Transactions of the Royal Society of Tropical Medicine and Hygiene 106 (2012) 613–618

Contents lists available at SciVerse ScienceDirect

Transactions of the Royal Society of Tropical Medicine and Hygiene

journal homepage: http://www.elsevier.com/locate/trstmh

Protective role of humoral immune responses during an outbreak of hepatitis E in Egypt☆

Mohamed T. Shata[a,*], Enas A. Daef[b], Maysaa E. Zaki[c], Sayed F. Abdelwahab[d,e], Naglaa M. Marzuuk[b], Maha Sobhy[e], Marwa Rafaat[b], Liala Abdelbaki[b], Mohamed A. Nafeh[b], Mohamed Hashem[e,f], Samer S. El-Kamary[f], Michelle D. Shardell[f], Nabiel N. Mikhail[g], George T. Strickland[f], Kenneth E. Sherman[a]

[a] *Digestive Diseases Division, University of Cincinnati College of Medicine, Cincinnati, OH, USA*
[b] *Microbiology and Tropical Medicine Departments, Faculty of Medicine, Assiut University, Assiut, Egypt*
[c] *Clinical Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt*
[d] *Microbiology and Immunology Department, Faculty of Medicine, Minia University, Minia, Egypt*
[e] *EgyBlood, VACSERA, Giza, Egypt*
[f] *Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, MD, USA*
[g] *Biostatistics and Cancer Epidemiology Department, South Egypt Cancer Institute, Assiut University, Assiut, Egypt*

ARTICLE INFO

Article history:
Received 3 February 2012
Received in revised form 9 July 2012
Accepted 9 July 2012
Available online 28 August 2012

Keywords:
Hepatitis E
Waterborne outbreak
Humoral immune response
Morbidity
Viral hepatitis
Egypt

ABSTRACT

Although the seroprevalence of hepatitis E virus (HEV) is approximately 80% in adult Egyptians living in rural areas, symptomatic HEV-caused acute viral hepatitis (AVH) is sporadic and relatively uncommon. To investigate the dichotomy between HEV infection and clinical AVH, HEV-specific immune responses in patients with symptomatic and asymptomatic HEV infection during a waterborne outbreak in Egypt were examined. Of 235 acute hepatitis patients in Assiut hospitals screened for HEV infection, 42 (17.9%) were acute hepatitis patients confirmed as HEV-caused AVH; 37 (88%) of the 42 patients were residents of rural areas, and 14 (33%) were from one village (Kom El-Mansoura). Another 200 contacts of AVH cases in this village were screened for HEV and 14 (7.0%), all of whom were family members of AVH cases, were asymptomatic HEV IgM-positive. HEV infections in this village peaked during the summer. Asymptomatic HEV seroconverters had significantly higher levels of epitope-specific neutralising ($p=0.006$) and high avidity ($p=0.04$) anti-HEV antibodies than the corresponding AVH cases. In conclusion, naturally acquired humoral immune responses appear to protect HEV-exposed subjects from AVH during an HEV outbreak in Egypt.

1. Introduction

Acute viral hepatitis (AVH) is caused by a variety of hepatitis viruses. Among them, hepatitis A virus (HAV) and hepatitis E virus (HEV) are both enteric infections that are transmitted faeco-orally. The prevalence of infection and morbidity of HEV differs greatly across different geographic regions. It has at least two distinct epidemiological presentations: large outbreaks and epidemics resulting in varying morbidity, ranging from asymptomatic infections to death especially among pregnant women; and

☆ This work was partially presented at the 13th International Symposium on Viral Hepatitis and Liver Disease, 20–24 March 2009, Washington, DC, USA.

* Corresponding author. Present address: Viral Immunology Laboratory, MSB 6466, Department of Internal Medicine, Division of Digestive Diseases, 231 Albert B. Sabin Way, University of Cincinnati Medical Center, Cincinnati, OH 45267-0595, USA. Tel.: +1 513 558 6110; fax: +1 513 558 1744.
E-mail address: mohamed.shata@uc.edu (M.T. Shata).

0035-9203/$ – see front matter © 2012 Royal Society of Tropical Medicine and Hygiene. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.trstmh.2012.07.004

few sporadic cases of HEV-caused AVH along with many asymptomatic cases that become seroreactive.[1,2] The reasons for the difference in clinical presentation are unclear. However, differences in dose of infection, virulence of viral strains including among HEV genotypes,[2] host responses including those associated with previous exposures to the virus, and the immunological profile of the susceptible population[3,4] could influence HEV clinical presentation. For example, in the Indian subcontinent, HEV hepatitis outbreaks frequently occur, accounting for 30–60% of sporadic hepatitis, and have been characterised by high morbidity especially among infected pregnant women.[5,6] In Egypt, despite a high prevalence of antibodies to HEV (anti-HEV),[7,8] no HEV hepatitis outbreaks have been reported. The profile of anti-HEV seroprevalence in Egypt is typical for a food borne disease. It is not common in infants and young children, but is as high as 60% in those aged 5–10 years and reaches a plateau of 80–90% in adults.[7] The incidence of asymptomatic HEV seroconversion has been reported as 42/1000 person-years among Egyptians.[9] Nevertheless, the incidence of symptomatic HEV-caused AVH was only 3/100 000 person-years,[10] with HEV infection reported to account for 12–42% of all cases of AVH.[11] The reasons for the discrepancy between the incidences of HEV exposure/infection and clinical illness/AVH in Egypt are largely unknown and are the subject of this study. We recently demonstrated that early cell-mediated immunity following infection may reduce HEV morbidity.[4] Here we report the first description of an outbreak of HEV in Egypt and examine the role of avidity and neutralising capacity of the anti-HEV in determining whether the infected subjects had AVH or were asymptomatic.

2. Methods

2.1. Study participants and informed consent

In total, 235 acute hepatitis patients from the Assiut Fever Hospital and Assiut University Hospital (Assiut, Egypt) were prospectively enrolled from March 2007 to August 2008. Criteria for enrolment in the study included fever, yellow sclera and/or jaundice, dark urine, pale stool, a tender liver for <2 weeks and an alanine aminotransferase (ALT) level that was ≥2.5 times the upper limit of normal (100 IU/ml). All patients with known pre-existing chronic liver disease or suspected liver damage caused by non-viral causes, e.g. ingestion of hepatotoxic drugs or alcohol, were excluded. Patients were serologically screened for HAV, hepatitis B virus (HBV) and hepatitis C virus (HCV) using commercially available tests. Patients without serological evidence of HAV, HBV or HCV infection were tested for HEV infection. Forty-two AVH cases meeting the criteria for HEV-caused AVH were identified. Fourteen cases were from a single village (Kom El-Mansoura) in Assiut Governorate. Another 200 inhabitants of Kom El-Mansoura village who were family members or lived near the HEV-caused AVH cases at the same time period (within 1–4 months) were invited and agreed to participate in the study. They were all screened for anti-HEV IgM.

The Ethics Committee of the Egyptian Ministry of Health's National Hepatology and Tropical Medicine Research Institute, the Local Ethics Committee at Assiut University and the University of Cincinnati Institutional Review Board approved the study protocol. All eligible patients signed an informed consent/assent form.

2.2. Clinical evaluation

Demographic characteristics and clinical examination findings were recorded using standardised forms with inquiries about past history of jaundice, hepatotoxic drug use and alcohol ingestion. All subjects completed a detailed questionnaire regarding potential exposure risks including food, water, animal and blood exposures as well as undergoing clinical examinations.

2.3. Sample collections and laboratory testing

Stool and 15–20 ml blood samples were collected from all subjects. Immunological tests were performed by the research team in the EgyBlood immunology laboratory on the Holding Company for Biological Products and Vaccine (VACSERA) campus at Giza, Egypt and/or at the Digestive Diseases Division's Viral Immunology Laboratory at the University of Cincinnati (Cincinnati, Ohio, USA). Liver function tests including ALT, aspartate aminotransferase (AST), total and direct bilirubin, and alkaline phosphatase were performed at local clinical laboratories using standard techniques.

2.4. Antigens

2.4.1. HEV open reading frame 2 (ORF2) protein

Recombinant ORF2 protein covering amino acids 452–617 (neutralising region of ORF2) was purchased from GenWay Biotech (San Diego, California, USA) and was used in the neutralising ELISA assay as described below.

2.4.2. Viral hepatitis serological tests and diagnostic criteria

Sera from 235 patients who met the clinical and ALT criteria for acute hepatitis were screened using rapid tests for anti-HAV IgM (CTK Biotech Inc., San Diego, California, USA), anti-HBV core IgM (anti-HBc IgM) (IND Diagnostic, Delta, BC, Canada) and anti-HCV IgG (ClinPro International, Union City, California, USA). For HEV testing, HEV IgM (Adaltis, Milano, Italy) and IgG ELISA (MP Diagnostics, formerly Genelabs Diagnostics, Singapore Science Park, Singapore) were used as specified by the manufacturers.

Acute HAV and HBV infections were diagnosed if serum samples were positive for anti-HAV IgM or anti-HBc IgM (irrespective of hepatitis B surface antigen status), respectively, and were excluded from the study. Diagnosis of acute HCV infection was made if there was seroconversion from anti-HCV-negative to anti-HCV-positive in acute and convalescent samples, irrespective of HCV-RNA. Samples that had initial test results positive for anti-HCV and HCV-RNA with no other viral markers were considered to be chronic HCV infections and were excluded from the study. A patient was considered to be positive for acute HEV infection if anti-HEV IgM antibodies were positive or if they had a >3-fold rising titre of anti-HEV IgG antibodies