Seroepidemiology and Molecular Characterization of Hepatitis E Virus in Jilin, China


Abstract

Background: In order to develop region-specific diagnostic assays and prevent hepatitis E virus (HEV), it is essential to understand epidemiology and genotypic variation within different populations. However, the epidemiological data of HEV infections in Jilin is shortage.

Methods: The seroepidemiological study was conducted by enzyme-linked immunosorbent assay, and the conserved genomic sequences of open reading frame 2 (348 bp) was detected using reverse transcription-PCR.

Results: Overall, 1,289 serum samples were positive to HEV-specific immunoglobulin G (IgG), and 180 serum samples were positive to HEV-specific immunoglobulin M (IgM). The seroprevalence of HEV-specific IgG was 26.3%, 27.9%, 25.3%, 32.7% in 2003, 2004, 2005, 2006, respectively. Acute HEV infection mainly occurred in male, people aged from 20 to 69 were more susceptible to infection, and cases with IgM anti-HEV reaction mainly occurred from July to November. HEV RNA was detectable in the serum samples or stool suspension of 15 patients with HEV-specific IgM, and all of these belonged to genotype IV.

Conclusions: Our results indicate that HEV is widely spread in Jilin and confined to genotype IV.

Introduction

Hepatitis E virus (HEV), a member of the genus Hepevirus, is a non-enveloped virus with a positive sense, single-stranded RNA genome approximately 7.2 kb in length [1]. Although a single serotype has been proposed, based on sequence analyses, HEV isolates identified worldwide have been classified into four genotypes. These include genotypes I (several countries from Asia and Africa), II (Mexico, Nigeria), III (US, Japan, Argentina, and Europe), and IV (Taiwan, Japan, and China) [2–5]. Areas in which HEV is endemic are often located in developing nations where people live without an adequate water supply [6, 7]. HEV infections also occur in some individuals living in industrialized countries who have no history of travel to areas of endemcity [8]. Hepatitis E (HE) caused by HEV previously is typically characterized as a self-limiting acute hepatitis with low mortality. However, the infections were the high mortality seen among pregnant women with HEV infection, especially those in the third trimester [9, 10]. Moreover, natural antibodies or RNA of HEV have been detected in numerous animal species (swine, sheep, goat, cattle, horse, donkey, deer, cat, rodent, and mongooses), suggested the possibility of zoonotic spread of the virus [11–17]. In addition, several cases of acute HE have been epidemiologically linked to eating undercooked pork liver or wild boar meat [18–20]. These cases provide convincing evidence of zoonotic food-borne HEV transmission.

About 10–20% of people in China have signs of past HEV infection [21]. A prolonged outbreak of HEV in Xinjiang Autonomous Region between July 1986 and April 1998 infected approximately 120,000 individuals and killed 707 [22]. There are two main genotypes in China, I and IV. However, genotype III HEV has been identified on the Chinese mainland in 2006 [23]. According to result of Li et al., in the 98 HEV strains discovered in patients from 18 cities in China, 63.3% were genotype I and 36.7% genotype IV [24]. A recent study showed that genotype IV HEV freely circulates among swine and humans, the
risk for human HEV infection was associated with occupational contact with swine and swine sewage [25].

The epidemiological data about HEV in Jilin province (in northeastern China) is shortage. Furthermore, the HEV genotypes in Jilin have not been previously characterized. Thus, we conducted a survey to ascertain the present state of HEV epidemiology in Jilin, analyzed the partially conserved nucleotide sequences of HEV strains isolated from patients with acute sporadic HE, and discussed the epidemiological features of the disease.

Materials and Methods

Serum Samples

We conducted a seroepidemiologic study of HEV infection from 2003 to 2006 in conjunction with annual health examinations among residents in Jilin province in northeastern China. These exams involved routine clinical examination, routine biochemical testing. In the study, we collected 4,654 serum samples randomly from individuals in seven different areas, including 1,631 from Changchun (ages 10–69), 584 from Siping (ages 11–86), 477 from Baishan (ages 11–83), 463 from Yanji (ages 18–73), 415 from Baishan (ages 14–81), 566 from Tonghua (ages 18–77), and 518 from Meithe (ages 12–90). There were 180 acute HE patients in this study. The serum samples were stored at –70 °C prior to use. All individuals were enrolled in the study after providing informed consent; parental consent was obtained for individuals <18 years of age. We also recorded clinical data including age, gender, month of infection, and symptom, such as jaundice, fatigue, anorexia, and abdominal discomfort. The distribution of the seven study areas in Jilin province is showed in figure 1.

Detection of HEV-specific Antibodies

Serum samples were tested for HEV-specific IgG and IgM by using commercial ELISA kit (Wan Tai Pharmaceutical Co., Beijing, China), produced with two recombinant peptide corresponding to amino-acid residues 396–606 of the major structural protein specified by open reading frame 2 (ORF2) of the HEV genome [26]. The sensitivity and specificity of the ELISA kits had been identified by Jiang and Zhang et al. [26–28]. Serum samples were tested according to manufacturer’s instructions, with three negative and two positive control wells included on each plate. The samples with optical density less than the cutoff value (Cutoff value of IgG was equal to the mean optical density for the three negative controls on each plate plus 0.12, and cutoff value of IgM was equal to the mean optical density for the three negative controls on each plate plus 0.26) were considered negative. Samples with optical density greater than or equal to the cutoff value were tentatively considered reactive and then retested in duplicate to confirm the result.

Statistical Analysis

Statistical analysis was performed with SPSS (version 11) statistical software (Scientific Package for Social Sciences, Chicago, IL, USA). Descriptive statistics were reported. The chi-square test were used to compare the proportions between groups. A p-value < 0.05 was considered statistically significant.

RNA Extraction and Reverse Transcription-nested PCR

Total RNA was extracted from 100 μl of serum samples or stool suspension, negative and positive controls with TRIZOL reagent (Invitrogen, Carlsbad, CA, USA), reverse transcribed into cDNA with 10 units of AMV Reverse Transcriptase (Promega, Madison, WI, USA) at 42 °C for 60 min. The primers used in this study were synthesized as previously described [29]. The primer positions indicated below are relative to the HEV strain “JYI-Chisi01C” (GenBank accession AB197674): P1, 5’-AAC(T)TATGCAACGTACCGGGTTG-3’ (forward, position 5725–5746); P2, 5’-CCCTTATCTCTGAGCATTTCTC-3’ (reverse, position 6433–6455); P3, 5’-GTC(T)ATGC(T)TC(T)TGACATACTGGCT-3’ (forward, position 6010–6031); P4, 5’-AGCCGAGCAAATCTAGATTCTGTC-3’ (reverse, position 6336–6357) (Table 1). The first rounds PCR was carried out in a 50 μl reaction volume with 8 μl cDNA, 25 pmol of each primer (P1, P2), 1 μl of 10 mM dNTPs, and 0.5 μl Takara Ex Taq polymerase (Takara biotechnology Co., Ltd., Dalian, China). PCR was conducted for 35 cycles of 94 °C for 1 min, 55 °C for 45 s, and 72 °C for 1 min, with a final extension time of 72 °C for 7 min. Second-round reactions were carried out in a 50 μl volume with 8 μl of the first-round product, 25 pmol of each primer (P3, P4), and 0.5 μl of Takara Ex Taq polymerase. The parameters were the same as the first round, except that only 30 cycles of amplification were used.

Sequence and Phylogenetic Analysis

The second-round PCR products were separated by 1% agarose gel electrophoresis and purified using the QIAquick gel extraction kit (Qiagen, Chatsworth, CA, USA). The purified PCR products were ligated into pMD18-T vectors (Takara Biotech-