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Antibody detection and sequence analysis of sporadic HEV in Xiamen region *

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INTRODUCTION

Hepatitis E virus (HEV) is transmitted through a fecal-oral route[1]. HEV induces acute hepatitis and is responsible for a significant portion of the fulminant hepatitis in epidemic and sporadic cases, especially in the mixed infection patients and women in their third trimester of pregnancy[1]. It has been reported that HEV infection is more prevalent in underdeveloped and develo ping countries in Asia, Africa, and Central America, but is rare in developed countries[1]. In China, a large outbreak occurred between 1986 and 1988 in Xinjiang, and sporadic spread was often found in other regions.

HEV is a non-enveloped virus, approximately 27 nm-34 nm in diameter and has a positive-sense, single-stranded RNA genome of approximately 7.2 kb. The viral genome consists of three discontinuous open reading frames (ORFs). Since the molecular cloning and sequencing of HEV were described[2], s everal genomic analyses of HEV strains obtained from different geographic areas have been reported[3]. The existing variations on the gene structure of HEV strains from some regions of China was reported by us[4]. In this study, after the collection of the serum samples of patients with acute hepatitis in Xiamen, anti-HEV antibody and HEV RNA in serum were detected, further HEV RN A was cloned and sequenced. The results are described and discussed.

PATIENTS AND METHODS

Patients

From September 1996 to March 1997, 81 samples of serum of patients (71 male and 10 female, aged 13-69 years) with acute hepatitis at clinic and admitted to the Infections Disease Hospital in Xiamen were collected. These serum samples were provided by professor LIAO Mian-Chu and were stored at -25℃ before test. The sera showed elevated ALT levels. The serum of patients with acute hepatitis was tested for detection of serum IgM anti-HAV, HBsAg, IgM anti-HBc, anti-HCV and anti-HEV antibodies. According to above detections, all samples suspected of hepatitis E were taken to our laboratory and were further studied. One case (sample No.3, Zhou, male aged 52 years) used in the determination of sequence was diagnosed as fulminant hepatitis. He had complained of tiredness, anorexia, urine-yellow, jaun dice in skin and sclera and spider nevus, with ALT 20420 nmol • s^{-1}/L and SB-205.2 μmol/L, and virus markers of anti-HAV IgM and anti-HEV IgM positive.

Detection of anti-HEV antibody

Anti-HEV IgG and IgM antibodies were further detected by ELISA with recombinantantigens (Institute of Virology, Chinese Academy of Preventive Medicine) accord ing to the manufacturer's instructions.

Cloning and sequencing of HEV RNA

Two sets of pair primers were synthesized at Institute of Microbiology of Chines e Science Academy according to the Burmese HEV sequence[3]. The sequence of each oligonucleotide primer was: outer primers (F1) 5'-GCT ATT ATG GAG GAG TGT GG 3' and (R1) 5'-CAG GGC CCC AAT TCT TCT 3', inner primers (F2) 5'-G CG TGG ATC TTG CAG GCC 3' and (R2) 5'-TTC AAC TTC AAG CCA CAG CC 3'. HEV RNA was extracted from-200 μL serum by proteinase K (10 g/L) guanidine thioc yanate buffer (4.2M guanidine thiocyanate and 5 g/L N-lauroyl sar cosine and 0.025 mol/L Tris-HCl, pH 8.0)phenol/choroform[5] . All viral RNA were used to be transcribed and nested-PCR[4]. The am plified PCR products were ligated into pGEM-T vector (Promega products) according to the manufacturer's instructions. The ligation mixture was transformed to *E. coli* strain JM 109. The positive clones were picked up and cultured in LB medium. The positive

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recombinant clones were identified and sequenced. The nucl eotide sequence (location 4522-4761) of X-S1 isolate of HEV was compared with those of other known HEV strains. Percentage of similarity and divergence were calculated as described previously[4].

RESULTS

Detection of anti-HEV and HEV RNA in serum samples of patients with acute hepatitis

Twelve of 81 serum samples of acute hepatitis in Xiamen were positive for anti- HEV IgG, of them, 11 were also positive for anti-HEV IgM. Eight of 12 serum sam ples of positive anti-HEV IgG were used to detect HEV RNA, 2 samples were found positive for HEV RNA. The results are shown in Table 1.

Cloning and sequencing of HEV

The amplified PCR positive products obtained from X-S1 and X-S6 samples were purified and ligated into pGEM-T vector, then transformed to *E.coli* JM 109. One of two white specks obtained from X-S1 had a band of about 239 bp in gel electrophoresis. Two white specks of the X-S6 were positive and a band of 239 bp was found after digested by *EcoR* I and *Hind* III. One positi ve clone of X-S1 was sequenced. HEV sequence of the X-S1 isolate could be reco gnized, this HEV X-S1 strain was neither lost nor inserted in length 239 bp compared with Burma strain of HEV. Aberrance occurred in 48 bases, 5 of them took place at the first codon position, 4 at the second position and 39 at the third codon position. The nucleotide sequence of X-S1 isolate is shown in Figure 1.

Table 1 Results of anti-HEV antibody and HEV RNA detection in seru m of patients from Xiamen

Patients	Name	Sex	Age	Collection time	Anti-HEV[a]		Results of HEV RNA detection
					IgG	IgM	
1	Ling JD	M	31	97-03-05	+	+	ND[b]
2	Du YM	M	24	97-02-05	+	+	-
3	Zhou SL	M	52	97-02-15	+	+	+
4	Huang JC	M	50	97-02-19	+	-	ND
5	Qiu SS	M	53	97-02-19	+	+	-
6	Huang JZ	M	45	97-01-28	+	+	-
7	Mei SP	F	31	96-02-17	+	+	ND
8	Zhen MC	M	68	96-12-09	+	+	-
9	Zhang MS	M	60	97-12-09	+	+	-
10	Zhong M	M	29	96-11-13	+	+	+
11	Kang CR	M	24	96-10-10	+	+	N
12	Jiang XW	M	29	97-02-19	+	+	-

[a]A>0.30 +$\bar{x}$ of negative samples: positive.[b]Not done.

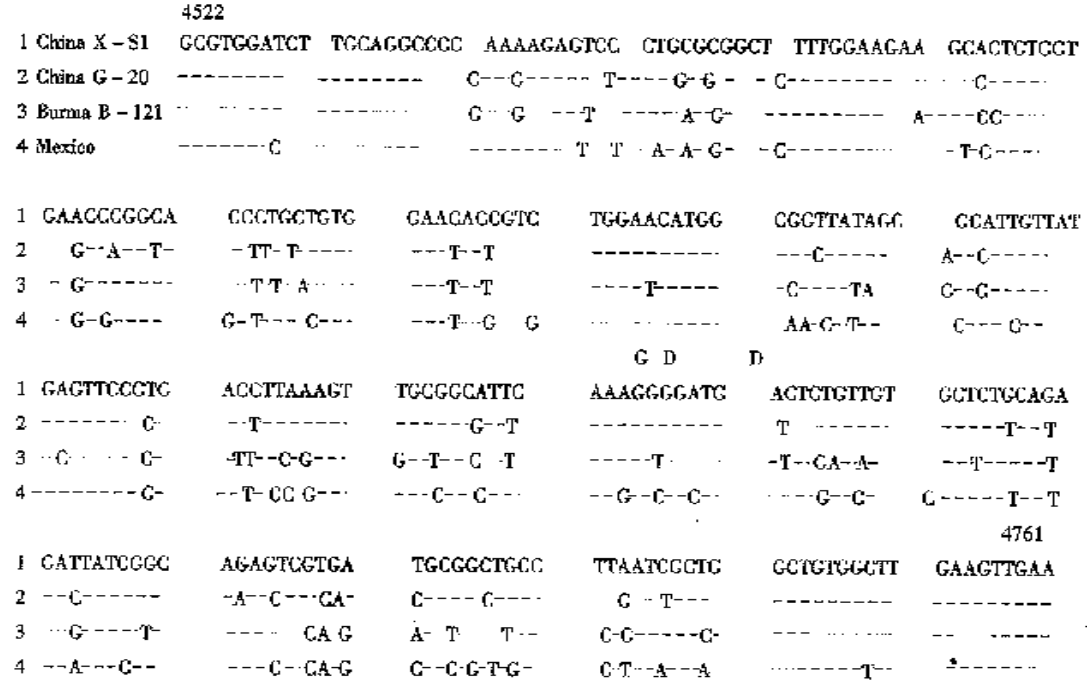

Figure 1 Comparison of the nucleotide sequences amon g four strains of HEV.

Comparison of the nucleotide sequence of HEV

The sequence of HEV X-S1 strain was compared with the Chinese (G-20), Burmese (B-121) and Mexican strains of HEV. The similarity of nucleotide sequences was 85.4%, 79.2% and 76.4% respectively. The divergence of nucleotide sequences was 14.2%, 19.9% and 22.3% respectively.

DISCUSSION

This paper reports the results of serological survey on hepatitis E virus infection in Xiamen population. The serum samples from 81 patients with acute hepatitis were tested for anti-HEV IgG and IgM antibodies with HEV recombination antigen by ELISA. Twelve (14.8%) of 81 patients with acute hepatitis had antibody to HEV, 11 were positive for IgM anti-HEV and 2 positive for HEV RNA. The results show that there is hepatitis E virus infection in Xiamen again, but there has been no documented report of detection of HEV RNA in anti-HEV positive patients from this area.

The mixed infection of HEV and HBV were described previously[4]. This paper reports the co-infection of HEV and HAV. This case is positive for both antibodies of HAV and HEV. The patient presented as severe hepatitis in the clinical characteristics, with very high ALT (20420 nmol • s^{-1}/L). This shows that both the HEV and HAV are transmitted primarily through a fecal-oral route. In this study, the partial nucleotide sequence of Xiamen X-S1 isolate of sporadic HEV was described and compared. It is shown that Xiamen (X-S1) strain and Guangzhou (G-20) strain[4] are most identical to each other (85.4%), with a lower range of identities to the Burmese strain[2] and Mexican strain[3] (80.1%-77.3%). The nucleotide sequences of the X-S1 strain and the G-20 strain may belong to a novel and unique branch. Similar results have been reported by other investigators[6,7]. Recently the HEV-US-1 strain was discovered by George G. Schlauder[8], it is significantly divergent from other human HEV isolates, which may be the fourth genotype. The discovery of these HEV variants may be important in understanding the worldwide distribution of HEV infection.

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