Dig Dis Sci (2009) 54:2512–2518
DOI 10.1007/s10620-008-0657-4

ORIGINAL ARTICLE

Clinical Significance of Anti-HEV IgA in Diagnosis of Acute Genotype 4 Hepatitis E Virus Infection Negative for Anti-HEV IgM

Shujun Zhang · Deying Tian · Zhengang Zhang · Junhui Xiong · Quan Yuan · Shengxiang Ge · Jun Zhang · Ningshao Xia

Received: 11 August 2008 / Accepted: 28 November 2008 / Published online: 1 January 2009

Abstract Anti-HEV IgM is a diagnostic for recent or ongoing HEV infection. However, some patients with acute hepatitis E (AHE) negative for anti-HEV IgM in acute period were often observed in clinical practice. In this study, we constructed the anti-HEV IgA indirect ELISA assay to evaluate the significance of anti-HEV IgA. The specificity of anti-HEV IgA was 99.6%. Among 245 AHE patients, 84 samples from 84 patients were positive for HEV RNA. The positive rate of anti-HEV IgA, anti-HEV IgM and anti-HEV IgG in 84 samples positive for HEV RNA was 96.3, 97.6, and 88.1%, respectively, and no sample was negative for anti-HEV IgA and anti-HEV IgM simultaneously. Among 245 AHE patients, we found nine samples collected from nine patients in acute period were negative for anti-HEV IgM but positive for anti-HEV IgA and two samples were positive for HEV RNA. Detection of anti-HEV IgA can be a useful supplement for diagnosis of acute HEV infection especially in patients negative for anti-HEV IgM.

Keywords Hepatitis E virus · Acute hepatitis E · Anti-HEV IgA · Anti-HEV IgM · Anti-HEV IgG

S. Zhang · D. Tian (✉) · Z. Zhang
Department of Infectious Disease, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jie Fang Avenue, Wuhan 430030, Hubei Province, China
e-mail: deyingtian@163.com

J. Xiong · Q. Yuan · S. Ge · J. Zhang · N. Xia
National Institute of Diagnostics and Vaccine Development of Infectious Diseases, Xiamen University, Xiamen 361005, China

Introduction

Acute hepatitis E (AHE) is caused by hepatitis E virus (HEV) in developing countries where sanitation is suboptimal, however, epidemiological investigation indicated HEV infection also occurs among individuals in industrialized countries with no history of travel to epidemic regions [1–3]. Like hepatitis A, it is transmitted by the fecal–oral route and contaminated water or food supplies have been implicated in major outbreaks. However, mortality rate of AHE is 0.5–4% in the general population and up to 20% among pregnant women [4].

The HEV genome is a single-stranded, positive-sense RNA, approximately 7.2 kb in length. It contains a short 5′ untranslated region (UTR), three open reading frames (ORFs: ORF1, ORF2 and ORF3), and a short 3′ UTR that is terminated by a poly(A) tract [5]. Although HEV sequences have been classified into four genotypes according to either the complete genome sequence or the nucleotide 80–450 of ORF1 [1, 6], only the single serotype was identified until now [4]. Genotype 1 is distributed in various developing countries in Asia and Africa; genotype 2 has been found in Mexico and Africa; genotype 3 is widely distributed and has been isolated from sporadic cases of an acute HEV infection and/or domestic pigs in the United States, several European countries, and Japan; genotype 4 is found mainly in Asian countries and contains strains from human and domestic pigs in China [7].

Clinical diagnosis of AHE is mainly done by a blood test that detects specific antibodies to HEV [8]. The immunoglobulin M (IgM) class of antibody against HEV (Anti-HEV IgM) is a diagnostic for recent or ongoing HEV infection for its short duration [9]. But the anti-HEV IgM alone should not be seen as evidence for infection because false-positive results are often caused by rheumatoid

factors and immunoglobulin G (IgG.). Although the IgG class of antibody against HEV (Anti-HEV IgG) is generally only used as the post-infectious index for its long duration, detection of newly elicited anti-HEV IgG also can be the proof to diagnose AHE [10]. Therefore, the diagnosis of AHE should be based on both the serological evidence and clinical manifestation.

Commercially available assays were produced with synthetic or recombinant peptides specified by open reading frames 2 and 3 of different strains of the HEV genome. P239 antigen is a bacterially expressed recombinant peptide corresponding to aa368–aa606 of HEV ORF2 of a genotype 1 strain of HEV. The outstanding features of this peptide are that it naturally interacts with one another to form homodimers under physiological conditions and that such is strongly recognizable by HEV reactive human sera [11].

In our previous study, we found that the duration of anti-HEV IgA is longer than anti-HEV IgM, but the clinical and epidemiological implications of anti-HEV IgA in finding HEV infection remain to be clarified. In this study, we constructed the indirect ELISA assay with p239 antigen to detect anti-HEV IgA and further demonstrated the value of anti-HEV IgA in diagnosis of AHE.

Materials and Methods

Serum Samples

About 245 patients with AHE in this study were recruited from the Department of Infectious Disease of Tongji Hospital in Wuhan, China, during the period of July 2006 to July 2008. All of the 245 patients showed clinical manifestation (jaundice or elevated serum aminotransferase levels) of the acute hepatitis. They were positive for HEV RNA and (or) anti-HEV (verified by monoclonal antibody and antigen blocking test) but negative for IgM antibody to hepatitis A virus (anti-HAV), hepatitis B virus surface antigen (HBsAg), IgM antibody to hepatitis B virus core antigen (anti-HBc), and antibody to hepatitis C virus (anti-HCV). Drug-induced hepatitis and autoimmune hepatitis were also excluded. Among these 245 patients, 84 serum samples from 84 patients were positive for HEV RNA. The patients were 14–92 years of age (mean = 51 ± 13 years), male/female = 5:1, alanine aminotransferase (ALT) = 2,327 ± 1,520 U/l. In addition, 2,210 control serum samples were collected from Tongji Hospital and Xiamen Blood Center. All 2,210 control samples were negative for HEV RNA and normal for serum ALT, aspartate aminotransferase (AST) and total serum bilirubin (T-Bil). Samples testing false-positive for anti-HEV IgM or anti-HEV IgA were verified by antigen and monoclonal antibody blocking test, respectively. Among them, 1,313 serum samples were negative for both anti-HEV IgM and anti-HEV IgG. Of the remaining 897 serum samples, 15 were positive for anti-HEV IgM and 868 were positive for anti-HEV IgG. All serum samples were stored at −80°C before detection.

RNA Extraction and RT-PCR

Viral RNA was extracted from 200 μl of serum samples with Trizol LS reagent (Invitrogen). Reverse transcription of the extracted RNA was carried out in a 20-μl reaction mixture containing 20 U of RNAsin (Promega), 10× RT buffer (Promega), 1 mM each dNTP (Takara), 5 U of AMV reverse transcriptase (Promega), and 2.5 uM of reverse transcription primer E5:5′-ctacacgaaaccgaragw-3′ (r = a OR g, w = a OR c). The mixture was incubated at room temperature for 5 min, then at 42°C for 60 min, and at 95°C for 5 min. About 2 μl of the obtained cDNA was added to a 20-μl reaction mixture containing 0.5 mM each of the primers E5 and E1:5′-ctgtttaaycttgctgacac-3′ (y = c OR t), 1 U of Taq DNA polymerase (Takara), and 10× PCR buffer (Takara), overlaid with 20 μl of mineral oil, and subjected to 35 cycles of PCR in a thermo-cycler (94°C, 40 s; 53°C, 40 s; 72°C, 40 s). About 2 μl of the first-round PCR product was amplified for a further 25 cycles (94°C, 40 s; 53°C, 40 s; 72°C, 40 s) using the internal primers E2:5′-gacagaattgatttcgtcg-3′) and E4:5′-gtcctaatactrttggttgt-3′ (r = a OR g). The length of product corresponding to ORF2 sequence is 189 bp (6,298 nt–6,486 nt).

ELISA for Anti-HEV IgM and Anti-HEV IgG

Anti-HEV IgM (capture method) and anti-HEV IgG were detected by using commercially available kits (BeiJing Wantai Biological Pharmacy Enterprise Co., LTD.) based on a recombinant HEV antigen, pE2. This is a structural peptide coded by an ORF2 sequence derived from a Chinese genotype 1 strain of HEV. For anti-HEV IgM, 100 μl of each samples was added to each well at a dilution of 1:10 in sample diluent. The microplates were incubated at 37°C for 30 min and then were washed five times with wash buffer. About 100 μl of horseradish peroxidase (HRP) conjugated pE2 antigen was added to each well. The microplates were incubated at 37°C for 30 min and then washed five times with washing buffer. Then, 100 μl tetramethyl benzidine buffer was added to each well. The microplates were incubated at 37°C for 15 min in the dark and then 50 μl of stop buffer was added to each well. For anti-HEV IgG, the procedures were the same as that of anti-HEV IgM except that HRP-labeled pE2 antigen against human IgG replaced by the HRP conjugated monoclonal antibody.

Absorbance (A) value was measured at 450 nm. According to the protocols provided by the manufacturer, anti-HEV IgM cut-off value = 0.26+ negative control mean A value and anti-HEV IgG cut-off value = 0.16+ negative control mean A value. Samples with A values higher than the cut-off value were considered positive, and samples with other A values were considered negative.

ELISA for Anti-HEV IgA

The well of the microplates was coated with 100 μl p239 antigen (2.66 μg/ml in carbonate buffer PH 9.6) and incubated at 4°C overnight after 37°C for 2 h. After removal of the coating buffer, 200 μl of the 3%PBS-BSA was added to every well and incubated at 37°C for 2 h. Then the blocking buffer was discarded and the microplates were dehumidified for 2 h. To test the anti-HEV IgA, 100 μl of each sample was added to each well at a dilution of 1:10 in 1% PBST-BSA. The microplates were incubated at 37°C for 30 min and then were washed five times with 1% PBST. A total of 100 μl of horseradish peroxidase conjugated goat monoclonal anti-human IgA (Wuhan Boster Biological Technology, LTD.) at a dilution of 1:4000 was added to each well. The microplates were then incubated at 37°C for 30 min and then washed five times with washing buffer. Then, 100 μl of tetramethyl benzidine buffer was added to each well. The microplates were incubated at 37°C for 15 min in the dark and then 50 μl stop buffer (2 mol/l sulphuric acid) was added to each well. The OD value of each well was read at 450 nm.

Monoclonal Antibody and Antigen Blocking Test

The specificity of anti-HEV IgA and anti-HEV IgM assay was verified by monoclonal antibody against p239 antigen (8H3 and 8C11) [12] and unlabeled pE2 antigen. The serum samples were diluted to adjust its OD value to below 1.5. For the anti-HEV IgA, the solid surface was incubated with saturating levels (1:100) of 8H3 and 8C11 at 37°C for 30 min prior to the addition of serum samples. Then the microplates were washed five times with washing buffer and added to the serum samples. The follow-up procedures were the same as above in ELISA for anti-HEV IgA. For the anti-HEV IgM, the microplates were incubated at saturating levels (100 μg/ml) of unlabeled pE2 antigen at 37°C for 30 min after the addition of serum samples. Then the microplates were washed five times with washing buffer and added to the HRP-labeled pE2 antigen. The follow-up procedures were the same as above in ELISA for anti-HEV IgM. The OD value of the tested sample was reduced by no less than 70%. All samples positive for anti-HEV IgA and (or) anti-HEV IgM were verified by blocking test.

Statistical Analysis

Chi-square test was performed using SPSS version 13.0. All tests were two-tailed and P-values of $P < 0.05$ were considered significant.

Results

Determination of Cut-Off Value and Specificity of Anti-HEV IgA Assay

To determine the cut-off value of anti-HEV IgA assay, 1,313 serum samples with normal ALT and negative for both anti-HEV IgM and anti-HEV IgG were used as a panel in the present study. The OD values of anti-HEV IgA ranged from 0.001 to 0.33. The OD value of 0.211 (mean + 7SD) was used as the cut-off value for anti-HEV IgA assay. Using the cut-off value, the remaining 897 serum samples considered not to have been infected recently with HEV were tested anti-HEV IgA, anti-HEV IgM, and anti-HEV IgG. Among the total 2,210 serum samples, anti-HEV IgA, anti-HEV IgM, and anti-HEV IgG were detected in nine (Table 1), 15, and 868 samples, respectively. Among the nine serum samples testing positive for anti-HEV IgA, no sample was positive for anti-HEV IgM and seven samples were positive for anti-HEV IgG. All nine samples positive for anti-HEV IgA and 15 samples positive for anti-HEV IgM were false-positive, confirmed by monoclonal antibody blocking test and antigen blocking test, respectively. The specificity of anti-HEV IgA assay was 99.6% (2201/2210) (95% CI: 99.2–99.8%).

Positive Rate of Anti-HEV IgA, Anti-HEV IgM, and Anti-HEV IgG in 84 Serum Samples Positive for HEV RNA

About 84 serum samples positive for HEV RNA from 84 patients with AHE were collected in 40 days after disease onset (Table 2). All the samples were genotype 4 HEV infection verified by sequencing. The positive rate of anti-HEV IgA, anti-HEV IgM, and anti-HEV IgG in serum samples positive for HEV RNA was 96.3% (81/84) (95% CI: 89.8–98.8%), 97.6% (82/84) (95% CI: 91.7–99.3%) and 88.1% (74/84) (95% CI: 79.5–93.4%), respectively. OD value of three samples negative for anti-HEV IgA was 0.007, 0.009, and 0.048, respectively. The difference between anti-HEV IgA and anti-HEV IgM was not statistically significant ($P = 0.65$). However, the difference between total positive rate of anti-HEV IgA and anti-HEV IgM compared to anti-HEV IgG were statistically significant ($P = 0.043$ and 0.017, respectively). Not only anti-HEV IgA but also anti-HEV IgM may be negative in serum

Table 1 Nine serum samples positive for anti-HEV IgA among 2,210 control serum samples

Sample ID no.	Anti-HEV IgM		Anti-HEV IgG		Anti-HEV IgA		8H3 and 8C11 (1:100) blocking rate (%)
	OD value	Reactivity	OD value	Reactivity	OD value	Reactivity	
X175	0.082	−	0.541	+	0.495	+	43.2
X394	0.024	−	0.643	+	0.284	+	63.7
X474	0.112	−	0.391	+	0.225	+	59.1
X504	0.006	−	0.008	−	0.296	+	44.0
X1032	0.008	−	0.064	−	0.33	+	50.3
X1136	0.013	−	3.431	+	0.438	+	67.6
X1172	0.126	−	3.505	+	2.621	+	62.5
X1390	0.037	−	3.321	+	0.247	+	46.6
X2476	0.065	−	0.847	+	0.546	+	39.6

Table 2 Positive rate of anti-HEV IgA, anti-HEV IgM and anti-HEV IgG in 84 serum samples positive for HEV RNA

Days after disease onset	No. of samples positive for HEV RNA	No. (%) of samples with		
		Anti-HEV IgA	Anti-HEV IgM	Anti-HEV IgG
0–10	28	27 (96.42)	27 (96.42)	24 (85.71)
10–20	40	39 (97.5)	39 (97.5)	36 (90.0)
20–30	13	12 (92.31)	13 (100)	11 (84.62)
30–40	3	3 (100)	3 (100)	3 (100)
Total	84	81 (96.34)[a]	82 (97.62)[a]	74 (88.1)

[a] $P < 0.05$ compared with the positive rate of anti-HEV IgG (Chi-square test)

sample positive for HEV RNA, but no samples were negative for anti-HEV IgA and anti-HEV IgM simultaneously.

Detection of Anti-HEV IgA in AHE Patients Negative for Anti-HEV IgM and Anti-HEV IgM in AHE Patients Negative for Anti-HEV IgA in Acute Period

Among 245 patients diagnosed with AHE, nine were negative for anti-HEV IgM in acute period (within 20 days after disease onset) but positive for anti-HEV IgA and two were positive for HEV RNA (Table 3). The two patients were infected with genotype 4 HEV verified by sequencing. For patient No. 46, anti-HEV IgG were detected at 10 days after disease onset, but the anti-HEV IgM was still negative. Anti-HEV IgM was positive at 3 days after disease onset and could not be detected at 9 days after disease onset in patient No. 97. For patient No. 156, anti-HEV IgM, anti-HEV IgG, and HEV RNA all were positive at 3 days after disease onset, but anti-HEV IgM was negative at 15 days after disease onset. For patient No. 219, anti-HEV IgM switched from positive to negative at 13 days after disease onset. Both anti-HEV IgM and anti-HEV IgG

Table 3 Nine serum samples collected at acute period negative for anti-HEV IgM

Sample ID no.	Anti-HEV IgM		Anti-HEV IgG		Anti-HEV IgA		HEV RNA	ALT (U/l)	T-Bil (μmol/l)	Days after disease onset
	OD value	Reactivity	OD value	Reactivity	OD value	Reactivity				
46	0.026	−	0.022	−	1.345	+	−	1195	74.6	3
97	0.013	−	1.219	+	1.12	+	−	75	13.1	9
156	0.128	−	1.417	+	2.41	+	+	221	24.1	15
157	0.015	−	1.702	+	3.045	+	−	1961	227	5
167	0.056	−	3.599	+	1.556	+	−	1037	79.2	7
217	0.049	−	1.211	+	1.329	+	−	147	95.8	18
219	0.074	−	3.232	+	2.298	+	−	574	19.4	13
391	0.038	−	0.026	−	2.613	+	+	1980	81.4	6
402	0.017	−	0.045	−	2.31	+	−	1243	198.4	15

Table 4 Four serum samples collected at acute period negative for anti-HEV IgA

Sample ID no.	Anti-HEV IgM		Anti-HEV IgG		Anti-HEV IgA		HEV RNA	ALT (U/l)	T-Bil (μmol/l)	Days after disease onset
	OD value	Reactivity	OD value	Reactivity	OD value	Reactivity				
11	1.254	+	1.278	+	0.007	−	+	582	9.5	6
21	0.879	+	0.016	−	0.009	−	+	127	27.3	11
78	2.398	+	1.879	+	0.103	−	−	394	10.7	16
249	1.895	+	1.652	+	0.117	−	−	1227	31.6	13

of patient No. 391 and patient No. 402 switched from negative to positive at 15 and 20 days after disease onset, respectively.

We also found four serum samples from above 245 patients with AHE were negative for anti-HEV IgA but positive for anti-HEV IgM in acute period. Among the four samples, HEV RNA was detected in two samples (Table 4). Anti-HEV IgA of patients No. 11, 21, and 78 switched from negative to positive at 15, 20, and 20 days after disease onset, respectively. But anti-HEV IgA of patient No. 249 was still negative at 40 days after disease onset. For patient No. 21, anti-HEV IgG was detected at 20 days after disease onset.

Among the 245 AHE patients, 13 samples collected at acute period with anti-HEV IgM or anti-HEV IgA alone were found and 4 samples of them were positive for HEV RNA. No sample was detected only positive for anti-HEV IgG.

Discussion

Although HEV RNA is the gold standard for the diagnosis of AHE, it has not been widely available for its complex procedure. In addition, not only is the duration of HEV viremia very short (about 2 weeks on average) in most of the AHE patients but also the low HEV viral loads, so that the patients negative for HEV RNA can not be ruled out for the diagnosis of AHE [13, 14]. Currently, the diagnosis of AHE is mainly based on the serological detection. The presence of anti-HEV IgM is the marker of recent HEV infection, but the sandwich and indirect ELISA methods for detecting anti-HEV IgM have two disadvantages. One is the reduced sensitivity due to competition among virus-specific IgM, IgA, and IgG for antigen-binding sites. Another is that IgM-rheumatoid factor in sera from patients with rheumatoid arthritis presumably induced false-positive results. Yu et al. [15] constructed the class-capture enzyme immunoassay that eliminates the competing IgG antibodies, IgA antibodies at the beginning of the assay so as to enhance the reaction between anti-HEV IgM and HEV antigen. But sensitivity of the capture system depends on the capacity of solidified antibodies against the total human IgM antibodies containing the IgM specific against the HEV antigen. In addition, there is no agreement about the duration of anti-HEV IgG and that the duration from 6 months to 14 years was reported by different investigators [16–18]. Anti-HEV IgG is generally used in epidemiological investigation and as the post-infectious index for its long duration, but anti-HEV IgG does not exist throughout the lifetime, thus the positive rate of anti-HEV IgG in a population cannot completely reflect the post-infection rate.

Chau, Tokita and Takahashi et al. [19–24] reported that anti-HEV IgA can be a useful supplementary marker for recent HEV infection. In a previous study [13], we found that the duration of anti-HEV IgA was longer than that of anti-HEV IgM. The positive rate of anti-HEV IgA in AHE patients was 100, 100, 97, 93, 63, and 30% in the second week and the 1–5 month after the disease onset, respectively. The positive rate of anti-HEV IgM was 100, 100, 77, 57, 20, and 3% in the second week and the first 1–5 month, respectively. In the present study, we constructed the anti-HEV IgA indirect ELISA assay with p239 antigen and determined the cut-off value. The specificity of anti-HEV IgA assay was 99.6% (2201/2210) (95% CI: 99.2–99.8%) and no samples were found to be false-positive for either anti-HEV IgA or anti-HEV IgM. Takahashi et al. [22] had the same findings that anti-HEV IgM and anti-HEV IgA may be false-positive but specificity of combined detection of anti-HEV IgM and anti-HEV IgA was 100%. Single anti-HEV IgM, anti-HEV IgA, and anti-HEV IgG could not confirm whether the diagnosis of AHE for either anti-HEV IgM or anti-HEV IgA may be false-positive, and anti-HEV IgG can be positive at both ongoing infection and post-infection. In 84 serum samples positive for HEV RNA, whether anti-HEV IgA or anti-HEV IgM may be negative, but no samples negative for both anti-HEV IgA and anti-HEV IgM. Using the combined detection of anti-HEV IgA and anti-HEV IgM, the diagnosis sensitivity of AHE was improved to 100% in the PCR positive group. However, 84 serum samples positive for HEV RNA in this study all were of genotype 4 HEV infection. The significance of anti-HEV IgA in other genotype HEV infection

remains to be clarified. Herremans et al. [25] reported IgA responses were more prominent in the patients with genotype 1 HEV infection compared to those with the genotype 3 infection, but the differences between genotype 1 and genotype 3-infected patients could be explained by the use of the homologous genotype 1 antigens in the assay. Although only one serotype of HEV was reported, the immunoreactivities of polypeptides from various HEV genotype isolates were different [26]. Therefore, it is necessary to synthesize various polypeptides from various HEV genotype isolates to clarify the significance of anti-HEV antibodies.

In clinical practice, the clinical manifestation of many patients was similar with acute hepatitis, but the indexes of HAV, HBV, HCV, HEV, EBV, CMV and autoimmune hepatitis and drug-induced hepatitis were negative. In this study, all nine serum samples collected from nine patients diagnosed as AHE in acute period (within 20 days after disease onset) were negative for anti-HEV IgM and two samples were infected verified by HEV genotype 4 by sequencing. All nine serum samples positive for anti-HEV IgA showed that some patients were negative for anti-HEV IgM in acute period and anti-HEV IgA assay can be a supplementary for diagnosis of AHE especially in patients negative for anti-HEV IgM in acute period.

IgA is the major secretory immunoglobulin and is widely distributed in all mucosal secretions as a dimeric molecule linked by a joining chain and a third molecule, the secretory piece. In the circulation, IgA occurs in both monomeric and polymeric forms. There are two serologically and structurally distinct IgA subclasses. IgAl makes up 90% of circulating IgA while the IgA found in secretions comprises 50% IgA1 and 50% IgA2. IgA deficiency is the most common reason for the primary immunodeficiency. There is a marked variability in the prevalence in different ethnic groups, with a frequency of 1/4000 among Chinese [27, 28]. Therefore, anti-HEV IgA of AHE patients may be a false-negative caused by IgA deficiency in the circulation.

Usually, mean plus 2(or 3) SD is used as the cut-off value for normal distribution data providing a specificity of 95% (or 99%). Using a higher cut-off value would increase the specificity at the expense of sensitivity. In this study, an OD value of three samples negative for anti-HEV IgA in HEV RNA positive group was 0.007, 0.009, and 0.048, respectively. Therefore, the sensitivity of anti-HEV IgA in HEV RNA-positive group was 96.3% using the cut-off value of both mean plus 2SD (0.101) and mean plus 7SD (0.211). However, the cut-off value of mean plus 7SD (0.211) can improve the specificity from 94.5% (122/2210) to 99.6% (9/2210) compared to mean plus 2SD (0.101). However, an OD value above 0.211 has little improvement to specificity of anti-HEV IgA. Moreover, an OD value of 0.211 (mean + 7SD) made both the sensitivity and specificity of combined detection of anti-HEV IgA and anti-HEV IgM were 100% so that 0.211 (mean + 7SD) was used as the cut-off value in this study. Certainly, more control samples and patient samples need to be detected to further optimize the cut-off value of anti-HEV IgA assay.

In conclusion, based on the results obtained in this study, anti-HEV IgA assay can be a useful supplement for diagnosis of acute HEV infection especially in patients negative for anti-HEV IgM in acute period.

Acknowledgments This project was supported by the Science and Technology Projects of Hubei Province (No. 2007AA301B26) and Science and Technology Projects of Wuhan City (No. 200761023424).

References

1. Schlauder GG, Dawson GJ, Erker JC, et al. 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.
2. Wang Y, Levine DF, Bendall RP, Teo CG, Harrison TJ. Partial sequence analysis of indigenous hepatitis E virus isolated in the United Kingdom. *J Med Virol*. 2001;65:706–709. doi:10.1002/jmv.2094.
3. Mizuo H, Suzuki K, Takikawa Y, et al. Polyphyletic strains of hepatitis E virus are responsible for sporadic cases of acute hepatitis in Japan. *J Clin Microbiol*. 2002;40:3209–3218. doi:10.1128/JCM.40.9.3209-3218.2002.
4. Emerson SU, Purcell RH. Hepatitis E virus. *Rev Med Virol*. 2003;13:145–154. doi:10.1002/rmv.384.
5. Tam AW, Smith MM, Guerra ME, et al. Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. *Virology*. 1991;185:120–131. doi:10.1016/0042-6822(91)90760-9.
6. Schlauder GG, Mushahwar IK. Genetic heterogeneity of hepatitis E virus. *J Med Virol*. 2001;65:282–292. doi:10.1002/jmv.2031.
7. Okamoto H. Genetic variability and evolution of hepatitis E virus. *Virus Res*. 2007;127:216–228. doi:10.1016/j.virusres.2007.02.002.
8. DeGuzman LJ, Pitrak DL, Dawson GJ, Shin DC, Holzer TJ, Layden TJ. Diagnosis of acute hepatitis E infection utilizing enzyme immunoassay. *Dig Dis Sci*. 1994;39:1691–1693. doi:10.1007/BF02087777.
9. Koshy A, Grover S, Hyams KC, et al. Short-term IgM and IgG antibody responses to hepatitis E virus infection. *Scand J Infect Dis*. 1996;28:439–441. doi:10.3109/00365549609037935.
10. Clayson ET, Myint KS, Snitbhan R, et al. Viremia, fecal shedding, and IgM and IgG responses in patients with hepatitis E. *J Infect Dis*. 1995;172:927–933.
11. Li SW, Zhang J, Li YM, et al. A bacterially expressed particulate hepatitis E vaccine: antigenicity, immunogenicity and protectivity on primates. *Vaccine*. 2005;23:2893–2901. doi:10.1016/j.vaccine.2004.11.064.
12. Zhang J, Gu Y, Ge SX, et al. Analysis of hepatitis E virus neutralization sites using monoclonal antibodies directed against a virus capsid protein. *Vaccine*. 2005;23:2881–2892. doi:10.1016/j.vaccine.2004.11.065.
13. Tian DY, Chen Y, Xia NS. Significance of serum IgA in patients with acute hepatitis E virus infection. *World J Gastroenterol*. 2006;12:3919–3923.

14. Ma Y, Lin SQ, Gao Y, et al. Expression of ORF2 partial gene of hepatitis E virus in tomatoes and immunoactivity of expression products. *World J Gastroenterol*. 2003;9:2211–2215.
15. Yu C, Engle RE, Bryan JP, Emerson SU, Purcell RH. Detection of immunoglobulin M antibodies to hepatitis E virus by class capture enzyme immunoassay. *Clin Diagn Lab Immunol*. 2003;10:579–586. doi:10.1128/CDLI.10.4.579-586.2003.
16. Bryan JP, Tsarev SA, Iqbal M, et al. Epidemic hepatitis E in Pakistan: patterns of serologic response and evidence that antibody to hepatitis E virus protects against disease. *J Infect Dis*. 1994;170:517–521.
17. Khuroo MS, Kamili S, Dar MY, Moecklii R, Jameel S. Hepatitis E and long-term antibody status. *Lancet*. 1993;341:1355.
18. Goldsmith R, Yarbough PO, Reyes GR, et al. Enzyme-linked immunosorbent assay for diagnosis of acute sporadic hepatitis E in Egyptian children. *Lancet*. 1992;339:328–331. doi:10.1016/0140-6736(92)91647-Q.
19. Chau KH, Dawson GJ, Bile KM, Magnius LO, Sjogren MH, Mushahwar IK. Detection of IgA class antibody to hepatitis E virus in serum samples from patients with hepatitis E virus infection. *J Med Virol*. 1993;40:334–338. doi:10.1002/jmv.1890400414.
20. Tokita H, Harada H, Gotanda Y, Takahashi M, Nishizawa T, Okamoto H. Molecular and serological characterization of sporadic acute hepatitis E in a Japanese patient infected with a genotype III hepatitis E virus in 1993. *J Gen Virol*. 2003;84:421–427. doi:10.1099/vir.0.18802-0.
21. Mitsui T, Tsukamoto Y, Suzuki S, et al. Serological and molecular studies on subclinical hepatitis E virus infection using periodic serum samples obtained from healthy individuals. *J Med Virol*. 2005;76:526–533. doi:10.1002/jmv.20393.
22. Takahashi M, Kusakai S, Mizuo H, et al. Simultaneous detection of immunoglobulin a (IgA) and IgM antibodies against hepatitis E virus (HEV) is highly specific for diagnosis of acute HEV infection. *J Clin Microbiol*. 2005;43:49–56. doi:10.1128/JCM.43.1.49-56.2005.
23. Takahashi M, Nishizawa T, Tanaka T, Tsatsralt-Od B, Inoue J, Okamoto H. Correlation between positivity for immunoglobulin A antibodies and viraemia of swine hepatitis E virus observed among farm pigs in Japan. *J Gen Virol*. 2005;86:1807–1813. doi:10.1099/vir.0.80909-0.
24. Elkady A, Tanaka Y, Kurbanov F, et al. Evaluation of anti-hepatitis E virus (HEV) immunoglobulin A in a serological screening for HEV infection. *J Gastroenterol*. 2007;42:911–917. doi:10.1007/s00535-007-2109-x.
25. Herremans M, Duizer E, Jusic E, Koopmans MP. Detection of hepatitis E virus-specific immunoglobulin a in patients infected with hepatitis E virus genotype 1 or 3. *Clin Vaccine Immunol*. 2007;14:276–280. doi:10.1128/CVI.00312-06.
26. Wang Y, Zhang H, Li Z, et al. Detection of sporadic cases of hepatitis E virus (HEV) infection in China using immunoassays based on recombinant open reading frame 2 and 3 polypeptides from HEV genotype 4. *J Clin Microbiol*. 2001;39:4370–4379. doi:10.1128/JCM.39.12.4370-4379.2001.
27. Morgan G, Levinsky RJ. Clinical significance of IgA deficiency. *Arch Dis Child*. 1988;63:579–581.
28. Hammarstrom L, Vorechovsky I, Webster D. Selective IgA deficiency (SIGAD) and common variable immunodeficiency (CVID). *Clin Exp Immunol*. 2000;120:225–231. doi:10.1046/j.1365-2249.2000.01131.x.