Serological and molecular study of hepatitis E virus among illegal blood donors

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Abstract

AIM: To investigate the seroprevalence and molecular characteristics of hepatitis E virus (HEV) in the illegal blood donors (IBDs) of central China in the early 1990s.

METHODS: A total of 546 blood samples were collected from the IBDs in Maanshan city, a questionnaire was completed by each subject, detailing the age, sex, and periods of blood or plasma donation. Anhui Province and tested for the anti-HEV antibodies. The seropositive samples were subjected to nested reverse transcription-polymerase chain reaction and sequencing to analyze HEV partial genome.

RESULTS: The prevalence of IgG and IgM HEV antibody in IBDs was 22.7% and 1.8%, and genotype 4 was the dominant circulating HEV type in IBDs. The prevalence of anti-HEV IgG was significantly related to sex (OR = 4.905, P = 0.004) and increased with age (OR = 2.78, P = 0.022), which ranged from 13.0% in those < 40 years old to 30.6% among older persons aged > 60 years. Moreover, frequency of blood donation was significantly associated with HEV seropositivity (OR = 2.06, P = 0.006). HEV partial sequences of ORF2 and obtained 3 sequences in serum samples of 10 IBDs which developed HEV specific IgM.

CONCLUSION: This study helps define one of the possible routes of transmission of sporadic HEV infection and provides guidance to screen HEV in the blood donors so as to guarantee safe blood banks in China.

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Key words: Molecular; Sero-epidemiology; Hepatitis E; Hepatitis E virus; Commercial blood donors

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INTRODUCTION
Hepatitis E virus (HEV) infection is an important public-health concern as a major cause of enterically transmitted hepatitis worldwide. Epidemiological studies have shown that HEV is prevalent in most developing countries, such as southeast Asia, northern and central Africa, India and central America. In addition, a high incidence of sporadic HEV infection has been observed in several industrialized countries, including the United States, European countries and Japan. Although the ingestion of contaminated drinking water contributes mainly to the spread of HEV, other routes of transmission should be considered, because some studies implicated that blood transfusion was the possible route of sporadic HEV infection in non-endemic developed countries.

Between 1992 and 1995, illegal blood donation (IBDs) occurred frequently in several provinces in central China, including Henan, Anhui and Shanxi provinces. Although commercial blood donation was eradicated by the Chinese government by the end of 1995, the practice of using contaminated blood collection equipment caused the spread of some viruses, such as hepatitis C virus (HCV) and human immunodeficiency virus (HIV).

While many studies have reported the prevalence of HEV infection and the HEV genome characteristics in different groups in China, to date, there has been no report on the prevalence of HEV infection among the IBDs. The aim of this study was to investigate whether HEV can be transmitted by the blood transfusion route and analyze the partially conserved nucleotide sequences of HEV strains among the IBDs in Maanshan city in Anhui Province, one of the provinces with the illegal blood collection in the early 1990s.

MATERIALS AND METHODS

Ethics
This work has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The study was initiated after the study protocol was approved by the Institutional Review Board (IRB) of China Center for AIDS/STD Control and Prevention and the IRB of Maanshan Center of Disease Control and Prevention.

Study population
A total of 546 samples were collected between January and August in 2005 from those who donated their blood or plasma frequently from 1992 to 1995. All participants were from Dangtu District in Maanshan city. A questionnaire was completed by each subject, detailing the age, sex, and periods of blood or plasma donation.

Detection of antibodies against hepatitis E virus
Serum samples were tested by enzyme-like immuno-sorbent assay (ELISA) for IgG and IgM with anti-HEV activity described previously. All serum samples were assayed at a 1:20 dilution. The absorbance of each sample was read at 450 nm. The cutoff value used for the anti-HEV IgG and IgM assay was 0.152. Then the serum positive for IgM antibody against HEV were tested for HEV RNA. All participants were previously tested for HBsAg (Diasorin, United States) and anti-HCV (third generation assay, Diasorin, United States) by ELISA.

Extraction of RNA and reverse transcription-polymerase nested chain reaction
Viral RNA was extracted from serum samples using Qia-gen viral RNA kit (Qiagen) according to the manufacturer's instructions. The viral RNA was finally dissolved in 20 μL RNase-free water. Reverse transcription-polymerase chain reaction (RT-PCR) was performed using TaKaRa RNA PCR kit (TaKaRa, Japan). The primers and the PCR protocol used were adapted from a previous study.[13] The external primers were P1 (5’-CCGAGAATTTGAATTCGCCGACATGGCGAGCC-3’) and P4 (5’-CGTAGGCGGGGATCTCTACGCGTACTC-3’). The internal primers were P2 (5’-GTTGTCTCGGGCAATGGCGAGCCGACC-3’) and P3 (5’-TGGGCGGGGCCGTTGAGAGAGCCAGGCA-3’). The first-round and the second-round amplifications were carried out according to the following cycling program denaturation at 9 °C for 45 s, annealing at 52 °C for 60 s, extension at 72 °C for 60 s, for 35 cycles. The size of the first-round PCR product was 307 bp, and that of the second-round PCR was 236 bp.

Sequencing and analysis of sequences
The PCR products were visualized on a 10 mL/L agarose gel, excised and purified using a gel extraction kit (Qiagen). The sequences were aligned using ClustalX v1.8 ([http://www.ncbi.nlm.nih.gov/GenBank/ClustalX/top.html](http://www.ncbi.nlm.nih.gov/GenBank/ClustalX/top.html)), and the phylogenetic analysis was performed using the MEGA program, version 3.1 (Pennsylvania State University).

Statistical analysis
The Pearson χ² test was used to evaluate the difference in the prevalence between groups in the univariate analyses. Odds ratios (OR) with 95% confidence intervals were used to determine whether the prevalence of HEV infection was associated.

RESULTS

Hepatitis E virus seropositivity in illegal blood donors
A total of 546 IBDs in the 1990s were enrolled to this study. The IBDs were aged from 29 to 75 years with a mean of 51 ± 9 years. Among them, 156 (28.6%) were males and 390 (71.4%) were females. While 124 IBDs developed HEV IgG antibody, only 10 IBDs developed HEV IgM antibody; therefore, the prevalence of IgG and
IgM HEV antibody in this group was 22.7% and 1.8%, respectively.

The prevalence of HEV IgG seropositivity in the 546 IBDs is shown in Table 1. In the male group, 30.8% (48/156) were positive as against 19.5% (76/390) in the female group. The prevalence of HEV IgG seropositivity was significantly higher in men than in women (OR = 4.905, \( P = 0.004 \)). In addition, subjects over 60 years of age had a higher prevalence of HEV IgG seropositivity than those aged < 40 years (OR = 2.780, \( P = 0.022 \)). The frequency of plasma donation was also associated with HEV infection. The odds ratio was 2.06 among those who donated more than 20 times compared with those who donated 10 or fewer times.

The sex specific prevalence of HEV IgG in IBDs with different frequency of donation is shown in Table 2. HEV infection prevalence was significantly correlated with the increasing age in total participants (\( \chi^2 = 2.91, P = 0.048 \)) and female participants (\( \chi^2 = 1.97, P = 0.048 \)).

We also examined the relationship between HEV infection and HBV or HCV coinfection in these IBDs. The results showed no significant difference in HBsAg positive status (3.2% vs. 3.1%) and HCV positive status (11.3% vs. 11.1%) between HEV IgG positive and negative IBDs (Table 3).

### Phylogenetic analysis of hepatitis E virus strains

We detected HEV partial sequences of ORF2 and obtained 3 sequences in serum samples of 10 IBDs which developed HEV specific IgM. Sequence analysis demonstrated that these 3 strains were 83.3%-93.6% identical to each other. When compared with the HEV reference isolates, the strains were closely related to Chinese strain T1 with an 82.6%-89.4% nucleotide homology, and demonstrated a 91.1% sequence homology to a Japanese strain JAK-Sai. The nucleotide homology of other Japanese HEV strains with the strains from IBDs ranged from 4.905-91.1%.

In the phylogenetic tree generated, the Bur82 strain (genotype 1), the Mexican strain (genotype 2), the US1/swine strain (genotype 3), and the Chinese strain T1 (genotype 4) represent major branches. Phylogenetic analyses clearly illustrate that all HEV sequences except avian strain can be divided into these four distinct genotypes and the three HEV sequences isolated in our study were genotype 4. The IBDs 1 sequence we analyzed formed an exclusive cluster, and was bound to a new subgenotype within genotype 4, which was supported by the bootstrap values obtained from 1000 replicates resampling analysis (Figure 1).

### DISCUSSION

HCV and HIV infections were prevalent among the IBDs who donated blood in the early 1990s in China. However, data on HEV infection in this population have been unavailable so far. To our knowledge, the present study is the first seroepidemiological and molecular study on HEV infection in this unique population. Our results demonstrated that HEV infection had been introduced into this population in this area and that the prevalence was much higher (22.5%) than that in the normal population (4.76%) in this area.

Our data indicated that 30.7% of males were HEV positive compared to 19.5% of females and the difference was statistically significant (OR = 1.84, \( P = 0.004 \)). In addition, we found that males with a history of blood transfusion had a high HEV seropositivity than females, suggesting that male IBDs are more likely to get infected by HEV than female donors. Our study also showed that HEV seropositivity increased with age of the first donating blood, consistent with previous studies demonstrating that age may be an important risk associated with HEV infection in this population.

Although no statistically significant association was observed between HEV seropositivity and blood-borne hep-

| Frequency | Hepatitis E virus positive IgG | \( P \) value |
|-----------|-------------------------------|---------------|
| <10       | 15/54 (27.8)                  | 0.052         |
| 10-20     | 18/57 (31.5)                  | 0.097         |
| ≥ 20      | 15/45 (33.3)                  | 0.648         |

**Table 3 Relationship between hepatitis E virus infection and hepatitis B virus or hepatitis C virus coinfection in commercial blood donors**

| Characteristics | HEV positive IgG \((n = 124)\) | HEV negative IgG \((n = 422)\) |
|-----------------|-------------------------------|-------------------------------|
| HBsAg positive  | 14 (11.3)                     | 47 (11.1)                     |
| HCV antibody positive | 13 (3.1)                  | 0.935                     |

**HEV: Hepatitis E virus.**
Hepatitis E virus (HEV) infection is an important public-health concern as a major cause of enterically transmitted hepatitis worldwide. Epidemiologic studies have shown that HEV is prevalent in most developing countries and some industrialized countries. Although commercial blood donation was eradicated by Chinese government by the end of 1995, the practice of using contaminated blood collection equipment caused the spread of some viruses such as hepatitis C virus and hepatitis A virus, but there has been no report on the prevalence of HEV infection in the illegal blood donors (IBDs).

**Innovations and breakthroughs**

This is a first serological and molecular study on HEV infection in IBDs. The results showed that the prevalence of HEV IgG antibody was higher (22.5%) in the IBDs than in general population, and the risks of HEV infection in IBDs were multi-fold. This is a first serological and molecular study on HEV infection in IBDs. The results showed that the prevalence of HEV IgG antibody was higher (22.5%) in the IBDs than in general population, and the risks of HEV infection in IBDs were multi-fold. The study also indicated that HEV is widely spread in the IBDs and the new possible modes of transmission of sporadic HEV infection in this population and provide guidance to screen HEV in the donors to guarantee safe blood banks in China.

**Applications**

As it was indicated in this study that HEV is wildly spread among the IBDs, and age, gender and times of blood donation are the risk factors of HEV infection. Therefore, HEV should be detected regularly among the IBDs for the safety of transfusion.

**Terminology**

Hepatitis E virus (HEV); Hepatitis E Virus 1 has a particle diameter of 32-34 nm, a buoyant density of 1.29 g/mL in KTr/Gly gradient, and is very labile. Serologically related smaller (27-30 nm) particles are often found in feces of patients with hepatitis E and are presumed to represent degraded viral particles. HEV has a single-stranded polyadenylated RNA genome of approximately 8 kb. Based on its physicochemical properties, it is presumed to be a calicivirus.

**Comments**

Hepatitis E virus (HEV) infection is an important public-health concern as a major cause of enterically transmitted hepatitis worldwide. Epidemiologic studies have shown that HEV is prevalent in most developing countries and some industrialized countries. Although commercial blood donation was eradicated by Chinese government by the end of 1995, the practice of using contaminated blood collection equipment caused the spread of some viruses such as hepatitis C virus and hepatitis A virus, but there has been no report on the prevalence of HEV infection in the illegal blood donors (IBDs).

**Innovations and breakthroughs**

This is a first serological and molecular study on HEV infection in IBDs. The results showed that the prevalence of HEV IgG antibody was higher (22.5%) in the IBDs than in general population, and the risks of HEV infection in IBDs were age, gender and times of donation. Additionally, phylogenetic analysis showed that 3 HEV strains isolated from IBDs belong to genotype 4. Therefore, the present study indicated that HEV is widely spread in the IBDs and the new possible modes of transmission of sporadic HEV infection in IBDs should be defined.
screening of HEV in the donors to guarantee safe blood banks. The manuscript is well presented and of interest and the design of this study is appropriate. The study was done well and their results can contribute to knowledge of this topic.

REFERENCES

1 Emerson SU, Purcell RH. Running like water—the omnipresence of hepatitis E. N Engl J Med 2004; 351: 2367-2368
2 Ijaz S, Arnold E, Banks M, Bendall RP, Cramp ME, Cunn
3 ningham R, Dalton HR, Harrison TJ, Hill SF, Macfarlane L, Meigh RE, Shafi S, Sheppard MJ, Smithson J, Wilson MP, Teo CG. Non-travel-associated hepatitis E in England and Wales: demographic, clinical, and molecular epidemiological characteristics. J Infect Dis 2005; 192: 1166-1172
4 Mansuy JM, Peron JM, Abravanel F, Poisson H, Dubois M, Miedouge M, Vischi F, Alric L, Vinel JP, Izopet J. Hepatitis E in the south west of France in individuals who have never visited an endemic area. J Med Virol 2004; 74: 419-424
5 Widdowson MA, Jaspers WJ, van der Poel WH, Verschoor F, de Roda Husman AM, Winter HL, Zaaier HL, Koopmans M. Cluster of cases of acute hepatitis associated with hepatitis E virus infection acquired in the Netherlands. Clin Infect Dis 2003; 36: 29-33
6 Boxall E, Herborn A, Kochethu G, Pratt G, Adams D, Ijaz S, Teo CG. Transfusion-transmitted hepatitis E in a ‘non-endemic’ country. Transfus Med 2006; 16: 79-83
7 Tamura A, Shimizu YK, Tanaka T, Kuroda K, Arakawa Y, Takahashi K, Mishiro S, Shimizu K, Moriyama M. Persistent infection of hepatitis E virus transmitted by blood transfusion in a patient with T-cell lymphoma. Hepatol Res 2007; 37: 113-120
8 Matsubayashi K, Kang JH, Sakata H, Takahashi K, Shindo M, Kato M, Sato S, Kato T, Nishimori H, Tsuji K, Maguchi H, Yoshida J, Maekubo H, Mishiro S, Ikeda H. A case of transfusion-transmitted hepatitis E caused by blood from a donor infected with hepatitis E virus zoonotic food-borne route. Transfusion 2008; 48: 1368-1375
9 Wu Z, Rou K, Detels R. Prevalence of HIV infection among former commercial plasma donors in rural eastern China. Health Policy Plan 2001; 16: 41-46
10 Xu JQ, Wang JJ, Han LF, Xu C, Ruan YH, Xu ZL, Chen X, Liu ZD, Wang J, Su B, Ding XP, Gao B, Gu YB, Cao XY, Xing H, Hong KX, Peng H, Zhao QB, Yuan L, Feng Y, Zhang GY, Ma LY, Wu L, Shao YM. Epidemiology, clinical and labora-
tory characteristics of currently alive HIV-1 infected former blood donors naive to antiretroviral therapy in Anhui Province, China. Clin Med J (Engl) 2006; 119: 1941-1948
11 Lu J, Dai X, Meng JH. Application of p166 recombinant proteins derived from different genotypes of hepatitis E virus (HEV) in anti-HEV antibody detection. Chin J Microbiol Immunol 2006; 26: 369-374
12 Dong C, Dai X, Shao JS, Hu K, Meng JH. Identification of genetic diversity of hepatitis E virus (HEV) and determination of the seroprevalence of HEV in eastern China. Arch Virol 2007; 152: 739-746
13 Zhai L, Dai X, Meng J. Hepatitis E virus genotyping based on full-length genome and partial genomic regions. Virus Res 2006; 120: 57-69
14 Chen XF, Chen J, Zhan SW, Chen DL, Xiang XK, Zhu M, Fang DC, Wen YF. Epidemiological characteristics of hepatitis E and genotypes of hepatitis E virus in Maanshan area of Anhui province. Chin J Public Health Jun 2010; 26: 710-712
15 Taremi M, Khoshbaten M, Gachkar L, Ehsan\'i Ardakani M, Zali M. Hepatitis E virus infection in hemodialysis patients: a seroepidemiological survey in Iran. BMC Infect Dis 2005; 5: 36
16 Li RC, Ge SX, Li YP, Zheng YJ, Nong Y, Guo QS, Zhang J, Ng MH, Xia NS. Seroprevalence of hepatitis E virus infection, rural southern People’s Republic of China. Emerg Infect Dis 2006; 12: 1682-1688
17 Mitsui T, Tsukamoto Y, Yamazaki C, Masuko K, Tsuda F, Takahashi M, Nishizawa T, Okamoto H. Prevalence of hepatitis E virus infection among hemodialysis patients in Japan: evidence for infection with a genotype 3 HEV by blood transfusion. J Med Virol 2004; 74: 563-572
18 Dalton HR, Bendall R, Ijaz S, Banks M. Hepatitis E: an emerging infection in developed countries. Lancet Infect Dis 2008; 8: 698-709
19 Li K, Zhuang H, Zhu W. Partial nucleotide sequencing of hepatitis E viruses detected in sera of patients with hepatitis E from 14 cities in China. Chin Med J (Engl) 2002; 115: 1058-1063
20 Wei S, Xu Y, Wang M, To SS. Phylogenetic analysis of hepatitis E virus isolates in southern China (1994-1998). J Clin Virol 2006; 36: 103-110
21 Chen Y, Tian DY, Xia NS. Epidemiology and genotypes of HEV in Wuhan. Chin J Dig Dis 2005; 6: 182-188
22 Schlaudger GG, Dawson GJ, Erker JC, Kwo PY, Knigge MF, Smallley DL, Rosenblatt JE, Desai SM, Mushahwar IK. The sequence and phylogenetic analysis of a novel hepatitis E virus isolated from a patient with acute hepatitis reported in the United States. J Gen Virol 1998; 79 (Pt 3): 447-456
23 Chobe LP, Lole KS, Arankalle VA. Full genome sequence and analysis of Indian swine hepatitis E virus isolate of genotype 4. Vet Microbiol 2006; 114: 240-251

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