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Original article

Hepatitis E virus seroprevalence among schistosomiasis patients in Northeastern Brazil

Ana Maria Passos-Castilho[a], *Anne de Sena*[a,b], *Ana Lucia Coutinho Domingues*[d], *Edmundo Pessoa Lopes-Neto*[d], *Tibério Batista Medeiros*[d], *Celso Francisco Hernandez Granato*[a,b], *Maria Lúcia Ferraz*[c,*]

[a] *Division of Infectious Diseases, Universidade Federal de São Paulo (Unifesp), São Paulo, SP, Brazil*
[b] *Fleury SA Group, Brazil*
[c] *Division of Gastroenterology, Universidade Federal de São Paulo (Unifesp), São Paulo, Brazil*
[d] *Division of Gastroenterology, Universidade Federal de Pernambuco (UFPE), Recife, PE, Brazil*

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ABSTRACT

Background: Hepatitis E virus (HEV) can cause chronic infection with rapid progression to liver cirrhosis in immunocompromised patients. HEV seroprevalence in patients with *Schistosoma mansoni* in Brazil is unknown. We evaluated the prevalence of past or present HEV infection in schistosomiasis patients in Recife, Pernambuco, Brazil. A total of 80 patients with *Schistosoma mansoni* were consecutively enrolled in a cross-sectional study. Serum samples were tested for the presence of anti-HEV IgG antibodies by enzyme immunoassay (Wantai anti-HEV IgG, Beijing, China) and for the presence of HEV RNA using real time reverse transcriptase-polymerase chain reaction with primers targeting the HEV ORF2 and ORF3. Clinical and laboratory tests as well as abdominal ultrasound were performed at the same day of blood collection.

Results: Anti-HEV IgG was positive in 18.8% (15/80) of patients with SM. None of the samples tested positive for anti-HEV IgM or HEV-RNA. Patients with anti-HEV IgG positive presented higher levels of alanine aminotranferase ($p = 0.048$) and gama-glutamil transferase ($p = 0.022$) when compared to patients without anti-HEV IgG antibodies.

Conclusion: This study demonstrates that the seroprevalence of HEV is high in patients with *Schistosoma mansoni* in Northeastern of Brazil. Past HEV infection is associated with higher frequency of liver enzymes abnormalities. HEV infection and its role on the severity of liver disease should be further investigated among patients with *Schistosoma mansoni*.

* *Corresponding author.*
E-mail address: marialucia.ferraz@uol.com.br (M.L. Ferraz).
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Introduction

Hepatitis E virus (HEV) presents as large epidemics and sporadic cases in endemic areas, including genotype 1 in Asia and Africa, genotype 2 in Mexico and Africa, and genotype 4 in Asia. Sporadic cases of genotype 3 occur in Europe, Japan and the Americas. Genotypes 1 and 2 are restricted to primates and are transmitted predominantly by the fecal-oral route. Genotypes 3 and 4 infect numerous mammalian species and can be transmitted through the ingestion of raw or undercooked meat from infected animals.[1]

HEV infection usually presents as an acute self-limiting hepatitis, but in immunocompromised patients it can cause chronic infection with rapid progression to liver cirrhosis.[2] Brazil has been classified as moderately endemic for HEV, with seroprevalence ranging from 1% to 4% in the general population and 15% in renal transplant recipients.[3,4]

Schistosoma mansoni (SM) is endemic in up to eight countries and islands in Latin America and the Caribbean, including Brazil. Up to 6 million people are infected, most of whom live in Northeastern Brazil and Venezuela, and 25 million are at risk for the infection.[5] Schistosomiasis mansoni may progress to the most advanced form of disease, which is commonly observed in endemic areas. When this hepatosplenic form occurs in association with other hepatic disease, such as viral hepatitis, hepatic fibrosis can progress into cirrhosis within a few years.[6]

The seroprevalence of HEV in patients with SM in Brazil is unknown. The aim of this study was to evaluate the prevalence of past or present HEV infection in a sample of schistosomiasis patients in Recife, an endemic region of Northeastern, Brazil, and to associate the positivity to HEV infection to clinical and laboratory abnormalities.

Patients and methods

Study area

Recife is the seventh largest metropolitan area in Brazil with approximately 3.9 million inhabitants, the second largest metropolitan area of the Northern/Northeastern Regions, and the capital and largest city of the state of Pernambuco.[7] In addition, the hospital where the study was carried out is a reference center for patients with the most severe forms of schistosomiasis mansoni and receives patients from both Recife metropolitan region as well as from the endemic zone of Pernambuco State.[8]

Study design

A cross-sectional study was carried out involving patients with SM who consecutively underwent an ultrasound exam over a nine-month period at the Division of Gastroenterology, of the Federal University of Pernambuco, Brazil. The diagnosis of SM was based on their clinical history of contact with contaminated water, positive parasitological stool examination for SM and/or reports of prior treatment for this parasite. In addition, to be included patients ought to have periportal fibrosis (PPF) on ultrasound evaluation of the liver.

Male and female patients aged 14 years or older with SM diagnosis were included. Those with the following criteria were excluded: presence of markers for hepatitis B or C (anti-HBc and anti-HCV); alcohol intake >210 g/week; and ultrasound evidence of other liver disease, as expressed by the presence of steatosis or fine fibrosis diffused throughout the parenchyma.

During the study period 122 patients were evaluated; however, 42 patients (32.7%) were excluded due to co-infection with HBV and/or HCV, alcohol abuse, or other liver diseases as shown on liver ultrasound. Therefore, 80 patients were included in this study.

Ultrasound exams were performed by a single operator (ALCD) using the Siemens Acuson X 150® device with a 3.5 MHz convex transducer for the evaluation of periportal fibrosis based on the Niamey classification, which has six pre-established patterns of fibrosis (PPF) intensity, ranging from Pattern A (normal) to Pattern F (very advanced fibrosis).[9]

According to the pattern of PPF by ultrasound, patients were divided into three groups: (1) mild group PPF: A + B; (2) moderate PPF: C + D; and (3) advanced PPF E + F.

Sample and data collection

Serum samples from these patients were collected for laboratory analysis. These included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma-glutamyl transpeptidase (GGT), total protein and albumin, total bilirubin, hemoglobin, leukocyte, and platelet count.

Normal levels of liver enzymes were ALT $\leq$ 31 UI/L; AST $\leq$ 31 UI/L, AP $\leq$ 105 UI/L; and GGT $\leq$ 41 UI/L.

Anti-HEV antibodies detection

The presence of anti-HEV IgG antibodies was investigated through enzyme immunoassay using the WANTAI HEV-IgG ELISA kit (Beijing Wantai Biological Pharmacy Enterprise, Beijing, China), strictly according to the manufacturer's recommendations. Specimens with positive results were tested for anti-HEV IgM antibodies using a specific kit from the same manufacturer.

RNA extraction and quantitative RT-PCR

HEV RNA was extracted from fecal samples using QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany), strictly according to the manufacturer's instructions.

Quantitative RT-PCR was performed according to a modified 1-step triplex real time protocol previously described[10] with a set of primers and probe targeting a highly conserved 70 nt long sequence within overlapping parts of ORF2 and ORF3,[11] and another set specific for a 113 nt sequence of ORF2.[12] A third set of primers and probe targeting the human RNAseP gene was used as endogenous internal amplification control to certify specimen quality and RNA extraction.[13]

A plasmid clone from a Brazilian human HEV strain previously characterized (GenBank accession number KF1528840)[14]