a false-positive result (10). Virus-specific IgA class antibodies have been detected during the acute stage of infection with hepatitis A virus (HAV) (57) or hepatitis B virus (HBV) (28). The IgA class of antibodies has also been detected in sera from patients with hepatitis E (2, 14, 47). Although a few previous studies reported that anti-HEV IgA could be utilized as an additional confirmatory antibody for recent HEV infection (2, 47), the clinical and epidemiological implications of positivity for anti-HEV IgA remain to be clarified.

Therefore, in the present study, we compared the sensitivity and specificity of the anti-HEV IgA and anti-HEV IgM assays and evaluated their ability to diagnose hepatitis E by using serum samples from 68 patients who were subsequently molecularly diagnosed as having hepatitis E and from 2,781 individuals who were assumed to not have been recently infected with HEV as negative controls in an attempt to improve the serological diagnosis of recent HEV infection that is occurring epidemically in developing countries and more frequently than previously thought in industrialized countries, including Japan.

MATERIALS AND METHODS

Serum samples. The present study included serum samples obtained from 68 patients (56 males and 12 females; age, mean ± the standard deviation [SD], 56.3 ± 12.8 [range, 25 to 86] years) at admission who had detectable HEV RNA and who were subsequently diagnosed as having sporadic acute or fulminant hepatitis E. Thirty of the 68 patients had been included in our previous studies for detection of HEV RNA and phylogenetic analysis of HEV isolates (1, 17, 25, 40, 41, 55, 56). The present study also included periodic serum samples collected from 15 of the HEV-infected patients, from whom one or more serum samples had been obtained during each of the following periods: between 0 and 10 days, between 20 and 40 days, and between 50 and 70 days after the onset of the illness. In addition, serum samples obtained from 2,781 individuals who were assumed to not have been recently infected with HEV (1,282 males and 1,499 females; 52.9 ± 18.9 [0 to 97] years) were used as negative controls, and they included 675 samples from voluntary blood donors with normal alanine aminotransferase level (328 males and 347 females; 39.0 ± 16.0 [16 to 64] years), 127 samples from patients with type A, type B, or type C acute hepatitis (77 males and 50 females; 35.7 ± 12.4 [16 to 78] years), 274 samples from patients with type B or type C chronic liver disease (158 males and 116 females; 55.0 ± 13.6 [23 to 83] years), 472 samples from patients on maintenance hemodialysis (262 males and 210 females; 59.0 ± 12.4 [24 to 94] years), 147 samples from patients with primary biliary cirrhosis (27 males and 120 females; 59.7 ± 10.7 [31 to 79] years), 186 samples from patients with rheumatoid arthritis (21 males and 165 females; 63.8 ± 13.2 [26 to 91] years), and 900 samples from patients (409 males and 491 females; 58.5 ± 20.7 [0 to 97] years) who received a routine health examination or care for various disorders at one of our hospitals.

The presence of IgM class antibodies to HAV (anti-HAV IgM), antibodies to HBV core IgM, hepatitis B surface antigen (HBsAg), and antibodies to hepatitis C virus (HCV) (anti-HCV) was determined by commercially available kits (HAVAB-M and CORZYME-M [Abbott Laboratories, Abbott Park, Ill.], Mycell II HBsAg [Institute of Immunology Co., Ltd., Tokyo, Japan], and Abbott HCV PHA Second Generation [Abbott Japan, Tokyo, Japan]). The presence of HBV DNA and HCV RNA was determined by the methods described previously (29, 43). The study protocol conformed to the ethical guidelines and was approved by the ethics committees of the institutions. Informed consent was obtained from each patient.

ELISAs for detecting anti-HEV antibodies. Our previously described in-house enzyme-linked immunosorbent assays (ELISAs) methods for detection of IgM and IgA anti-HEV antibodies using purified recombinant ORF2 protein (25) were performed with the following modifications. Wells of microplates (part no. 762071; Greiner Bio-One GmbH, Frickenhausen, Germany) were coated with 50 μl of the recombinant ORF2 protein (5 μg/ml in phosphate-buffered saline [pH 7.5]) and incubated at room temperature overnight. After removal of the coating buffer, 100 μl of 10 mM Tris-buffered saline (pH 7.5) containing 2.5% (vol/vol) Block Ace (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) and 0.18% Tween 20 was added. The microplates were incubated at room temperature for 4 h. The blocking buffer was discarded, and each well was washed five times with saline containing 2% lactose (Kanto Chemical Co., Inc., Tokyo, Japan) and then freeze-dried. To test for anti-HEV IgM, 50 μl of each sample was added to each well at a dilution of 1:100 in 10 mM Tris-buffered saline containing 40% Block Ace, 0.18% Tween 20, and a mock protein (optical density [OD] at 280 nm = 0.1) that had been obtained from the pupae of silkworm infected with nonrecombinant baculovirus. The microplates were incubated at room temperature for 1 h and were then washed five times with washing buffer (saline with 0.05% Tween 20). A total of 50 μl of phosphate-buffered saline containing 25% (vol/vol) fetal bovine serum (Sigma Chemical, St. Louis, Mo.) and peroxidase-conjugated mouse monoclonal anti-human IgM (M-49; Institute of Immunology Co., Ltd.) (50) was added to each well. The microplates were incubated at room temperature for 1 h and then washed five times with washing buffer. Then, 50 μl of tetramethylbenzidine-soluble reagent (BioFX Laboratories, Inc., Owings Mills, Md.) as a substrate was added to each well. The plate was incubated at room temperature for 30 min in the dark, and then 50 μl of tetramethylbenzidine stop buffer (BioFX Laboratories, Inc.) was added to each well. The OD value of each sample was read at 450 nm. For the anti-HEV IgA assay, peroxidase-labeled mouse monoclonal anti-human IgA (A-13; Institute of Immunology Co., Ltd.) (28) was used in place of the enzyme-labeled anti-human IgM. Test samples with OD values equal to or greater than the cutoff value were considered to be positive for anti-HEV IgM or anti-HEV IgA.

In addition, anti-HEV IgG was assayed according to the method described previously, and the cutoff value used for the anti-HEV IgG assay was 0.152 (25).

The specificity of the anti-HEV assays was verified by absorption with the same recombinant ORF2 protein (50 μg/ml at the final concentration for anti-HEV IgG or anti-HEV IgA assay; 150 μg/ml at the final concentration for anti-HEV IgM assay) that was used as the antigen probe. Briefly, prior to testing, the serum sample was diluted 1:100, 1:300, 1:1,000, 1:3,000, 1:10,000, or 1:30,000 to adjust its OD value to <1.5. If the OD value of the tested sample was reduced by ≥50% in the anti-HEV IgM assay or ≥70% in the anti-HEV IgA or IgG assay after absorption with the recombinant ORF2 protein, the sample was considered to be positive for anti-HEV.

Detection of HEV RNA. Reverse transcription-PCR (RT-PCR) was performed for detection of HEV RNA in serum. Total RNA was extracted from 100 μl of serum, reverse transcribed, and then subjected to nested PCR with ORF2 primers as described previously (25, 42). The size of the amplification product of the first-round PCR was 506 bp and that of the second-round PCR was 457 bp. The nested RT-PCR assay used had the capability of amplifying all four known genotypes of HEV strains reported thus far (25, 42, 56). The RT-PCR assay was performed in duplicate, and the reproducibility was confirmed. The specificity and sensitivity of the RT-PCR assay were assessed as described previously (25, 42).

RESULTS

Determination of the cutoff values for the anti-HEV IgM and anti-HEV IgA assays. Since the prevalence of HEV infection in the southern part of Japan is low (8, 30), it was assumed that voluntary blood donors in Yamaguchi Prefecture, which is located in the southern part of mainland Honshu of Japan, were highly unlikely to have been infected with HEV in the period just prior to their donating blood. Therefore, to determine the cutoff values for the anti-HEV IgM and anti-HEV IgA assays, serum samples from 675 donors with a normal alanine aminotransferase level who donated blood at the Japanese Red Cross Blood Center in Yamaguchi Prefecture were used as a panel in the present study. In the anti-HEV IgM assay, the OD values ranged from 0.001 to 0.542, and the value of 0.440, which was calculated as 7 SD above the mean value (0.072), was used as the tentative cutoff value. In the anti-HEV IgA assay, OD values ranging from 0.000 to 1.754 were obtained from the 675 control sera; the OD value of 0.642 (mean + 7 SD) was used as the cutoff value for anti-HEV IgA.

Although 16 (2.4%) of the 675 serum samples were positive for anti-HEV IgG (Table 1), the 16 samples tested negative for HEV RNA, and their OD values ranged from 0.036 to 0.161 (below the cutoff value) in the anti-HEV IgM assay and from 0.019 to 0.180 (below the cutoff value) in the anti-HEV IgA

TABLE 1. Prevalence of anti-HEV IgM and anti-HEV IgA among various groups of subjects

Group	No. of subjects studied	Age (yr) (mean ± SD)	No. (%) of subjects with:			
			Anti-HEV IgG[a]	Anti-HEV IgM	Anti-HEV IgA	Both anti-HEV IgM and anti-HEV IgA
Blood donors with normal ALT	675	39.0 ± 16.0	16 (2.4)	1 (0.1)	1 (0.1)	0
Patients with acute hepatitis	127	35.7 ± 12.4	11 (8.7)	4 (3.1)	2 (1.6)	0
Type A	57	35.1 ± 9.3	7 (12.3)	4 (7.0)	1 (1.8)	0
Type B	61	34.6 ± 12.9	3 (4.9)	0	1 (1.6)	0
Type C	9	47.6 ± 20.4	1 (11.1)	0	0	0
Patients with chronic liver diseases	274	55.0 ± 13.6	26 (9.5)	2 (0.7)	0	0
Chronic hepatitis	182	51.2 ± 13.4	15 (8.2)	1 (0.5)	0	0
Liver cirrhosis	57	62.9 ± 10.2	7 (12.3)	1 (1.8)	0	0
Hepatocellular carcinoma	35	61.7 ± 11.2	4 (11.4)	0	0	0
Hemodialysis patients	472	59.0 ± 12.4	60 (12.7)	2 (0.4)	0	0
Patients with primary biliary cirrhosis	147	59.7 ± 10.7	15 (10.2)	4 (2.7)	0	0
Patients with rheumatoid arthritis	186	63.8 ± 13.2	6 (3.2)	3 (1.6)	0	0
Hospital patients[b]	900	58.5 ± 20.7	24 (2.7)	0	1 (0.1)	0
Total of control subjects[c]	2,781	52.9 ± 18.9	158 (5.7)	16 (0.6)	4 (0.1)	0
Patients with hepatitis E	68	56.3 ± 12.8	68 (100)	68 (100)	68 (100)	68 (100)

[a] Positivity for anti-HEV IgG was confirmed in all 226 samples by the absorption test (see Materials and Methods).
[b] They received a routine health examination or care for various disorders at one of our hospitals.
[c] They were assumed not to have been recently infected with HEV.

assay, suggesting the absence of present HEV infection in the studied population.

Detection of anti-HEV IgM and anti-HEV IgA in individuals who were assumed not to have been infected recently with HEV. Among the serum samples obtained from the above-mentioned 675 donors, only one sample was positive for anti-HEV IgM with an OD value of 0.542, and a different sample was positive for anti-HEV IgA alone with an OD value of 1.754 (Table 2). However, these two serum samples were negative for anti-HEV IgG and HEV RNA, suggesting that the anti-HEV IgM or IgA was falsely detected in these two samples.

Using the cutoff values described above, the remaining 2,106 serum samples obtained from 127 patients with type A, type B, or type C acute hepatitis, 274 patients with type B or type C chronic liver disease, 472 patients on maintenance hemodialysis, 147 patients with primary biliary cirrhosis, 186 patients with rheumatoid arthritis, and 900 patients who received routine health examination or medical care for various disorders

TABLE 2. Serum samples that were falsely positive for anti-HEV IgM or IgA in the in-house ELISAs used in the present study

Sample ID no.	Diagnosis	Age (yr)/sex[a]	OD at 450 nm[b]			HEV RNA
			Anti-HEV IgM	Anti-HEV IgA	Anti-HEV IgG	
389	Blood donor	59/M	**0.542 (8)**	0.038	0.075	–[c]
674	Blood donor	55/F	0.024	**1.754 (5)**	0.025	–
868	Health check-up	62/M	0.078	**0.946 (9)**	0.034	–
1614	Hemodialysis	55/F	**2.541 (8)**	0.056	0.089	–
1761	Hemodialysis	84/M	**1.018 (23)**	0.194	0.259 (83) (+)	–
2110	Acute hepatitis (type A)	21/F	**1.986 (−7)**	0.173	1.824 (92) (+)	–
2136	Acute hepatitis (type A)	28/F	**1.509 (−6)**	0.042	0.036	–
2113	Acute hepatitis (type A)	43/F	**0.559 (25)**	0.046	0.089	–
2102	Acute hepatitis (type A)	31/F	**0.445 (5)**	0.079	**0.388 (−1)**	–
2099	Acute hepatitis (type A)	34/M	0.286	**0.731 (6)**	0.037	–
2061	Acute hepatitis (type B)	55/M	0.055	**0.692 (−6)**	0.028	–
2201	Primary biliary cirrhosis	48/F	**1.215 (20)**	0.068	0.049	–
2229	Primary biliary cirrhosis	61/F	**0.545 (−6)**	0.072	0.054	–
2232	Primary biliary cirrhosis	60/F	**0.470 (9)**	0.083	0.068	–
2257	Primary biliary cirrhosis	52/F	**0.462 (17)**	0.081	0.023	–
2463	Rheumatoid arthritis	70/M	**0.933 (8)**	0.106	0.071	–
2496	Rheumatoid arthritis	83/F	**0.785 (22)**	0.038	0.022	–
2545	Rheumatoid arthritis	71/F	**0.522 (18)**	0.103	0.024	–
2919	Liver cirrhosis (type C)	61/M	**0.594 (28)**	0.325	0.265 (86) (+)	–
3007	Chronic hepatitis (type B)	44/M	**0.488 (2)**	0.020	0.008	–

[a] M, male; F, female.
[b] If the OD value of the tested sample was reduced by only <50% in the anti-HEV IgM assay or < 70% in the anti-HEV IgA or IgG assay after absorption with the recombinant ORF2 protein, the result was considered to be false positive, and such samples are indicated in boldface. Numbers in parentheses are percent values.
[c] —, no HEV RNA was detected.

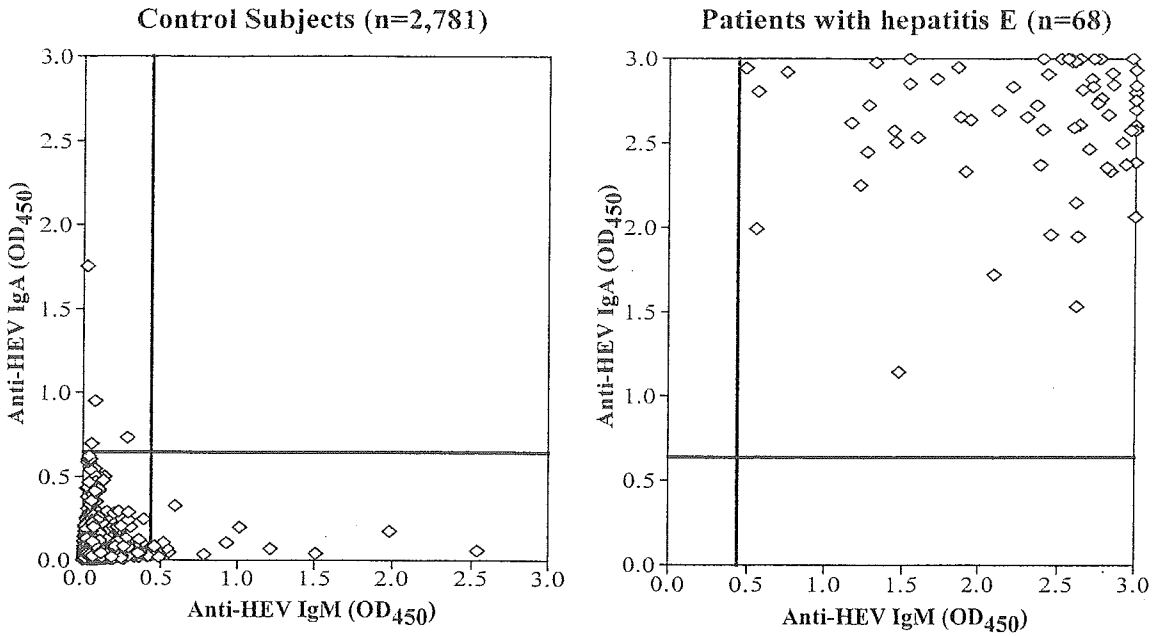

FIG. 1. Distribution of OD values from two ELISAs for anti-HEV IgM and anti-HEV IgA among patients with hepatitis E and among the control subjects. Serum samples from 2,781 subjects who were assumed not to have been infected recently with HEV and from 68 patients with hepatitis E were concurrently tested by the solid-phase ELISAs for anti-HEV IgM and anti-HEV IgA. Horizontal and vertical lines represent the cutoff values for anti-HEV IgA and anti-HEV IgM, respectively.

at one of our hospitals, were tested for anti-HEV IgM and anti-HEV IgA (Table 1). Among the 2,781 subjects who were assumed not to have been infected recently with HEV, including the 675 blood donors described above, anti-HEV IgM was detected in the serum samples from 16 subjects (0.6% or 16 of 2,781), including 4 patients with acute hepatitis A (7.0% or 4 of 57). Anti-HEV IgA was detected in the serum samples from four other patients (0.1% or 4 of 2,781) (Table 1), the difference being statistically significant ($P = 0.0139$ [χ^2-test]). Although the 16 samples had OD values of anti-HEV IgM greater than the cutoff value, with the OD value ranging from 0.445 to 2.541, and the other four samples had OD values of anti-HEV IgA greater than the cutoff value, with the OD value ranging from 0.692 to 1.754, positivity for HEV antibodies could not be confirmed by the absorption test in any of the 20 samples (Table 2). Furthermore, none of these 20 serum samples with anti-HEV IgM or anti-HEV IgA alone had detectable HEV RNA, indicating that these serum samples were falsely positive for anti-HEV IgM or anti-HEV IgA in the ELISAs used.

Of note, among the 2,781 samples from subjects who were assumed not to have been recently infected with HEV in the present study, no serum sample was positive for both IgM and IgA anti-HEV antibodies (Fig. 1).

Detection of anti-HEV IgM and anti-HEV IgA in patients with hepatitis E. Serum samples obtained from 68 patients with sporadic acute or fulminant hepatitis E were tested for the presence of IgM and IgA anti-HEV antibodies. All 68 patients had anti-HEV IgM with OD values ranging from 0.486 to >3.0 and anti-HEV IgA with OD values ranging from 1.146 to >3.0 (Fig. 1). The presence of anti-HEV IgM and anti-HEV IgA was confirmed by the absorption test in the serum samples from all 68 patients, indicating that patients with virologic evidence of the early phase of HEV infection are positive for both anti-HEV IgM and anti-HEV IgA, in sharp contrast to the 20 patients in the control group who had anti-HEV IgM or IgA alone. This finding suggests that the combinatorial detection of both classes of antibodies (IgM and IgA) is efficient for serological diagnosis of hepatitis E with increased accuracy. Among the 68 patients with hepatitis E, four patients (5.9%) had an OD value of <1.000 in the anti-HEV IgM assay, and only one patient had an OD value of <1.500 in the anti-HEV IgA assay. All 68 patients had high levels (1.235 to >3.000) of anti-HEV IgG, 59 (86.8%), of whom had an OD value of >2.000.

Detection of anti-HEV IgM, anti-HEV IgA, and HEV RNA in follow-up serum samples from infected patients. Figure 2 shows the HEV RNA, anti-HEV IgM, and anti-HEV IgA profiles associated with the HEV infection in 15 patients (patients 1 to 15). From these 15 patients, in addition to the serum sample obtained at admission, 3 to 30 other serum samples, including those obtained between 20 and 40 days and between 50 and 70 days after the disease onset, were available. HEV RNA remained detectable in the serum until 7 to 40 (21.4 ± 9.7) days but disappeared 15 to 59 (32.7 ± 13.4) days after the onset of the disease. Anti-HEV IgM and IgA antibodies were both detectable up through the end of the observation period (50 to 144 [72.8 ± 28.5] days after disease onset) in 12 of the 15 patients. In the remaining three patients (patients 1, 3, and 5), both IgM and IgA anti-HEV antibodies were detectable until 37, 55, and 62 days, respectively, after disease onset, but either the IgM or IgA class of anti-HEV antibodies disappeared at 44, 62, and 104 days, respectively, after the disease onset. The presence of anti-HEV IgM and anti-HEV IgA was

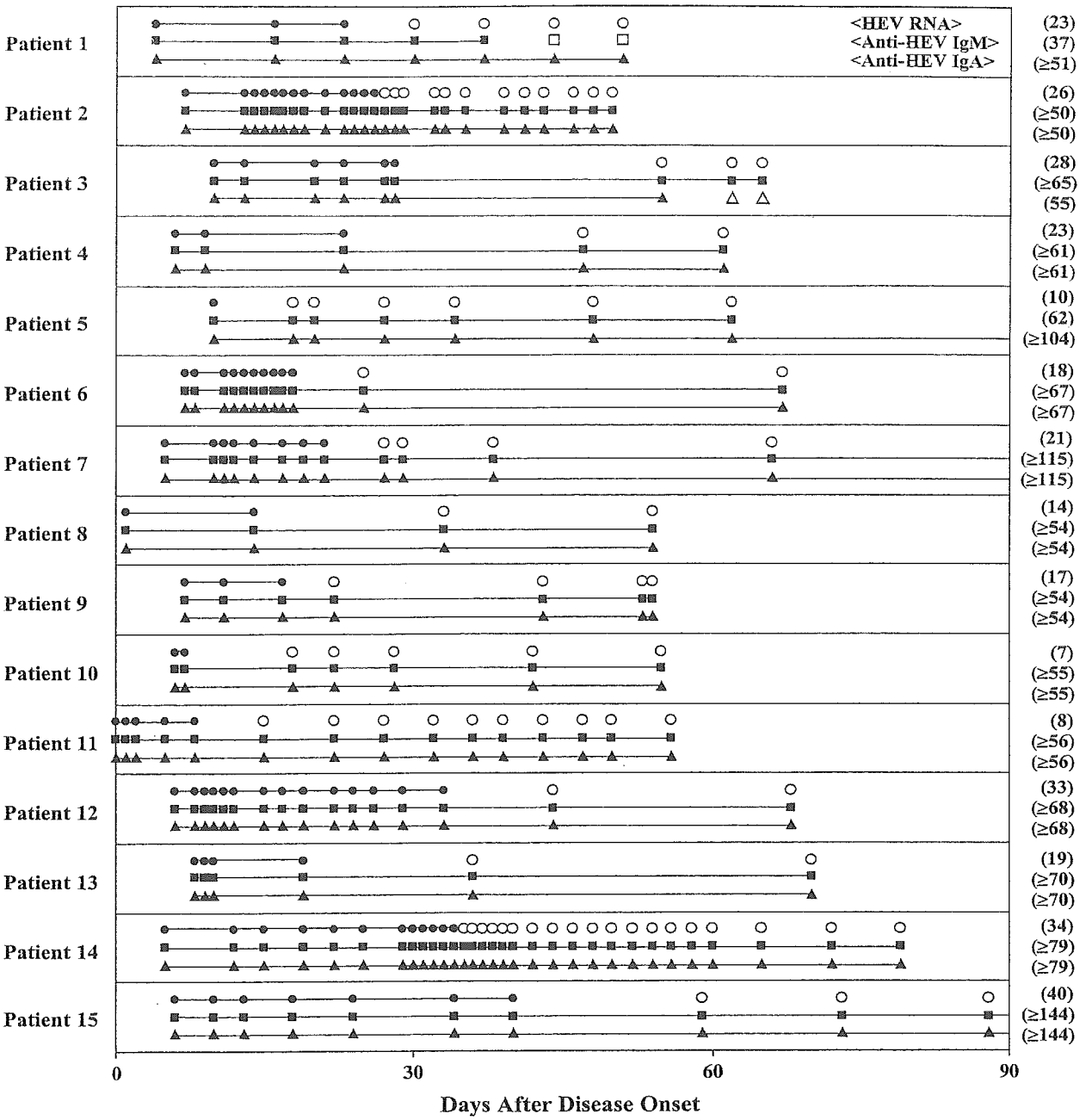

FIG. 2. Detection of HEV RNA, anti-HEV IgM and anti-HEV IgA in initial and follow-up serum samples from 15 patients (patients 1 to 15) with hepatitis E. For each patient, closed and open circles in the top row represent positivity or negativity for HEV RNA, respectively; closed and open boxes in the middle row represent positivity or negativity for anti-HEV IgM, respectively; and closed and open triangles in the bottom row represent positivity or negativity for anti-HEV IgA, respectively. The number in parentheses at the end of each row indicates the final day on which HEV RNA, anti-HEV IgM, or anti-HEV IgA was detectable. Patients 1 and 9 contracted fulminant hepatitis E and died 56 and 54 days, respectively, after onset of the illness.

also confirmed by the absorption test in the follow-up serum samples from all 15 patients, including the last two specimens from patients 1 and 3, who became positive for only anti-HEV IgA and IgM, respectively. The IgG antibody level was as high as 3.0 OD at admission in all 15 patients and persisted at a high level. There was no discernible reduction in the IgG antibody level up through the end of the observation period.

DISCUSSION

The diagnosis of acute or fulminant hepatitis E is based on detection of the HEV genome in serum or feces by RT-PCR (3, 5, 13, 25) or detection of newly elicited antibodies to HEV (3, 4, 14, 19, 25, 38, 49, 59). The presence of a specific antibody of the IgM class is diagnostic of recent or ongoing infection. As

is our in-house ELISA, a solid-phase (sandwich or indirect) ELISA method for detecting anti-HEV IgM is simple and is currently used in the majority of reported in-house ELISAs (3, 4, 14, 19, 25, 38, 49, 59), as well as in a commercial kit marketed in Asia by Genelabs Diagnostics (Singapore). One of the weaknesses of the solid-phase ELISA format is reduced sensitivity due to competition among virus-specific IgM, IgA, and IgG for antigen-binding sites. It has been pointed out that sensitivity is compromised when corresponding IgG titers are disproportionately higher than those of the IgM antibodies (16). Another potential weakness of the solid-phase test for IgM antibody is that IgM-rheumatoid factor in sera from patients with rheumatoid arthritis may elicit a false-positive result (10). Recently, to overcome these weaknesses in the solid-phase ELISAs, an IgM class capture system was introduced by Yu et al. (58). In the class capture system, competing IgG antibodies (also IgA antibodies) in the sample are eliminated at the beginning of the assay, thus enhancing the reaction between anti-HEV IgM and the HEV antigen, although its efficiency depends on the capacity of solidified antibodies against total human IgM molecules containing anti-HEV IgM to capture the HEV antigen. The class capture assay developed by Yu et al. (58) provided a reliable method for detecting anti-HEV IgM and had specificities comparable to those determined by the solid-phase assay when acute-phase sera with high anti-HEV IgM levels were tested and had higher sensitivity for samples with a low anti-HEV IgM concentration or with a high anti-HEV IgG concentration. However, as described by Seriwatana et al. (38), we had to stop developing an IgM class capture ELISA after initial experiments demonstrated poor sensitivity despite the use of substantially greater amounts of the recombinant HEV antigen and several monoclonal antibodies raised against recombinant HEV antigen with distinct specificities to detect the recombinant HEV antigen captured by anti-HEV IgM (unpublished observations). Therefore, in the present study, we chose the solid-phase ELISA format for detecting anti-HEV antibodies.

It has been reported that anti-HEV IgA can be utilized as an additional confirmatory antibody for recent HEV infection (2). Although the presence of a specific antibody of the IgA class is diagnostic for recent infection in several viral or nonviral diseases, including type A, type B, or type C acute hepatitis (28, 35, 57), as well as *Chlamydia trachomatis* infection, *Chlamydia pneumoniae* infection, and cholera (26, 33, 51), the clinical and epidemiological significance of positivity for anti-HEV IgA remains to be fully verified. In the present study, we used the IgM and IgA anti-HEV tests together to characterize serum specimens from 68 patients with acute or fulminant hepatitis E and from 2,781 subjects who were assumed to not have been recently infected with HEV as negative controls. With this dual testing, we obtained the following results. (i) Both anti-HEV IgM and anti-HEV IgA were detectable in serum samples obtained at admission from all 68 patients tested who were subsequently diagnosed molecularly as having hepatitis E (estimated sensitivity rate of the assay: 100% and 100%, respectively). (ii) Among the 2,781 serum samples collected from subjects who were assumed to not have been recently infected with HEV as negative controls, 16 samples (0.6%) were falsely positive for anti-HEV IgM alone and four samples (0.1%) were falsely positive for anti-HEV IgA alone, indicating that the false-positive rate was significantly lower in the anti-HEV IgA assay than in the anti-HEV IgM assay used ($P = 0.0139$) (the estimated specificity rates of the assays were 99.4 and 99.9%, respectively). (iii) Of the 2,781 serum samples collected from the subjects who were assumed to not have been recently infected with HEV, none was positive for both anti-HEV IgM and anti-HEV IgA (estimated specificity rate of the dual assay: 100%), indicating that an erroneous diagnosis of hepatitis E based on serological assay can be minimized by performing the anti-HEV IgM assay on samples that show positive results by the anti-HEV IgA assay or by performing combinatorial assay for anti-HEV IgA and anti-HEV IgM.

Regarding the duration of seropositivity for anti-HEV IgM, it has been reported that sera collected from patients during various hepatitis E outbreaks 3 to 4 months and 6 to 12 months after the onset of jaundice, 50 and 40%, respectively, were positive for anti-HEV IgM (7). In three cases of imported hepatitis E in Japan, the duration of seropositivity for anti-HEV IgM was 66, 112, and 154 days, respectively, from disease onset (19). Little is known about the duration of seropositivity for anti-HEV IgA in HEV-infected patients. Although the duration of observation was limited in the present study, anti-HEV IgA was detectable up through the end of the observation period (50 to 144 days after disease onset) in 14 of the 15 patients with hepatitis E and until 55 days after disease onset in the remaining one patient, suggesting that the durations of seropositivity for anti-HEV IgA and anti-HEV IgM, as determined by the assays that were used, are similar (Fig. 2).

In the circulation, IgA occurs in both monomeric and polymeric forms. Antibodies of the IgA class are unique in that they are produced in response to antigenic stimuli applied locally (48) and have distinct molecular forms. As for anti-HEV IgA, it is unclear whether our assay is detecting both dimeric secretory IgA and monomeric IgA, since the monoclonal antibody to IgA (A-13) that is used as an enzyme-labeled antibody in the present study can bind to various IgA species, such as secretory IgA and two subclasses of IgA (IgA1 and IgA2) (11, 28). However, it seems likely that only polymeric IgA antibody of either the IgA1 or the IgA2 subclass against HEV can be detected as described for IgA antibodies to hepatitis B core in type B acute hepatitis (11). Although an individual may have IgA deficiency, which may elicit a false-negative result in the anti-HEV IgA test, it has been reported that absence or deficiency (<1/100 of the average of normal controls) of total IgA was observed in only 4 (0.004%) of 93,020 apparently healthy blood donors and in 1 (0.01%) of 6,800 hospital patients in Japan: the absence of IgA was found at a frequency of 0.001% (31), indicating that false-negative results in the anti-HEV IgA assay due to the absence or deficiency of IgA in the circulation may be negligible.

Based on the results obtained in the present study, we conclude that, in solid-phase ELISA, the anti-HEV IgA assay is significantly more specific than the anti-HEV IgM assay with regard to ability to diagnose hepatitis E; that anti-HEV IgA could be the first-choice marker as a diagnostic indicator of recent HEV infection when the solid-phase ELISA method is used; and that the diagnostic accuracy increases when positive

results obtained by the anti-HEV IgA assay are confirmed by additional or simultaneous detection of anti-HEV IgM. However, due to the limited number of patients with hepatitis E enrolled in the present study, further studies are needed to verify our conclusions in larger cohorts.

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はじめに

E型肝炎ウイルス（HEV）はE型急性肝炎（一部は劇症肝炎）の起因ウイルスである。HEVは衛生環境が整っていない熱帯・亜熱帯の発展途上国に常在するウイルスであり，現在でも発展途上国では時に汚染された飲料水などを介した大規模な流行発生がみられ，医学的にも公衆衛生学的にも大きな問題となっている[1]。一方，日本を含む先進国では従前，E型肝炎は輸入感染症としてのみ散発発生すると認識されていた。しかし，近年，流行国への渡航歴のないE型肝炎症例が日本を含む先進国で少なからず認められ，ブタなどの動物がreservoirとなり得る人獣共通感染症であることが明らかにされた[1)~8)]。

HEVの血清型は1種類であるが，現在，ウイルス遺伝子RNAの配列の違いによって四つの遺伝子型（遺伝子型Ⅰ～Ⅳ）に分類され，アジア・アフリカの流行地域のHEVはⅠ型に，メキ

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Evaluation of "Immunis IgA anti-HEV EIA" (a kit for detection of IgA-class antibodies to hepatitis E virus) for the serological diagnosis of acute hepatitis E

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Key words：E型肝炎，急性肝炎，IgAクラス抗HEV抗体，EIA

シコでの流行株はII型に，そして欧米での散発性E型肝炎患者から分離されるHEV株はIII型に分類され，さらに中国や台湾，ベトナムなどのアジアの国々では散発性E型肝炎患者からI型の他にIV型に分類され得るHEV株が分離されている[9]。

日本では2001年に初めて，海外渡航によらない国内感染型のE型肝炎患者の存在が認識され報告されたが[10]，その後の調査によって1979年にすでに国内土着HEVによる感染例が存在し[11]，かつてA型肝炎ウイルス，B型肝炎ウイルス，そしてC型肝炎ウイルスの関与が否定され（非A非B非C型），海外渡航歴がないことから「原因不明の急性肝炎，あるいは劇症肝炎」と診断されていた患者の中に少なからずE型肝炎の患者が含まれていたことも明らかにされている[12][13]。それらの患者からは日本固有の土着株と想定されるIII型，ないしIV型のHEVが分離されている。

最近国内では，飼育ブタや野生のイノシシやシカなどの動物とE型肝炎患者から互いに酷似した塩基配列を有するHEV株が分離され，それら動物の内臓や肉を生や加熱不十分な状態で摂取したあとの劇症肝炎を含むE型肝炎発症事例が北海道や兵庫県，長崎県などで報告されている[14]~[17]。兵庫県の発症事例では，ウイルスが感染したシカの生肉が感染源であることを示すウイルス学的な直接証拠も得られている[14]。このような事実を踏まえ，厚生労働省は平成15年8月19日に「食肉を介するE型肝炎ウイルス感染事例について」と題する健康危険情報を公開し（http://www.mhlw.go.jp/houdou/2003/08/h0819-2.html），国民に注意を呼びかけている。そして，平成15年11月5日から施行された改正感染症法においてE型肝炎は新4類感染症に分類され，届出が義務づけられている。しかし，E型肝炎を診断するための体外診断用医薬品は皆無であるのが現状であり，E型劇症肝炎による死亡例も散見される中，臨床現場ではE型肝炎に対する簡便で特異的な診断法の登場が切望されている。

かかる状況下で，カイコ蛹で発現されたHEVのORF2抗原蛋白質（キャプシド蛋白質）[12]を固相抗原として使用し，horseradish peroxidase標識抗ヒトIgMマウスモノクローナル抗体を2次抗体としたIgMクラス抗HEV抗体の血清学的測定法が開発され，研究室レベルでのE型肝炎の血清診断に用いられた[12]。しかし，IgMクラス抗HEV抗体測定系一般の問題点として，非特異的反応による偽陽性検体が存在することが指摘されていた[18]。最近，horseradish peroxidase標識抗ヒトIgAマウスモノクローナル抗体を2次抗体としたIgAクラス抗HEV抗体の測定系が検討され，IgMクラス抗HEV抗体よりも非特異的反応が少なく，E型肝炎の診断法としてより適していることが報告された[19]。

今回，この報告に準拠して開発されたIgAクラス抗HEV抗体測定試薬「イムニスIgA anti-HEV EIA」(㈱特殊免疫研究所，東京都)のE型肝炎の血清診断における臨床的有用性について検討し，良好な成績を得たので報告する。

I．測定原理

酵素免疫測定（enzyme immunoassay[EIA]）法を応用した本検出系は，2段階の抗原抗体反応と酵素呈色反応とからなる（**図1**）。1次抗原抗体反応は，プレートに固相された精製リコンビナントHEV ORF2抗原蛋白質（遺伝子型IV）[12]と被検検体中の抗HEV抗体との間で起こる。2次抗原抗体反応は，固相抗原に結合したIgAクラスの抗HEV抗体と酵素標識抗体（peroxidase標識抗ヒトIgAマウスモノクローナル抗体）との間で起こる。検体中にIgAクラスの抗HEV抗体が存在すれば1次，2次反応が成立し，酵素反応により発色する。検体中のIgAクラスの抗HEV抗体量に応じて呈色物質が生成され，反応を停止後，波長450 nmで吸光度を測定し，判定する。

なお，非特異的反応を減らすための工夫の一つとして，検体希釈液中にMock蛋白質を添加した。Mock蛋白質とは非組換えのバキュロウ

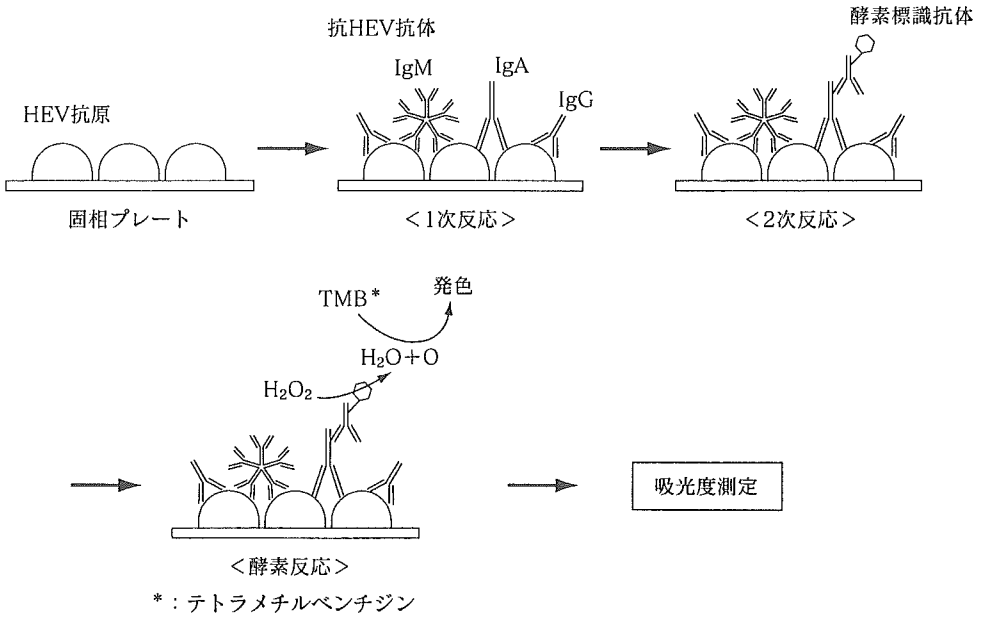

図 1 「イムニス IgA anti-HEV EIA」の測定原理

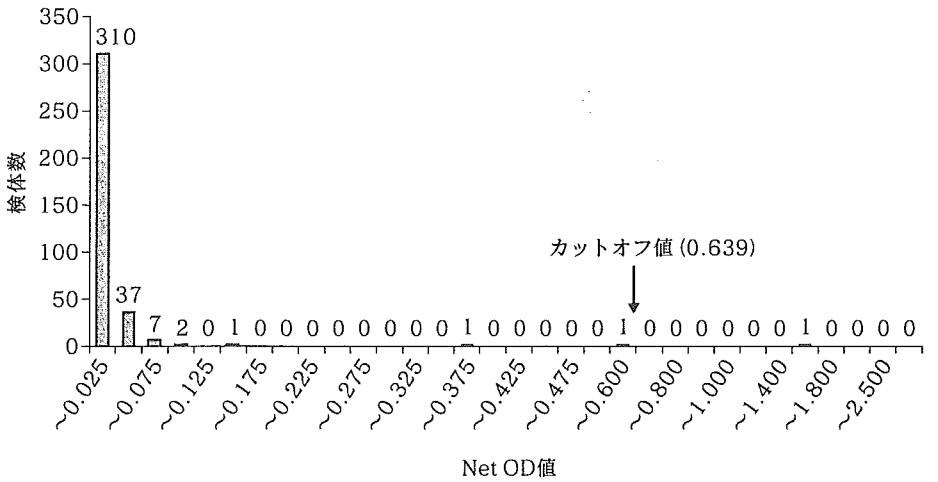

図 2 「イムニス IgA anti-HEV EIA」による健常人360検体でのIgAクラス抗HEV抗体測定：カットオフ値の設定

イルスを感染させたカイコ蛹の磨砕液である[19]。

II. カットオフ値の設定

「イムニス IgA anti-HEV EIA」キットを用いて健常人360名の血清検体についてIgAクラス抗HEV抗体を測定し，カットオフ値を求めた。カットオフ値の設定は，一般に用いられているカットオフ設定方法と同様，Takahashiらの報告に準じて[19]，「陰性(健常人)検体のNet OD値のMean+7SD」(Mean=0.023，SD=0.088)である0.639をカットオフ値とした(図

2)。360検体の中で，カットオフ値以上の吸光度を示した検体が1検体認められた。この検体はHEV RNAが陰性であり，リコンビナントHEV ORF2抗原蛋白質を用いた吸収試験により有意な吸収が認められなかったことから，この検体での反応は非特異的反応であると判定された。したがって，本カットオフ値での特異性は359/360×100%＝99.7%と良好であると判断された。

III. 対象および方法

1. 対象検体

本キットの性能を試験するための対象として，RT-PCR法によりHEV RNAが陽性であることが確認され，さらに感染HEVの遺伝子型も判定され，E型肝炎と確定診断された患者の初診時血清を用いた。その内訳は，I型HEVに感染したネパールのE型肝炎患者53例の血清，および日本国内のIII型HEVに感染した患者15例の血清とIV型HEVに感染した患者26例の血清である。また，非E型の対照検体として健常人（日本人）1,001名の血清を用いた。E型肝炎の94例については，A型肝炎ウイルス，B型肝炎ウイルス，およびC型肝炎ウイルスの重複感染はなかったことが確認されている。なお，本試験で用いた検体と，Takahashiら[19]が測定対象とした検体との重複はない。E型肝炎患者から提供された血清は，本試験への利用について各医療機関での十分な説明のうえで同意が得られたものである。

2. 方　法

1）「イムニス IgA anti-HEV EIA」を用いたIgAクラス抗HEV抗体の測定

㈱特殊免疫研究所が開発した「イムニス IgA anti-HEV EIA」キットを用いて，血清中のIgAクラス抗HEV抗体を測定した。測定手順，測定結果の判定はキットの添付文書に従って行った。吸収試験はTakahashiらの方法[19]に準拠して行った。

2）IgMクラスの抗HEV抗体の測定

血清中のIgMクラス抗HEV抗体の測定については，「イムニス IgA anti-HEV EIA」キットの酵素標識抗体をhorseradish peroxidase標識抗ヒトIgMマウスモノクローナル抗体（㈱特殊免疫研究所社製）に置き換えて測定した。カットオフ値は「II. カットオフ値の設定」で示したように，「イムニス IgA anti-HEV EIA」キットと同様，健常人360名の血清検体について測定した結果のMean+7SD（Mean＝0.044，SD＝0.051）の値から算定し，0.401とした。吸収試験はTakahashiらの方法[19]に従って行った。

3）IgGクラスの抗HEV抗体の測定

血清中のIgGクラス抗HEV抗体は，Mizuoらの方法[12]に従って測定し，カットオフ値を0.152とした。

4）HEV RNAの測定

特異性の確認のため，一部検体についてHEV ORF2領域のプライマーを用いたRT-PCR法[12]によってHEV RNAを測定した。

IV. 結　果

1,001検体の健常人検体において，IgAクラス抗HEV抗体はいずれもカットオフ値以下のOD値を示し非特異的反応は認められなかった。それに対して，IgMクラス抗HEV抗体は1検体（No. 759）がカットオフ値を大きく上回るOD値(1.320)を示した(**図3**)。このNo. 759検体はIgMクラス抗HEV抗体の吸収試験が陰性であり，IgGクラス抗HEV抗体とIgAクラス抗HEV抗体がともにカットオフ値以下のOD値を示した（それぞれOD値は0.028，0.021)。また，HEV RNAも陰性であったことから，IgMクラス抗HEV抗体測定の非特異的反応と判定された。したがって，1,001検体の健常人検体でのIgAクラス抗HEV抗体測定の特異性は100%であるが，IgMクラス抗HEV抗体測定の特異性は1検体の非特異的反応のため99.9%と算定された。

HEV RNA陽性のE型肝炎患者検体94検体についてIgAクラス抗HEV抗体とIgMクラス抗HEV抗体を測定し得られたOD値の分

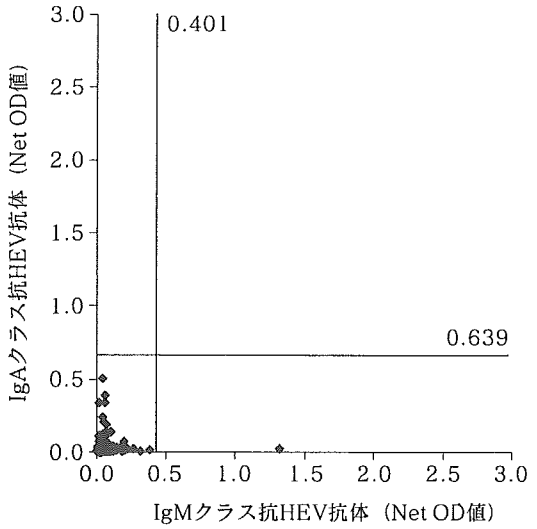

図3　健常人検体（1,001検体）について測定したIgAクラス抗HEV抗体とIgMクラス抗HEV抗体のOD値の分布図

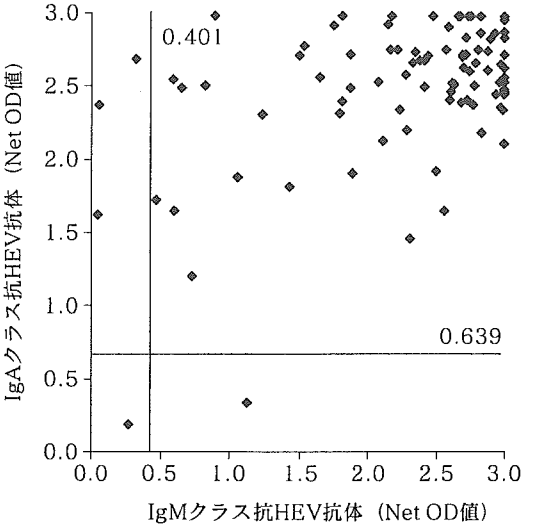

図4　E型肝炎患者検体（94検体）について測定したIgAクラス抗HEV抗体とIgMクラス抗HEV抗体のOD値の分布図

表1　E型肝炎患者検体におけるIgAクラス抗HEV抗体とIgMクラス抗HEV抗体の測定結果の比較

抗HEV抗体の種類	合　計 (n=94)	I型HEV陽性検体 (n=53)	III型HEV陽性検体 (n=15)	IV型HEV陽性検体 (n=26)
IgAクラス抗HEV抗体	92（97.9%）	53（100%）	14（93.3%）	25（96.2%）
IgMクラス抗HEV抗体	90（95.7%）	50（94.3%）	15（100%）	25（96.2%）

表2　抗HEV抗体がカットオフ値以下の測定値を示したE型肝炎患者検体

検体No.	遺伝子型	抗HEV抗体		
		IgGクラス（OD値）	IgAクラス（OD値）	IgMクラス（OD値）
1040（EN10）	I	**2.984**（+）	**2.690**（+）	0.318（−）
1072（EN42）	I	**2.980**（+）	**1.620**（+）	0.042（−）
1073（EN43）	I	**2.019**（+）	**2.374**（+）	0.049（−）
1089（EJ1）	III	**0.754**（+）	0.340（−）	**1.124**（+）
1117（EJ29）	IV	0.038（−）	0.188（−）	0.269（−）

カットオフ値以上のOD値を太字で示す。

布図を**図4**に示す。94検体のうち92検体（97.9%）でIgAクラス抗HEV抗体が陽性と判定された（**表1**）。その内訳は，I型HEV感染のE型肝炎患者検体では53例全例が陽性と判定され，III型HEV感染のE型肝炎患者からの15検体中14検体（93.3%）で，またIV型

HEV感染のE型肝炎患者からの26検体中25検体（96.2%）でIgAクラス抗HEV抗体が陽性と判定された。一方，IgMクラス抗HEV抗体は94検体中90検体（95.7%）で陽性であるに留まり，I型HEV感染患者では3検体，IV型HEV感染患者では1検体，合計4検体がカットオフ値以下のOD値を示し，陰性と判定された。

IgAクラス抗HEV抗体とIgMクラス抗HEV抗体のいずれか一方，あるいは両者が陰性と判定された5検体での遺伝子型と抗体クラス別の抗HEV抗体の測定値（OD値）を**表2**に示す。検体No. 1117はIgGクラス，IgAクラス，およびIgMクラスの抗HEV抗体のどれもが陰性であった。残りの4検体はいずれもIgGクラス抗HEV抗体が陽性であったが，3検体でIgMクラスの抗HEV抗体が陰性であり，1検体のみIgAクラスの抗HEV抗体が陰性であった。したがって，94検体についての検討結果からの評価ではあるが，感度の点でもIgAクラス抗HEV抗体（97.9%）はIgMクラス抗HEV抗体（95.7%）よりも優れていることがわかった。

V. 考　察

ウイルス感染における血清学的な抗体検査法として，受身血球凝集法や補体結合反応，中和試験などさまざまな方法が用いられるが，EIA法には測定が簡便で高感度である点と，IgG，IgM，IgAクラスに分けて測定できるという利点がある。

単一血清で急性期の感染を診断する場合には，A型肝炎の診断でのIgMクラス抗HAV抗体の測定やB型急性肝炎でのIgMクラス抗HBc抗体の測定などが具体例として挙げられるように，通常IgMクラスの抗ウイルス抗体の測定が行われている。E型肝炎の血清診断においても，文献的にはIgMクラス抗HEV抗体を測定する系が主として紹介されているが[20)~25)]，IgMクラスの抗体測定系一般の問題点として，リウマチ因子を含む臨床検体などでの非特異的反応による偽陽性が指摘されていた。

IgAクラス抗HEV抗体測定系については，1993年に流行地域でのHEV感染例，すなわちI型HEV感染患者を対象として最初にChauらによって報告され[26)]，2003年にTokitaらによってIII型HEV感染患者についての測定結果が報告されているが[27)]，ともにIgMクラス抗HEV抗体測定系の補足的な意義という評価に留まっていた。しかし，*Chlamydia pneumoniae*や*Chlamydia trachomatis*などの感染症の診断では，IgAクラス抗体測定系が保険適用の体外診断用医薬品として実際に臨床診断に応用されている[28)29)]。

最近Takahashiら[19)]は，IgMクラス抗HEV抗体測定系よりもIgAクラス抗HEV抗体測定系のほうが4倍も非特異的反応が少なかったということ（それぞれ，0.58% [16/2,781]，0.14% [4/2,781]），そして68例のE型肝炎患者からの初診時では両者に優劣はなく，ともに100%の感度でE型肝炎の血清学的な診断が可能であったことを報告した。

以上のような事実を踏まえ，今回，㈱特殊免疫研究所によってTakahashiらの方法に準拠したIgAクラス抗HEV抗体測定用試薬「イムニス IgA anti-HEV EIA」がキットとして開発されたことから，当該キットについてE型肝炎の血清診断を行ううえでの臨床的有用性について検討した。Takahashiらの論文[19)]に記載されている対象検体と，今回われわれが検討したE型肝炎症例94例の検体および対照検体1,001検体との重複は全くないことから，測定系の特異性と感度について新たな検体で評価できたことになる。

今回「イムニス IgA anti-HEV EIA」キットを用いてIgAクラス抗HEV抗体を測定し，その結果をキットの酵素標識抗体のみを抗ヒトIgAマウスモノクローナル抗体から抗ヒトIgMマウスモノクローナル抗体に置換してIgMクラス抗HEV抗体を測定した結果と比較検討した結果，特異性の点でも，さらに感度の点でもIgAクラス抗HEV抗体測定系のほ

表 3　既報の対照集団[19]についての「イムニス IgA anti-HEV EIA」キットの特異性の推定

検　体　群	IgA クラス抗 HEV 抗体陰性検体数	特異性（%）
A 型・B 型・C 型急性肝炎患者（127 例）	125	98.4
B 型・C 型慢性肝疾患患者（274 例）	274	100
原発性胆汁性肝硬変患者（147 例）	147	100
慢性関節リウマチ患者（186 例）	186	100
透析患者（472 例）	472	100
病院患者（900 例）	899	99.9
合計（2,106 例）	2,103	99.9

うが IgM クラス抗 HEV 抗体測定系よりも優れていることがわかった。

すなわち，IgA クラス抗 HEV 抗体を測定する「イムニス IgA anti-HEV EIA」キットでは，100%(1,001/1,001)の特異性と 97.9%(92/94) の感度が得られたのに対して，IgM クラス抗 HEV 抗体測定系では 99.9% (1,000/1,001) の特異性と 95.7% (90/94) の感度であった。

今回の試験対象には，一般に非特異的反応が出現しやすいとされている自己免疫性肝疾患患者や慢性関節リウマチ患者の血清検体は含まれていなかったが，「イムニス IgA anti-HEV EIA」キットは Takahashi ら[19]の方法に準拠して測定試薬として開発されたものであり，カットオフ値も全く別の検体を用いて測定し Mean+7SD から設定した値であるが，ともに近似していることから (0.639 vs 0.642)，カットオフ値として本キットの 0.639 を用い，特異性について検討した。その結果，「イムニス IgA anti-HEV EIA」キットの特異性は自己免疫性肝疾患患者や慢性関節リウマチ患者の血清検体を含む集団でも 99.9% (2,103/2,106) であると推定された（**表 3**）。

Takahashi らは 68 例の E 型肝炎患者の初診時血清を用いて IgA クラス抗 HEV 抗体を測定した結果，68 例全例で陽性であったことを報告している[19]。今回「イムニス IgA anti-HEV EIA」キットを用いて測定した 94 例では III 型 HEV 感染例の 1 例と IV 型 HEV 感染の 1 例で IgA クラス抗 HEV 抗体が陰性であった。それぞれ発症当日，発症から 3 日目に採取された検体であり，特に後者の検体では HEV RNA は陽性でありながら，IgA クラスの抗 HEV 抗体のみならず，IgG クラスおよび IgM クラスの抗 HEV 抗体も陰性であった。

他のウイルス感染症でも，例えば A 型肝炎ウイルス感染や B 型肝炎ウイルス感染，C 型肝炎ウイルス感染でも，感染初期にはウイルス核酸が検出されウイルス血症の状態にあってもまだウイルス関連抗原や抗体が検出されない空白期間があることはよく知られている[30)~32)]。したがって，今回の「イムニス IgA anti-HEV EIA」キットを用いて測定した結果で，94 例中 2 例で IgA クラス抗 HEV 抗体が陰性であったとしても，本キットの臨床的有用性を否定するものではないと考えられる。

現在国内では，保険適用にはなっていないが，E 型肝炎の血清診断用試薬として，Genelabs 社がアジア向けとしてマレーシアで販売している IgM クラス抗 HEV 抗体測定用試薬が試薬輸入業者を通じて入手可能である。しかし，IgA クラス抗 HEV 抗体がすべて陽性であった 68 例の E 型肝炎患者血清[19]のうち，Genelabs 社のキットで IgM クラス抗 HEV 抗体が陽性と判定できたのはわずか 61 例 (89.7%) にすぎなかった（未発表データ）。したがって，「イムニス IgA anti-HEV EIA」キットは現在入手可能な最も特異性および感度に優れた E 型肝炎

の血清診断試薬であると考えられる。

結　語

以上の結果から，「イムニス IgA anti-HEV EIA」キットは特異性，感度ともに優れ，臨床現場でのE型急性肝炎の血清診断に極めて有用な試薬であると判断された。

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ヒト

E型肝炎の重症例

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索引用語：E型肝炎ウイルス，劇症肝炎，急性肝炎重症型，亜急性肝炎，薬剤性肝障害

1 はじめに

E型肝炎は，E型肝炎ウイルス(HEV)によって引き起こされる肝炎である．これまでは，主に糞口感染(主に水系感染)によって感染する肝炎として知られ，その発生には地域差があり，赤道周囲の国や地域(東南アジア，アフリカ，中南米など)，いわゆる開発途上諸国で多く発生しているとされてきた[1,2]．そのため，わが国では，その流行地域へ旅行に行った際に感染して帰国後に発症する「輸入感染症」の一つとして考えられていた．しかし，近年，海外渡航歴のない人がE型肝炎を発症する国内感染もあることが次第に判明してきて，国内株のHEVも存在することが明らかとなり[3]，注目されている．さらに，われわれの検討により原因不明の劇症肝炎の中にE型劇症肝炎例が存在することが初めて明らかとなり[4]，その後の実態調査も進められている．一方，HEVの感染様式については，最近，人畜共通感染症(Zoonosis)を間接的あるいは直接的に証明する報告[5~8]が相継いでおり，また，輸血後E型肝炎の報告[9]もある．

本稿では，われわれの施設で経験した急性肝炎および劇症肝炎におけるE型肝炎の実態とE型肝炎の重症例(重症化および劇症化した症例)を中心に述べる．

2 E型急性肝炎および劇症肝炎の頻度

1998年から2002年までにわれわれの施設で経験した急性肝炎(重症型を含む) 166例について，入院時の保存血清を用いてHEV-RNAを測定した．A型肝炎，B型肝炎，C型肝炎，薬剤性肝炎，自己免疫性肝炎およびアルコール性肝炎と診断された例，EBウイルス，サイトメガロウイルス，TTウイルスなどの肝炎ウイルス以外のウイルスによる急性肝炎例を除くと，残りの25例が原因不明の急性肝炎であった．このうち4例(16%)が

Ichiro KUMAGAI et al: The severe form of hepatitis E in Japan
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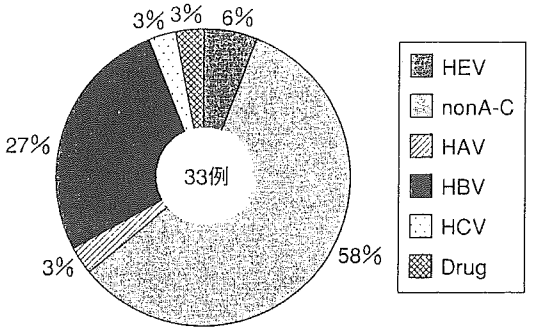

図1　劇症肝炎におけるHEVの頻度(岩手医科大学第一内科)

HEV-RNA陽性を示し，E型急性肝炎と診断された[10]．これらの遺伝子型はいずれもⅢ型であった．一方，当科で経験した劇症肝炎(1992～2001年) 33例のうち非A非B非C型(非A-C型)の劇症肝炎と診断されたのは22例であるが，入院時の血清が保存されていた19例についてHEV-RNAを測定したところ，2例(10.5％)が陽性を示した．したがって，劇症肝炎全体では6％の頻度となる(図1)[11]．

わが国における非A-C型の急性肝炎におけるE型急性肝炎あるいは劇症肝炎の頻度に関するこれまでの報告をみると，多施設共同による原因不明の急性肝炎例におけるE型肝炎の検討[12]では，北海道地域において25％と高い．大西ら[13]も過去5年間に経験した原因不明の急性肝炎の中で血清が保存されていた59例中16例(27.1％)にE型(血中HEV-RNA陽性)を認めている．また，松井ら[14]は非A-C型急性肝炎35例中5例(14％)に，劇症肝炎10例中1例(10％)にE型を認め，小島ら[15]は非A－C型急性肝炎104例中5例(4.8％)に，劇症肝炎21例中1例(4.8％)にE型を認めている．このように，HEV感染による急性肝炎の発生頻度には地域差がみられている．一方，劇症肝炎におけるE型の頻度については，難治性の肝疾患に関する研究班の2003年度総括研究報告書[16]によると，劇症肝炎，遅発性肝不全(LOHF)の全国集計に登録された症例中A，B，C型肝炎ウイルスの感染が明らかでない症例は，2000～2001年には124例が登録されており，そのうち血清供与などで測定可能であった38例でHEV-RNAを測定したところ2例が陽性(5.3％)であった．また，2002年には同様の症例が69例登録され，うち24例でHEV-RNAが測定され2例が陽性(8.3％)であった．そして，これら4例はすべて北海道からの登録症例であり，これらの遺伝子型はすべてⅣ型であった．

3　E型肝炎の重症例の臨床像

HEV感染の多くは不顕性に経過するが，感染者の一部が2～9週(平均6週)の潜伏期を経て急性肝炎を発症する．多くの症例は経過が良好とされている．

しかし，稀に重症化し，E型肝炎の重症化または劇症化例の報告もみられるが，その頻度は少ない[4,11,17]．特に，妊娠後期の妊婦が罹患すると高率に劇症化すると報告されている[18]が，これまでのところわが国の妊婦からの発症例は報告がない．

われわれの施設では，これまで2例の劇症肝炎[11]と3例の重症肝炎(1例は急性肝炎重症型，1例は亜急性肝炎，1例はLOHF)，および1例のHEVが関与して死亡に至った急性肝炎を経験している(表1)．6例中3例は中年の女性で，また，6例中3例は薬剤性肝障害の合併がみられ重症化には薬剤の関与も考えられた[11,19]．いずれの症例も過去1年以内の海外渡航歴はなく，感染経路は不明であった．遺伝子型は劇症化した1例はⅣ型であったが，他の5例はⅢ型であった．