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Motowo Mizuno[*] Gotaro Yamada[†] Yuzi Sakamoto[‡]

Takashi Nishihara[**] Yasuhiro Yumoto[††]

Yasuo Moritsugu[‡‡] Hideo Nagashima[§]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,
[††]National Shikoku Canter,
[‡‡]National Institute of Health,
[§]Okayama University,

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Abstract

Serum specimens from 12 patients with type A hepatitis were analyzed for immunoglobulin M-type antibody to hepatitis A virus (IgM anti-HA). A recently developed solid-phase radioimmunoassay kit for IgM anti-HA (HAVAB-M, Abbott Laboratories) and a competitive binding radioimmunoassay kit (HAVAB, Abbott Laboratories) with or without 2-mercaptoethanol treatment, as modified by Yano et al. (Acta Hepatol. Jpn. 21, 704-712, 1980) were used to obtain an M-index. All specimens obtained within 60 days of the onset of illness and specimens from 2 of 4 patients later than 60 days after the onset were positive with the HAVAB-M test. This test gave negative results to sera which were positive for anti-HA by a standard HAVAB test in the following: 3 patients with type B hepatitis; 5 with non-A, non-B hepatitis; 11 healthy adults; and 10 sera strongly positive for rheumatoid factor. The M-index for type A hepatitis in sera within 30 days of the onset (mean value of the M-index, m, = 1.52; standard deviation, SD, = 0.25) was significantly higher than that for non-A hepatitis (m = 1.05; SD = 0.15) and for healthy adults (m = 1.02; SD = 0.10). The simplicity and usefulness of the HAVAB-M test in diagnosis of acute type A hepatitis over those measuring the M-index by HAVAB tests were shown by direct comparison of the results.

KEYWORDS: type A hepatitis, IgM, anti-HA, radioimmunoassay.

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SERODIAGNOSIS OF TYPE A HEPATITIS BY DETECTION OF IMMUNOGLOBULIN M-TYPE ANTIBODY TO HEPATITIS A VIRUS

Motowo MIZUNO, Gotaro YAMADA, Yuzi SAKAMOTO, Takashi NISHIHARA, Yasuhiro YUMOTO*, Yasuo MORITSUGU** and Hideo NAGASHIMA

First Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan;
**Department of Internal Medicine, National Shikoku Cancer Center, Matsuyama 790, Japan and*
***Enterovirus Division, National Institute of Health, Tokyo 190-12, Japan*
(Director: Prof. H. Nagashima)

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Abstract. Serum specimens from 12 patients with type A hepatitis were analyzed for immunoglobulin M-type antibody to hepatitis A virus (IgM anti-HA). A recently developed solid-phase radioimmunoassay kit for IgM anti-HA (HAVAB-M, Abbott Laboratories) and a competitive binding radioimmunoassay kit (HAVAB, Abbott Laboratories) with or without 2-mercaptoethanol treatment, as modified by Yano *et al.* (Acta Hepatol. Jpn. 21, 704-712, 1980) were used to obtain an M-index. All specimens obtained within 60 days of the onset of illness and specimens from 2 of 4 patients later than 60 days after the onset were positive with the HAVAB-M test. This test gave negative results to sera which were positive for anti-HA by a standard HAVAB test in the following: 3 patients with type B hepatitis; 5 with non-A, non-B hepatitis; 11 healthy adults; and 10 sera strongly positive for rheumatoid factor. The M-index for type A hepatitis in sera within 30 days of the onset (mean value of the M-index, $\bar{m}$, = 1.52; standard deviation, SD, = 0.25) was significantly higher than that for non-A hepatitis ($\bar{m}$ = 1.05; SD = 0.15) and for healthy adults ($\bar{m}$ = 1.02; SD = 0.10). The simplicity and usefulness of the HAVAB-M test in diagnosis of acute type A hepatitis over those measuring the M-index by HAVAB tests were shown by direct comparison of the results.

Key words : type A hepatitis, IgM, anti-HA, radioimmunoassay.

In the early diagnosis of hepatitis A virus (HAV) infection, significant progress has been achieved through the development and application of several techniques for the detection of a specific immunoglobulin M (IgM)-type antibody to HAV (IgM anti-HA). Procedures of separation of IgM anti-HA from immunoglobulin G-type anti-HA (IgG anti-HA) used are sucrose density gradient ultracentrifugation (1), blocking of IgM anti-HA by anti-human IgM antibody (μ-chain specific) (2), absorption of IgG anti-HA by staphylococcal protein A (3) and treatment with 2-mercaptoethanol (2-ME) to inactivate IgM anti-HA (4). Because a commercially available competitive binding radioimmunoassay (CBRIA) kit for anti-HA (HAVAB, Abbott Laboratories) is relatively simple, we have employed it with treatment with 2-ME for the diagnosis of type A hepatitis.

Recently a solid-phase radioimmunoassay for IgM anti-HA (IgM-SPRIA)

was developed using anti-human IgM antibody (μ-chain specific) bound to the solid-phase for separating IgM antibody from specimens (5, 6). We used this IgM-SPRIA kit (HAVAB-M, Abbott Laboratories) for the diagnosis of type A hepatitis.

In this report we describe the results of the clinical application of the IgM-SPRIA kit and compare the results with those obtained with the treatment of 2-ME method.

MATERIALS AND METHODS

Serial specimens were obtained within 4 months of the onset of illness from 12 patients with type A hepatitis diagnosed by immune adherence hemagglutination (IAHA) (7). The elevation of antibodies to HAV in these cases was also ascertained on paired sera by the CBRIA test (HAVAB kit, kindly supplied by Abbott Laboratories, North Chicago, IL.) (8). As controls, various sera were collected from 8 patients with acute type B hepatitis, 9 patients with acute non-A, non-B hepatitis within 30 days of the onset of illness and from 50 healthy adults. All of these were tested for anti-HA by the CBRIA test. Sera positive for anti-HA by CBRIA from 3 patients with type B hepatitis, 5 patients with non-A, non-B hepatitis (4 cases of non-B posttransfusion hepatitis and 1 of sporadic non-B hepatitis without change in titers of anti-HA by IAHA between paired sera) and from 11 healthy adults were used for further specificity controls of IgM anti-HA. Thirteen serum specimens strongly positive for rheumatoid factor (RF) and sera from 3 patients with infectious mononucleosis were also prepared for detection of IgM anti-HA.

IgM anti-HA was estimated in the serial specimens of type A hepatitis and the control sera by the CBRIA test modified by incorporating a step of the treatment with 2-ME (4) and the IgM-SPRIA test (HAVAB-M kit, kindly supplied by Abbott Laboratories, North Chicago, IL.). The modified CBRIA test with 2-ME was performed and the ratio, M-index, of the cpm of the treated specimen with 2-ME to the cpm of the untreated one was calculated according to the method given in (4). The IgM-SPRIA test was performed following the instructions of the kit[1]. The activity of IgM anti-HA was expressed as the cpm ratio of a specimen to the cutoff value (S/CO). Specimens with S/CO more than 1.0 were considered positive for IgM anti-HA.

RESULTS

The results for anti-HA by IAHA and CBRIA on paired sera from the 12 patients with type A hepatitis are shown in Table 1. In 11 cases, significant rises in titers of anti-HA were demonstrated by IAHA. In case No. 12, anti-HA was not detected by IAHA up to 53 days after the onset of illness, whereas serum 14 days after the onset was already positive for anti-HA by CBRIA. In contrast to the results by IAHA, all 12 sera obtained within 30 days of the onset were already positive for anti-HA by CBRIA.

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1. Antibody to hepatitis A virus ^{125}I (Human) / Hepatitis A virus (Primate) / Antibody to human IgM (Goat) coated beads. HAVAB-M. Radioimmunoassay for the detection of IgM antibody to hepatitis A virus. Abbott Laboratories, North Chicago, IL., May 1980.

The control sera collected from the 8 patients with acute non-A hepatitis (3 of type B hepatitis and 5 of non-A, non-B hepatitis) within 30 days of the onset and from the 11 healthy adults were positive for anti-HA by CBRIA (Table 2). Anti-HA was also detected in 10 of the 13 sera strongly positive for RF by CBRIA (Data not shown).

The results of the modified CBRIA test with 2-ME for the detection of IgM anti-HA on the acute phase sera collected within 30 days of the onset from the 12 patients with type A hepatitis are presented in Table 1 and the control sera in Table 2. The M-index for type A hepatitis ranged from 1.23 to 2.12 (a mean value of the M-index, $\bar{m}$, = 1.52: standard deviation, SD, = 0.25) (Fig. 1). The M-index ranged from 0.81 to 1.28 ($\bar{m}$ = 1.05; SD = 0.15) for the 8 patients with non-A hepatitis and from 0.86 to 1.14 ($\bar{m}$ = 1.02; SD = 0.10) for the 11 healthy adults (Fig. 1). The M-index in the acute phase sera for type A hepatitis was

TABLE 1. ANTI-HA TESTED BY IAHA AND CBRIA IN PAIRED SERA FROM 12 PATIENTS WITH TYPE A HEPATITIS AND IgM ANTI-HA ASSAYED BY THE MODIFIED CBRIA WITH 2-ME (M-INDEX) AND THE IgM-SPRIA (S/CO) IN ACUTE PHASE SERA

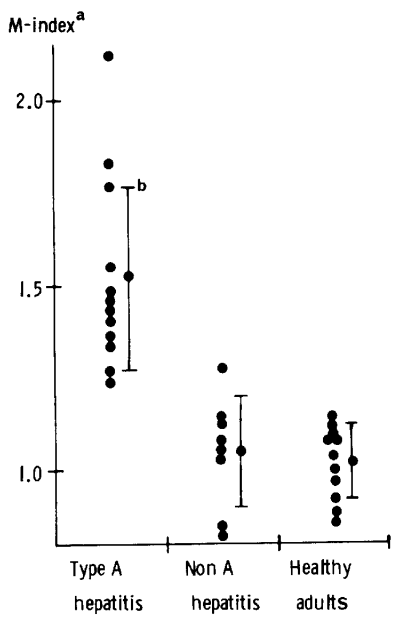
Case No.	Days after onset at serum collection	Anti-HA titers by IAHA	Anti-HA % inhibition by CBRIA	IgM anti-HA	
				M-index	S/CO
1	15	<8	92	1.15	4.5
	49	256	100	—	—
2	10	<8	88	1.47	5.3
	72	512	100	—	—
3	14	<8	97	1.46	3.6
	89	64	100	—	—
4	10	<8	85	1.45	3.4
	64	512	100	—	—
5	15	<8	95	1.77	4.4
	84	4096	100	—	—
6	7	<8	65	2.12	1.9
	42	32	96	—	—
7	12	<8	92	1.84	5.1
	50	256	100	—	—
8	14	<8	96	1.23	4.4
	53	1024	100	—	—
9	18	<8	94	1.33	4.0
	113	2048	100	—	—
10	12	<8	96	1.36	4.6
	160	512	99	—	—
11	7	<8	92	1.27	5.6
	32	32	99	—	—
12	14	<8	93	1.40	4.1
	53	<8	100	—	—

significantly higher than that for non-A hepatitis and the healthy adults ($p < 0.001$).

Fig. 2 shows the temporal profile of the M-index in the specimens from the 12 patients with type A hepatitis. In all the cases the M-index was dropped sharply between 30 and 60 days after the onset.

The sera obtained from the 12 patients with type A hepatitis were tested for IgM anti-HA by the IgM-SPRIA test. The S/CO ratios of the acute phase sera ranged from 1.9 to 5.6 (Table 1 and Fig. 3). No reactivity was shown toward the control sera, of which the S/CO ranged from 0.5 to 0.7 (Table 2 and Fig. 3). No false positive reaction was observed in the 13 strongly RF-positive sera including the 10 specimens also positive for anti-HA by a standard CBRIA test (Fig. 3).

The S/CO ratio for type A hepatitis was plotted relative to time up to 113 days after the onset of illness (Fig. 4). IgM anti-HA could be detected by the IgM-SPRIA test in all specimens collected within 60 days of the onset and in specimens from 2 patients up to 87 and 107 days.



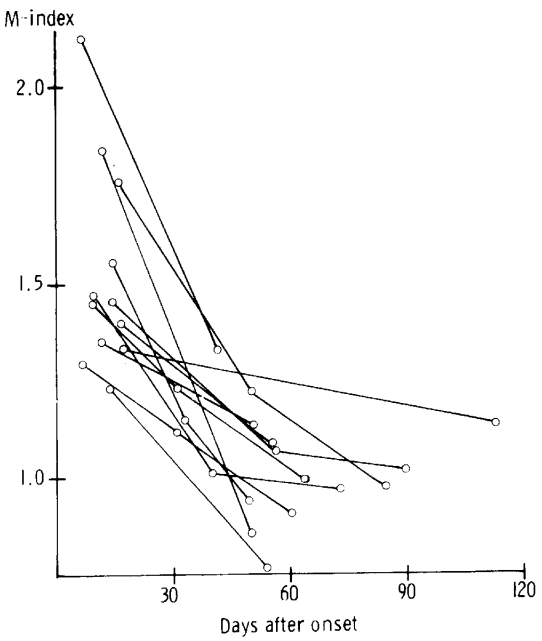


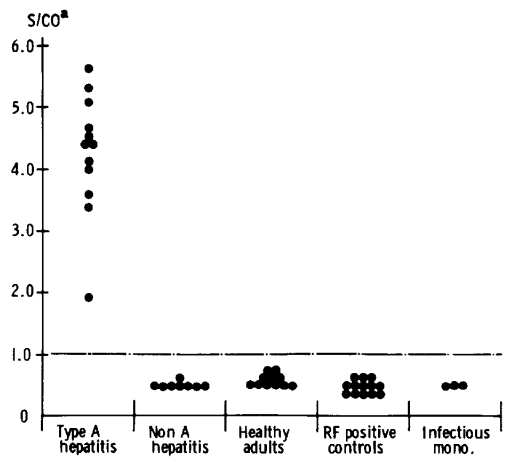
Fig. 1. *(Left)* The M-index by the modified CBRIA test with 2-ME in acute phase sera from 12 patients with type A hepatitis and in the control sera.

a; Definition of the M-index is described in text. b; A mean value of the M-index ± Standard deviation.

Fig. 2. *(Right)* Serial determinations of the M-index by the modified CBRIA test with 2-ME in sera from 12 patients with type A hepatitis.

TABLE 2. ANTI-HA TESTED BY CBRIA AND IgM ANTI-HA ASSAYED BY THE MODIFIED CBRIA WITH 2-ME (M-INDEX) AND THE IgM-SPRIA (S/CO) IN CONTROL SERA COLLECTED FROM 3 PATIENTS WITH ACUTE TYPE B HEPATITIS, 5 PATIENTS WITH ACUTE NON-A, NON-B HEPATITIS AND FROM 11 HEALTHY ADULTS

Cases		Days after onset at serum collection	Anti-HA % inhibition by CBRIA	IgM anti-HA	
				M-index	S/CO
Acute type B hepatitis	1	25	90	0.85	0.5
	2	12	100	1.08	0.6
	3	15	100	1.14	0.5
Acute non-A, non-B hepatitis	1	27	96	1.07	0.5
	2	14	99	1.15	0.5
	3	19	88	1.04	0.5
	4	14	75	0.81	0.5
	5	10	96	1.28	0.5
Healthy adults	1	—	99	1.04	0.6
	2	—	99	1.13	0.5
	3	—	99	1.08	0.6
	4	—	99	1.14	0.7
	5	—	100	1.08	0.7
	6	—	99	0.97	0.6
	7	—	98	1.00	0.5
	8	—	96	1.11	0.5
	9	—	99	0.92	0.5
	10	—	99	0.88	0.5
	11	—	98	0.86	0.5



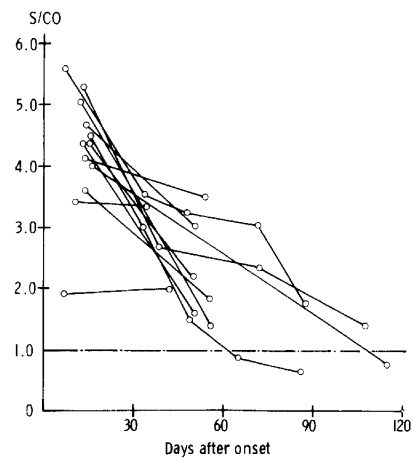


Fig. 3. *(Left)* Reactivity of the IgM-SPRIA test for IgM anti-HA toward acute phase sera from 12 patients with type A hepatitis and the control sera.

a ; Definition of the S/CO is described in text.

Fig. 4. *(Right)* Serial determinations of the S/CO by the IgM-SPRIA test in sera from 12 patients with type A hepatitis.

DISCUSSION

HAV infection has been routinely diagnosed by demonstrating a rise in the titer of anti-HA from the acute to the convalescent phase, IAHA being employed extensively to detect anti-HA (7, 9, 10). In many situations, however, both acute and convalescent phase sera taken at an appropriate intervals were not available, and in all situations it would be preferable to make a diagnosis on a single serum specimen obtained during acute illness without having to wait for a sample of convalescent serum. The illness caused by HAV infection, moreover, has been shown to be mild and of limited duration (11), not to progress to chronic liver disease (12), and timely administration of immune serum globulin prevents its spread to the contacts of patients with type A hepatitis (13). Early diagnosis of acute hepatitis A, therefore, is important to assessing the prognosis of the disease and to know what kind of ISG to give to contacts of the patient. Several promising approaches to early serodiagnosis have been developed for the detection of IgM anti-HA, accepted as an early antibody response of the host against HAV (14).

Attempts along this line have been to demonstrate negative results by IAHA in serum samples positive for anti-HA tested by RIA (8), IEM (15) or enzyme linked immunosorbent assay (ELISA) (16), taking advantage of the failure to detect IgM antibodies by IAHA (2, 11, 16). In our cases shown in Table 1, differences in the results for IAHA and CBRIA were observed in all the acute phase samples and in 1 convalescent sample from case No. 12 obtained 53 days after the onset. Negative results by IAHA were shown in specimens positive for anti-HA by CBRIA.

Reducing agents such as 2-ME tend to break the linking disulfide bonds of IgM and cause dissociation of IgM molecules into 5 subunits which are immunologically inactive (18). Breakage of the disulfide bonds of IgG seems unlikely, because the mean M-index in control subjects was close to 1.0. Although the M-indices for type A hepatitis (Fig. 1) were significantly higher than those for the control, only 4 of the 12 patients gave a positive result when 1.5 (Table 1) was used as the positive cut off boundary according to Yano *et al.* (4). Accordingly, an M-index of 1.2 seems more promising in making a better separation of cases for early diagnosis of type A hepatitis, although the number of cases studied in the present work was limited.

The IgM-SPRIA test showed no reactivity toward control sera positive for anti-HA by CBRIA (Fig. 3). The test could clearly distinguish anti-HA indicating recent HAV infection (IgM anti-HA) from pre-existing anti-HA reflecting past HAV infection (IgG anti-HA) which appears to persist for many years and make the diagnosis of type A hepatitis on the basis of anti-HA detected in a single serum specimen difficult (19). It is reported that RF may occasionally lead to a false positive result especially when anti-human IgM is used as the second labeled antibody (20, 21). In assays based on the same principle as the IgM-

SPRIA test where anti-human IgM is used as the first antibody bound to the solid-phase, 3 false positive results occurred in 11 RF positive sera (6), but in another study, no false positive results were found when 50 RF-positive sera were tested (5). In our study, no false positive reaction was observed in the 13 RF-positive sera (Fig. 3).

IgM anti-HA could be detected in sera from patients with type A hepatitis for 90 to 150 days (6) and for 12 months (5) after the onset of illness by assays based on the same principle. We determined IgM anti-HA serially in sera from 4 cases up to about 4 months after the onset by the IgM-SPRIA test. In 2 cases, IgM anti-HA was still detectable 87 and 107 days after the onset (Fig. 4). All specimens collected within 60 days of the onset were positive for IgM anti-HA by the IgM-SPRIA test (Fig. 4). The greater sensitivity of the IgM-SPRIA test makes a serological diagnosis of acute HAV infection possible in a single serum specimen obtained in the early convalescent phase up to at least 60 days after the onset of illness, even when clinical recognition of hepatitis is delayed and an acute phase serum is not available.

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