hepatosplenomegaly (13%).

Conclusions: This study highlights the occurrence of HEV infection among other etiological conditions causing congenital infections. Vertical transmission from mothers was common in our patients. Although HEV ran a milder course, more studies are needed.

Hepatitis E virus (HEV) is a small RNA virus and the etiological agent for hepatitis E. The disease is endemic in large parts of Asia, Africa, and Latin America, where epidemic and sporadic disease has been reported.1 In Egypt, HEV accounts for 20% to 40% of adult and pediatric hospitalized cases of acute viral hepatitis (AVH).2 Although no major HEV epidemics have been documented in Egypt, community surveillance revealed a high prevalence of antibody to HEV (anti-HEV) within several rural populations.3

The common feature in almost all the epidemics is the contamination of the water supplies with sewage, confirming the feco-oral transmission.4 Recent studies on the isolation of HEV-like viruses from animal species also suggest zoonotic transfer of the virus.1

An interesting and intriguing observation with HEV infection has been its relationship with pregnancy. Hepatitis
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E is usually a self-limiting disease, with a low rate of fulminating hepatic failure. However, when this infection occurs in pregnant women, the consequences are disastrous. Pregnant women, particularly those in the second and third trimesters, are more frequently affected during HEV outbreaks. The infections during pregnancy lead to congenital defects, spontaneous abortion, and even death. The definitive diagnosis and management of pregnancy-related viral infections may be challenging, especially in countries with less resources.

In Egypt, there are no reports to our knowledge about the effect of HEV infection on mothers and their infants. Moreover, there are no reports about the association of HEV with congenital infections. There is limited information in Egypt regarding (1) hepatitis E viremia among pregnant patients and their neonates, especially those with early signs of congenital infections, and (2) mother-to-infant transmission of HEV from infected pregnant women and the natural history of such an acquired infection. Thus, we studied these parameters.

Material and Methods

The present study included 29 neonates attending Mansoura University Children’s Hospital from January 2012 through December 2013 with clinical signs and symptoms suggesting congenital infections, such as respiratory distress starting more than four hours after birth, seizures, the need for mechanical ventilation in a term baby, signs of shock, abnormal heart rate (bradycardia or tachycardia), and hypoxia. Their mothers were also recruited and included in the study. Furthermore, 29 healthy neonates and their mothers were included in the study as a control group. The study was approved by the Mansoura Faculty of Medicine Ethics Committee, and signed written consent was obtained from each adult and the legal guardian of each neonate.

Each neonate had complete clinical examinations. Then, blood samples were obtained from each neonate for complete screening of toxoplasmosis-, herpes simplex-, rubella-, and cytomegalovirus-specific antibody detection. Moreover, blood samples were subjected to full liver function tests and complete virological serology, including studies of hepatitis A IgM (Dia-Pro), hepatitis B surface antigen (Bio-Rad, Segrate, Italy), HCV IgG (Dia-Pro), and hepatitis E IgG (DSI-EIA-ANTI-HEV-G, DSI) and IgM (DSI-EIA-ANTI-HEV-M, DSI). Moreover, positive samples for HEV antibodies were subjected to nested PCR for the detection of hepatitis E viremia.

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. 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.

Detection of Serum HEV RNA by Nested RT-PCR

Reverse transcription (RT)–PCR was performed using a QIAGEN (Hilden, Germany) One-Step RT-PCR kit according to the manufacturer’s instructions. The primers were adopted after Huang et al.

Briefly, a reaction tube contained 50 μL of the reaction solutions, including 10 μL of the 5x QIAGEN One-Step RT-PCR buffer, 2 μL of the dNTP mix (containing 10 mmol of each dNTP), 10 μL of the 5x Q-Solution, 2 μL of the external forward primer (100 pmol/μL), 2 μL of the external forward primer set (5′-AATTATGCCC(T)CAGTAC(T)CGG(A) GTTG-3′) and reverse primer set (5′-CCCTTA(G)TCC(T) TGCTGA(C)GCATTCTC-3′) (100 pmol/μL), 2 μL of the QIAGEN One-Step RT-PCR enzyme mix, 1 μL of the RNase Out RNA inhibitor (10 U/μL; Gibco BRL, Gaithersburg, MD), 10 μL of the template RNA, and 11 μL of RNase-free water.

The thermal cycling conditions included one step of RT for 30 minutes at 50 C and an initial PCR activation step for 15 minutes at 95°C. This was followed by 40 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 50°C, extension for 1 minute 15 seconds at 72°C, and a final incubation for 10 minutes at 72°C.

A nested PCR was conducted with the following components: 3 μL of the RT-PCR product, 5 μL of the 10x PCR buffer, 5 μL of MgCl2 (25 mg/mL), 4 μL of the dNTP mix (10 mmol of each dNTP), 1 μL of the nested forward primer (5′-GTT(A)ATGCTT(C)TGCTA(T)CATGCGT-3′) and 1 μL of the nested reverse primer (5′-AGCCGACGAAATCAATTCTGTC-3′) (100 pmol/μL), 0.5 μL of Takara Ex Taq polymerase (5 U/μL), and 30.5 μL of double-distilled water. The thermal cycling conditions for the nested PCR
included five cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 45°C, and extension for 1 minute 15 seconds at 72°C. This was followed by 35 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 53°C, extension for 1 minute 15 seconds at 72°C, and a final incubation for 7 minutes at 72°C.\textsuperscript{9}

Sterile distilled water was used as a negative control. The positive control was the prototype US strain of swine HEV. Positive and negative controls were included in each run with specific molecular weight markers.\textsuperscript{8} Strict sterile procedures were followed to avoid false-positive results, such as using sterile filter pipette tips and microcentrifuge tubes and avoiding the carryover of stock solutions.\textsuperscript{10}

The amplified PCR products were examined by agarose gel electrophoresis. The expected product of the universal nested RT-PCR was 348 base pairs.

### Results

The present study was carried out on 29 neonates with symptoms and signs of congenital infections, along with their mothers. \textbf{Table 1} summarizes the characteristics of mothers of affected neonates compared with the control group. Participants were mainly from rural areas (69%), with a mean ± SD age of 26.16 ± 3.9 years.

The most common viral markers were for hepatitis C IgG (41%), followed by hepatitis B surface antigen (34%) and HEV IgG (31%). An interesting finding was the presence of hepatitis E viremia in nine (31%) mothers. The liver function biochemical profile for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) had mild elevations.

None of the mothers had positive markers for other infectious agents, including toxoplasmosis, rubella, cytomegalovirus, or herpes simplex (data not shown). In the control group, none of the mothers had positive virological markers for hepatitis viruses.

\textbf{Table 2} presents a format used in previous studies\textsuperscript{11,12} that includes clinical, biochemical, and virological findings regarding HEV. All nine mothers had vaginal deliveries. Nine mothers and six babies from the cases had HEV viremia. All mothers affected by HEV viremia had a complicated obstetric history (eg, high blood pressure, gestational diabetes, pre-eclampsia, preterm labor, pregnancy loss), and six had elevated liver enzymes (ALT and AST) associated with HEV viremia.

Mothers and infants (cases 1, 3, 5, 6, and 8) had both positive IgG and HEV viremia. However, some mothers (cases 2, 4, and 7) had positive IgG and HEV viremia, but their neonates were negative for IgG and HEV RNA. An
interesting finding involved one mother (case 9) with HEV viremia whose neonate had a positive serological marker for both HEV IgG and IgM. All neonates with positive HEV viremia had RDS and an elevated bilirubin level, with one neonate having marked elevated liver enzymes. Four of the affected neonates with viremia were born prematurely.

Discussion

Hepatitis E is common in pregnant women in endemic areas, and clinicians are often faced with problems of defining the outcome of pregnancy and fetal and perinatal morbidity and mortality. Our present data show that intrauterine infection with hepatitis E was common and contributed significantly to perinatal morbidity.

We have not studied HEV-infected women in the first and second trimesters. However, a history of abortions was common in pregnant women with hepatitis E in the present study.

Our data suggest that significant proportions of neonates had congenital infections in the first month of life due to HEV viremia (17%), and 20% had antibodies to HEV. The clinical profile of HEV-infected babies varied from elevated liver enzymes alone, elevated bilirubin alone, and elevated bilirubin with increased liver enzymes. In another study, the elevated bilirubin was caused by physiological jaundice, which occurs in neonates. However, the increase in serum bilirubin in the neonates in this study occurred after the period of normal physiological jaundice, which suggests that HEV infection was the likely cause.

The liver disease in the babies born with vertically transmitted HEV infection was associated with severe clinical signs, including RDS, and had a significant association with HSM ($P = .004$). Other studies reported fatal acute liver failure syndrome in neonates who were unresponsive and had hypoglycemia and hypothermia.$^{11,14}$

In the present study, five neonates had evidence of vertical transmission of acute hepatitis E either by positive HEV PCR and/or IgM for HEV. Similarly, Singh et al$^{12}$ reported that of the six cord blood samples tested, three (50%) from neonates born to mothers affected by HEV viremia in India were positive for HEV RNA. Although all mothers were RNA positive, half of the babies were not infected in utero with HEV.

The current belief is that intrauterine infection is an important route of mother-to-infant transmission of HEV.$^{14,19}$ In the present study, five of nine infants had evidence of the vertical transmission of hepatitis E from the 26 symptomatic, HEV RNA-seropositive mothers to their 26 respective infants, who were also HEV RNA seropositive and soon became symptomatic with liver disease.

The clinical, biochemical, serological, and virological profile of babies who survived showed that HEV infection (mother-to-fetus transmission) in neonates is a self-limiting disease and does not cause chronic viremia or a prolonged clinical course, in contrast to the clinical course of hepatitis B virus (HBV) infection acquired by neonates through perinatal transmission. This outcome is possibly related to the different ways in which these two viruses cause hepatic injury. HBV

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Table 3
Clinical, Biochemical, and Virological Findings Regarding HEV for Mother-Infant Pairs

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Obstetric Diagnosis</th>
<th>HEV Liver Function Tests</th>
<th>Infant Liver Function Tests</th>
<th>Clinical Presentation</th>
<th>Age, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PROM, oligohydramnios, and fever</td>
<td>+ (1.490)</td>
<td>+</td>
<td>1.45 40 30 RDS</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>PROM</td>
<td>+ (0.754)</td>
<td>+</td>
<td>0.8 40 40 RDS and preterm</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>Polyhydramnios</td>
<td>+ (2.07)</td>
<td>+</td>
<td>1.1 3.8 60 50</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>PET</td>
<td>+ (1.002)</td>
<td>+</td>
<td>1.3 2.9 115 90</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>PET</td>
<td>+ (0.537)</td>
<td>+ (0.973)</td>
<td>1.5 3.1 60 60</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>PET</td>
<td>+ (0.646)</td>
<td>+</td>
<td>1.3 3.1 60 40</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>PROM and puerperal sepsis</td>
<td>+ (1.39)</td>
<td>+</td>
<td>2 3.0 145 130</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>Recurrent abortion</td>
<td>+ (1.36)</td>
<td>+</td>
<td>2 4.5 45 45</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>Recurrent abortion</td>
<td>+ (0.973)</td>
<td>+</td>
<td>1.1 3.0 50 45</td>
<td>25</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HEV, hepatitis E virus; HSM, hepatosplenomegaly; IgG, immunoglobulin G; IgM, immunoglobulin M; PCR, polymerase chain reaction; PET, preeclamptic toxemia; PROM, premature rupture of membranes; RDS, respiratory distress syndrome.
<table>
<thead>
<tr>
<th>HEV</th>
<th>Liver Function Tests</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Total Bilirubin,</td>
</tr>
<tr>
<td></td>
<td>mg/dL.</td>
</tr>
<tr>
<td>IgG</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+ (1.469)</td>
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<tr>
<td>–</td>
<td>–</td>
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<tr>
<td>+</td>
<td>–</td>
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<td>–</td>
<td>–</td>
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<tr>
<td>+</td>
<td>+ (0.576)</td>
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<tr>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>+</td>
<td>+ (2.173)</td>
</tr>
<tr>
<td>+</td>
<td>+ (2.01)</td>
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<tr>
<td>–</td>
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</table>

In endemic areas such as Egypt, confirming recent exposure to HEV is challenging since most of the population is anti-HEV IgG positive. The avidity of virus-specific antibodies has been associated with disease progression in patients with human immunodeficiency virus and measles. The avidity of anti-HEV changing over time after infection may influence susceptibility to reinfection and its outcome in endemic areas. Not surprisingly, neutralizing antibodies to certain epitopes protect against HEV infection in animals and humans. However, the effect of either anti-HEV avidity or epitope-specific neutralizing antibody levels on the modulation of HEV morbidity has not been reported except in a study carried out by Shata et al in Egyptian patients from our region. Anti-HEV avidity and ORF2 452-617 epitope-specific neutralizing capacity in HEV-infected AVH cases were significantly lower than in asymptomatic HEV-infected family members, supporting their roles in protection from symptomatic AVH. Moreover, maturation of anti-HEV IgG avidity has been reported in other HEV infections. However, there was no increase in ORF2 452-617 epitope-specific neutralizing antibodies, which may be due to the presence of human leukocyte antigen–specific dominant HEV epitopes in the host immune cells, with the contribution of these epitopes to neutralization affecting the outcome of the disease. These responses are not changed or boosted like the avidity of the anti-HEV responses. Previous reports from studies in Egyptian patients have confirmed the protective role of cell-mediated immunity and cytokines in the morbidity of HEV infection.

It is difficult to determine whether differences in antibody responses are causally related to reduction of the severity of liver damage or can be considered a surrogate marker for other protective factors. Additional studies are required, particularly in pregnant patients and their neonates, to elucidate the role of the humoral immune response in controlling disease severity in both groups.

Although our current data do not explain how HEV-specific antibody responses ameliorate HEV morbidity in neonates and their mothers, the most plausible explanation is that prior exposure/infection or exposure to a less virulent HEV viral strain leads to protective immunity that quickly clears subsequent viremia and prevents the development of severe, clinically AVH or fulminant hepatitis in both neonates and their mothers.

The present study highlights the occurrence of HEV infection among other etiological conditions causing congenital infections. Clinical presentations vary, with the presence of jaundice and viremia in neonates. Vertical transmission from mothers appears common in our patients. Although HEV runs a milder course in our patients, further extended studies are needed to explore this condition.
Address reprint requests to Dr El Sayed Zaki: may_s65@hotmail.com.

References