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High prevalence of hepatitis E virus antibodies in Sao Paulo, Southeastern Brazil: analysis of a group of blood donors representative of the general population

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ABSTRACT

Brazil is a non-endemic country for hepatitis E virus (HEV) infection with seroprevalence from 1% to 4% in blood donors and the general population. However, data on seroprevalence of HEV in the country are still limited. This study evaluated the prevalence of past or present HEV infection in a group of blood donors representative of the general population of the city of Sao Paulo, Southeastern Brazil. Serum samples from 500 blood donors were tested from July to September 2014 by serological and molecular methods. Anti-HEV IgG antibodies were detected in 49 (9.8%) subjects and categorized age groups revealed an age-dependent increase of HEV seroprevalence. Among the anti-HEV IgG positive subjects, only 1 had anti-HEV IgM while none tested positive for HEV-RNA. The present data demonstrate a higher seroprevalence of anti-HEV IgG than previously reported in the region.

Introduction

Hepatitis E virus (HEV) infection is among the most frequent causes of acute hepatitis worldwide. It is estimated that 2.3 billion people have already been infected with HEV with 70,000 deaths attributed to this virus every year.[1,2]

Hepatitis E 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 worldwide. HEV detected from Argentina and Brazil are more related to viruses from industrialized countries (North America and Europe), whereas the HEV in the Caribbean and Mexico include viral

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genotypes more closely related to outbreaks in Africa and Asia.[3,4]

The total prevalence of antibodies against HEV in endemic countries is variable (3%–27%).[5] In non-endemic areas with proper sanitary conditions and a well-controlled water supply, the prevalence of antibodies against HEV in the general population is relatively high (up to 7%–10%).[5]

Data on HEV seroprevalence in Brazil are still scarce. HEV is not routinely investigated in the country, even in cases of unexplained liver enzyme elevation or acute hepatitis, and only a few laboratories perform anti-HEV tests. Brazil is a non-endemic country for HEV, with rare cases confirmed by molecular methods. HEV seroprevalence in the country ranges from 1% to 4% in blood donors or the general population, 13% in individuals from an agricultural settlement in the Amazon Basin and 15% in renal transplant recipients.[6–9] Nonetheless, most available studies are limited, outdated and cannot be compared properly because of their small sample sizes and/or diverse methodology. Therefore, the occurrence and characteristics of hepatitis E in Brazil are poorly understood.

The aim of this study was to assess the prevalence of hepatitis E in Sao Paulo, Southeastern Brazil, through the analysis of anti-HEV antibodies in a group of blood donors representative of the general population.

Patients and methods

Study area

Sao Paulo, the most populous city in Brazil, is also considered the most multicultural city in the country, with approximately 12 million inhabitants and immigrants from all parts of the world.[10] The city is divided in eight zones, with distinct socioeconomic characteristics.

Study design

A sampling plan accounting for demographic zone, gender and age group was created to gather a sample representative of the general adult population in the city.

A prospective, cross-sectional study was carried out involving 500 blood donors who consecutively underwent blood donation from July to September 2014 in the Beneficent Association for Blood Collection (COLSAN) in Sao Paulo, Southeastern Brazil, strictly following the sampling plan.

This sample was calculated to be sufficient to successfully estimate HEV seroprevalence of approximately 10% in the city population at a 95% confidence interval (CI).

Sample and data collection

Five hundred milliliter aliquots of the serum samples routinely collected for serological testing were separated and stored at –80 °C until laboratory analysis. Data regarding date of birth and gender were collected during enrollment.

Anti-HEV IgG and IgM 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 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 the serum 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[11] with a set of primers and probe targeting a highly conserved 70 nt long sequence within overlapping parts of ORF2 and ORF3[12] and a set specific for a 113 nt sequence of ORF2.[13] 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.[14]

A plasmid clone from a Brazilian human HEV strain previously characterized (GenBank accession number KF152884)[15] was constructed with TOPO® TA cloning® kit (Invitrogen, Carlsbad, CA, USA) and the primers described. Plasmid DNA was purified using QIAprep spin miniprep kit (QIAgen, Hilden, Germany), linearized and quantified with the Nanodrop ND-1000 instrument (Wilmington, DE, USA) following transcription to RNA with T7 RNA polymerase (Promega, Madison, WI, USA). Standard curves were generated using 10^0 to 10^{10} copies of plasmid RNA. HEV viral loads were determined based on the standard curves. The limit of detection of the real-time RT-PCR was 5 copies of RNA per reaction, while the limit of quantification was set at 50 copies of RNA per reaction. All screening reactions were run in duplicates using with proper controls.

Statistical analysis

All data were entered and analyzed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics consisted of the characterization of the studied population and anti-HEV IgG seroprevalence through the respective percentages and 95% confidence intervals (CI) or mean/median values and standard deviation (SD) for continuous variables. The bivariate analysis consisted of Pearson's Chi-square test to compare categorical values. Non-conditional logistic regression was used to identify associations between dependent and independent variable reported as odds ratio (OR). Mann–Whitney *U* test was used to compare means of non-normally distributed variables. Linear regression analysis was used to evaluate the trends of anti-HEV positivity with respect to age group. Statistical significance level was $p < 0.05$. All reported values are two-tailed.