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Anti-HBc IgM and Anti-delta Screening by EIA Method*

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The clinical value of an enzyme-linked immunosorbent assay (ELISA) for the detection of anti-HBc IgM was evaluated by testing 202 sera from acute viral hepatitis B (AVHB), hepatitis B (HB), chronic hepatitis (CAH), chronic liver disease (CLD), cirrhosis, primary hepatoma, HBsAg carrier, acute viral hepatitis A (AVHA), hepatitis A (HA), non-A, non-B (NANB) hepatitis and miscellaneous conditions other than hepatic disease, and 19 additional various hepatic disease cases were examined for anti-delta. In clinical situations the accurate diagnosis of HB is not always possible and the differential diagnosis seems to be very important especially in making decisions of treatment and estimation of prognosis. In overall cases the highest positive rate of anti-HBc IgM was found in AVHB as shown as 74.3% (26/35) comparing to other conditions in which the positive rate was extremely low (2.1%). The anti-HBc IgM appeared to be highly specific to AVHB (83.9%) as compared to the other. The positive rate of HBsAg was high in AVHB, CAH and HBsAg carrier (100.0%) followed by CLD, cirrhosis and HB (up to 70.8%). The ALT activities and ALP_{alb} fractions were significantly high in AVHB ($p < 0.005$). The correlation between the positivity of anti-HBc IgM and highly abnormal ALT appeared be high. AVHB was confined mostly to 10 – 20 age group and the male to female ratio was about 6 to 1. Subgroup of AVHB II with positive anti-HBc IgM appeared to have a greater chance being positive for HBsAg and ALP_{alb}. The S/N ratio of anti-HBc IgM was as high as 20 which was unique to AVHB. It was unremarkable in other conditions. A significant correlation was found between the highest range of S/N and ALT. No positive case was observed in anti-delta test for various hepatic diseases.

Key Words: *Anti-HBc IgM, anti-HBc, HBsAg, anti-HBs, HBe Ag, anti-HBe, anti-delta, HBV DNA, HBV DNA polymerase, S/N ratio*

INTRODUCTION

Despite the availability of very sensitive immunoassays for all serologic markers for diagnosing hepatitis B (HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc) it is often difficult to differentiate acute, recent, or remote infections from chronic hepatitis.

All of these hepatitis B (HB) may be characterized by persistence of the same set of serological markers (Mushahwar et al., 1981; Chang, 1983). Also the ability to distinguish acute viral hepatitis B (AVHB) from non-A, non-B (NANB) hepatitis in an asymptomatic HBsAg carrier is important in epidemiological and clinical aspects. However this is not resolvable with current HB virus (HBV) markers.

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Furthermore sometimes HBsAg and HBeAg are transient and may be undetectable in some cases of AVHB. It is difficult to make the diagnosis of AVHB in these occasions and anti-HBc would be a primary marker. But because this persist for many years anti-HBc does not distinguish AVHB from a past infection. Recently anti-HBc IgM, a new class of anti-HBc was discovered and has been proposed as a serological marker for differentiating sequential stages of AVHB (Lemon et al., 1981; Roggendorf et al., 1981; Feinman et al., 1982).

Although the results are still equivocal in relation to this antibody through convalescence, recovery or HBsAg carrier, the presence of anti-HBc IgM theoretically indicates AVHB (Lemon et al., 1981; Roggendorf et al., 1981; Feinman et al., 1982; Chau et al., 1983; Brzosko et al., 1975; Cohen, 1978; Kryger et al., 1981).

An attempt to screen the presence of anti-HBc IgM in various hepatic diseases and the other non-hepatic miscellaneous disease was made to establish a direction of further evaluation for the accurate diagnosis and differential diagnosis in hepatitis B. Also attempted is a partial screening of anti-delta test in similar conditions.

MATERIALS AND METHODS

Specimens

The serum specimens were collected from patients with various hepatic diseases and patients without hepatic disease. 35 cases with AVHB, 27 cases with HB, 19 cases with chronic active hepatitis (CAH), 7 cases with chronic liver disease (CLD), 26 cases with cirrhosis, 7 case with primary hepatoma, 8 cases of healthy HBsAg carrier, 10 cases of acute viral hepatitis A (AVHA), 3 cases of hepatitis A (HA), 3 cases of NANB hepatitis and 47 cases of miscellaneous conditions other than hepatic disease were used.

Serological methods

HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBe and anti-delta were measured by commercially available EIA (Auszyme II, Ausab-EIA, Corzyme, HBe-EIA, Abbott Laboratories, North Chicago III.).

Tests for anti-HBc IgM and its S/N ratio

Also commercially available Corzym-M EIA (Abbott Lab.) was used. It is a solid phase EIA which uses the sandwitch principle.

In the first incubation, antibody specific to human IgM coated on polystyren beads captures IgM in patient serum. In the second incubation, HBcAg is added to react with IgM antibody that is specific for HbcAg. In the third incubation, anti-HBc conjugated with horseradish peroxidase (anti-HBc: HRPO) is added to react with patient's anti-HBc IgM retained on the bead. The o-phenylene diamine (OPD) solution containing hydrogen peroxide is added. A yellow color develops in proportion to the amount of anti-HBc HRPO. The enzyme reaction is stopped by the addition of acid and the intensity of color is measured with spectrophotometer (Quantum II, Abbott Lab.). The absorbance at 492nm is proportional to the amount of anti-HBc IgM in the patient serum. A cut off value of 0.25 times the positive control mean ($PC_{\bar{x}}$) plus the negative control mean ($NC_{\bar{x}}$) is determined. The absorbance values which are equal to or greater than

Table 1. Anti-HBc IgM positivities in various hepatic diseases

Conditions	No. of cases tested	Anti-HBc IgM	
		Positive	Negative
AVHB	35	26 (83.9%)	9
Hepatitis B	27	.	27
CAH	19	.	19
CLD	7	1 (3.2%)	6
Cirrhosis	26	1	25
Hepatoma	7	.	7
Carrier	8	.	8
AVHA	10	2 (6.5%)	8
Hepatitis A	13	.	13
NANB	3	.	3
Miscel.	47	1	46
Total	202	31 (100.0%)	171

the cut off are reactive for anti-HBc IgM.

The ratio of sample absorbance to negative control absorbance (S/N) is determined by calculation.

Laboratory method

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were examined using routine clinical methods and the normal ranges are 7-27 U/L and 8-30 U/L respectively. LDH isoenzyme and alkaline phosphatase (ALP) isoenzyme were examined by cellulose acetate electrophoresis system (Helena Lab., Beaumont, Texas, U.S.A.).

RESULTS

Anti-HBc IgM positivities in various hepatic diseases:

Among positive anti-HBc IgM cases the positivity was high (83.9%) in AVHB followed by AVHA 6.5%, CLD 3.2%, cirrhosis 3.2% and miscellaneous conditions 3.2% as seen in Table 1.

Hepatitis B markers and other findings in various hepatic deseases and miscellaneous conditions:

Positivities, anti-HBc, IgM 26/35 (74.3%) in AVHB, 1/7 (14.3%) in CLD, 1/26 (3.8%) in cirrhosis, 2/10 (20.0%) in AVHA and 1/47 (2.1%), and the remaining conditions were all negative. HBeAg and anti-HBe were more highly positive in chronic hepatic disorders. ALT and AST activities were significantly higher in AVHB. The increase of LDH5 appeared to not be specific but ALP_{alb} was were more prominent in acute hepatitis. Jaundice was remarkable in most hepatic diseases. In age distribution 10-20 year old group was observed in acute hepatitis whereas the 30-49 year ald group belong to chronic hepatic diseases. The ratio of male to female was highest in CAH and AVHB as seen in Table 2.

Hepatitis B markers among AVHB with anti-HBc IgM:

In 9 cases of subclass I of AVHB with negative anti-HBc IgM and 26 cases of subclases II of AVHB with positive anti-HBc IgM, the positivities of HBs Ag and HBeAg were higher in the later as seen Table 3.

Enzyme activities and electrophoretic isoenzyme patterns in AVHB I and II:

Table 2. Hepatitis B markers and other findings in various hepatic diseases and miscellaneous condition

Dseases	No. of cases tested	Anti-HB_c-IgM	HBsAg	HBeAg	Anti-HBe	ALT (U/L)	AST	LDH_5	ALP_{alb}	Jaundice	Sex M/F	Age major
AVHB	35	26/35 (74.3%)	31/34 (91.2%)	15/35 (42.9%)	8/32 (25.0%)	547.1± 334.6 (n=23)	278.9± 178.08	25/29 (86.20%)	22/23 (95.7%)	21/21 (100.0%)	29/5	10-20
HB	27	0/27	17/26 (65.4%)	10/23 (43.5%)	7/20 (35.0%)	130.6± 122.38	85.61± 74.35	7/12 (58.3%)	6/8 (75.0%)	3/3 (100.0%)	15/9	30-49
CAH	19	0/9	19/19 (100.0%)	2/10 (20.0%)	5/10 (50.0%)	115.1± 109.22	74.9± 52.08	3/8 (37.5%)	4/6 (66.7%)	3/5 (60.0%)	16/1	30-49
CLD	7	1/7 (14.3%)	6/7 (70.8%)	5/7 (71.4%)	1/7 (14.3%)	114.2± 90.64 (n=24)	92.3± 89.31		2/7 (28.6%)	2/7 (28.6%)	7/1	10-29
Cirrhosis	26	1/26 (3.8%)	17/24 (70.8%)	11/22 (50.0%)	10/17 (58.8%)	69.4± 50.52	77.9± 53.27	16/24 (66.7%)	18/22 (81.8%)	12/18 (66.7%)	16/9	30-49
Hepatoma	7	0/7	1/7 (14.3%)	0/6	2/6 (33.3%)	61.3± 44.93 (n=7)	73.3± 50.50	6/6 (100.01%)	5/6 (83.3%)	4/4 (100.0%)	6/0	50-
Carrier	8	0/8	8/8 (100.0%)	4/7 (57.1%)	1/6 (16.7%)	29.1± 10.83	24.3± 13.86	1/3 (33.3%)	1/2 (50.0%)	0/6	4/2	10-29
AVHA	10	2/10 (20.0%)	0/10	0/5	0/4	221.9± 213.87	185.2± 188.20	3/9 (30.0%)	7/8 (87.5%)	8/9 (88.9%)	7/3	10-29
HA	13	0/13	0/12	0/11	0/11	129.2± 61.06	107.7± 54.09	1/7 (14.2%)	3/5 (60.0%)	4/6 (66.7%)		9
NANB	3	0/3	0/3	0/2	0/2	131.3± 48.01	58.5± 23.44	2/2 (100.0%)	1/2 (50.0%)	1/1 (100.0%)	3/3	10-
Miscle (Control)	47	1/47 (2.1%)	1/42 (2.4%)	1/36 (2.8%)	2/31 (6.5%)	92.9± 95.78 (n=39)	63.8± 83.09	7/12 (58.3%)	6/16 (37.5%)	4/44 (9.9%)	7/3	10-29
Total	202	31/171 (18.1%)	100/192 (52.1%)	48/164 (29.3%)	36/146 (24.7%)	147.5± 107.44	100.2± 78.21	71/172 (63.4%)	73/105 (69.5%)	62/122 (50.8%)	83/36	<9->50

As shown in Table 4 the average activities of ALT and AST were 547.1 and 278.9 U/L respectively and there were no difference between AVHB I and II. However in LDH5 and ALP_{alb} the subclass II showed a higher positive rate.

S/N ratio of anti-HBc IgM in various hepatic diseases and control:

As shown in Table 5 the S/N ratio was classified into 3 classes. AVHB showed the highest S/N ratio and the other conditions showed lower S/N ratios. Among 22 cases of AVHB with positive anti-HBc IgM 14 cases (63.6%) showed S/N greater than 20. The S/N ratio appeared to be a strong evidence for the diagnosis of AVHB because of the high specificity.

S/N ratio of anti-HBc IgM in various hepatic diseases and miscellaneous diseases by anti-HBc IgM positivity:

The S/N ratio was examined (Table 5) by anti-HBc IgM positivity and the higher S/N ratio was observed in patients with AVHB as shown in Table 6 while the other conditions showed low S/N ratio as below 9.9 and were negative for anti-HBc IgM. In HB one case was anti-HBc IgM positive and the S/N ratio was in the range of 10-19.9, whereas 2 cases were anti-HBc IgM negative and the S/N ratios were also in 10-19.9 range.

S/N ratio and aminotransferases activities in AVHB cases with positive anti-HBc IgM:

The ALT was significantly high in patients with high S/N ratio of more than 20 compared to the AST. No difference of ALT activities was found between 0-9.9 and 10-19.9 in S/N ratio group as shown Table 7.

Hepatitis B markers and the S/N ratio in patients with very high activities of ALT

As shown in Table 8, ALT activities as high as 1,000 U/L were found mostly in patient with AVHB and the S/N ratio of more than 20 was observed in 66.7% of patients with AVHB.

Anti-delta tests for various diseases:

No positive case of anti-delta was found in 19 cases of various disease conditions with positive HBsAg including 3 cases with AVHB and CAH each, 7 cases of cirrhosis and one case of primary hepatoma as

Table 3. Hepatitis B markers among acute viral hepatitis B (AVHB) by anti-HBc IgM

AVHB	Anti-HBc IgM	No. of cases	HBsAg	HBeAg	Anti-HBe	Anti-HBs	Anti-HBc
I	–	9	7/8* (87.5%)	3/9 (33.3%)	2/ (25.0%)	1/8 (12.5%)	7/8 (87.5%)
II	+	26	24/26 (92.3%)	12/26 (46.2%)	6/24 (25.0%)	3/24 (12.5%)	24/24 (100.0%)
Total		35	31/34 (91.2%)	15/35 (42.9%)	8/32 (25.0%)	4/32 (12.5%)	31/32 (96.9%)

* No. of positive cases/No. of cases tested

Table 4. Enzyme activities and electrophoretic isoenzyme patterns in acute viral hepatitis B I and II

AVHB		ALT (U/L)	AST (U/L)	LDH_5 abnormal	ALP_{alb}
I	9	537.6± 297.51	352.1± 221.10	6/8 (75.01)	5/6 (83.3%)
II	26	551.9± 353.17	242.4± 156.58	19/21 (90.51)	17/17 (100.0%)
	35	547.1± 334.62	278.9± 178.08	25/29 (86.2%)	22/23 (95.7%)

Numbers/Numbers, No. of positive cases/No. of cases tested

Table 5. S/N ratios of anti-HBc IgM in various hepatic diseases and control

Diseases	No. of cases	S/N ratio 0 - 9.9	10 - 19.9	>20
AVHB	22 (24.4%)	4	4	14 (63.6%)
HB	15	12	3	
CAH	6	6		
CLD	5	5		
Cirr.	10	10		
Hepatoma	1	1		
Carrier	2	2		
Bil. sys.	6	6		
H. (NS)*	7	7		
AVHA	2	2		
Control**	14	14		
Total	90	69	7	14

* 1 case of NANB 1 of CS, 2 cases of drug hepatitis and 3 cases of neonatal hepatitis are included.
** Control as non-specific disease other than hepatic disease

Table 6. S/N ratios of anti-HBc IgM in various hepatic diseases and control by anti-HBc IgM positivity

Diseases (No, cases tested)	Anti-HBc -IgM	S/N Ratio 0–9.9	10-19.9	>20	Total
AVHB	+	4	4	14	22
(24)	−	2	.	.	2
HB	+	.	1		1
(15)	−	13	2		15
CLD	+	1			1
(5)	−	4			4
CAH	+	.			.
(6)	−	6			6
Cirrhosis	+	1			1
(10)	−	9			9
Carrier	+	.			.
(2)	−	2			2
Bil. sys.	+	.			.
(6)	−	6			6
H (NS)	+	.			.
(7)	−	7			7
AVHA	+	.			.
(2)	−	2			2
Control	+	1			1
(14)	−	13			13
Total	+	7	5	14	26
	−	62	2	.	64
G. total		69	7	14	90

AVHA, acute viral hepatitis B CAH, chronic active hepatitis CLD, chronic liver disease

shown in Table 9, while by one reference 2 cases (1.4%) were positive in anti-delta among 70 cases of chronic hepatic diseases. The one case is a multi-transfused patient and the other case is a patient with cirrhosis.

DISCUSSION

At present time the serological tests facilitate the etiologic diagnosis of the most cases of clinical hepatitis into type A, type B, and by a exclusion process, type NANB. Acute hepatitis A is diagnosed when anti-HAV IgM is positive whereas acute hepatitis B is usually diagnosed whenever HBsAg detected during acute viral hepatitis. However acute NANB or delta-agent hepatitis superimposed on the HBsAg carrier is not diagnosed by anti-HAV IgM or HBsAg.

The discovery of NANB virus is anticipated. Very recently (Altman 1984) anti-delta test became available in the laboratory since Rizzetto et al. (1977) isolated the delta antigen. The delta infections only occur in patients with positive HBsAg. They can occur as "coinfection" (acute delta concurrent with acute type B infection) or as "superinfection" (acute delta superimposed on a chronic HBsAg carrier state). The coinfection is associated with fulminant hepatitis, while the superinfection is more likely to progress to chronic delta hepatitis (Schumacher, 1985), chronic active hepatitis (CDC, 1984) and cirrhosis (CDC, 1984). The epidemic of delta hepatitis occured among Yucpa Indians in the central mountains of Venezuela between 1979 and 1981 and showed an extremely high mortality. In the U.S.A. less than 5% of positive HBsAg have anti-delta marker and the incidence of HBsAg is only about 0.2%. About half of drug addicts and hemophiliacs who are positive for HBsAg (with 7% incidence) have a delta infection (CDC, 1984).

Among the positive cases with anti-HBc IgM the highest positivity was found in AVHB as 83.9% and anti-HBc IgM appeared to be highly specific to AVHB. The other anti-HBc IgM positive cases were observed in CLD, cirrhosis, AVHA and non-hepatic disease as 3.2%, 3.2%, 6.4% and 3.2% respectively. These results were very similar to the results of (Perrillo et

Table 7. S/N ratio and aminotransferases in acute viral hepatitis B cases with positive anti-HBc IgM

	S/N ratio		
	0-9.9	10-19.9	20
No. of cases	4	4	14
Aminotransferase			
ALT	486.0±202.56	489.9±389.46	985.8±596.38*
AST	256.5±171.36	352.5±447.23	298.3±239.52

*$p < 0.05$

Table 8. Hepatitis B markers and S/N ratios in high ALT activity hepatitis

Cases	Anti-HBc IgM	HBsAg	HBeAg	Anti-HBe	ALT	AST	LDH_5	ALP_{alb}	J	Sex	Age	S/N
AVHB	+	+	+	−	1,751.2	636.8	++	+	+	M	27	20.5
AVHB	+	+	+	−	1,400.0	640.0	.	.	+	M	13	12.58
AVHB	+	+	−	±	1,810.0	450.0	+++	+	+	M	25	25.24
AVHB	+	+	+	−	1,069.0	1,021.0	+++	+	+	F	31	10.29
AVHB	+	+	+	−	1,135.7	749.4	+++	+	+	M	46	22.4
AVHB	+	+	−	−	1,385.0	629.0	+++	+	+	M	34	20.5
CAH	−	+	−	−	1,098.6	561.4	+++	−	−	M	24	2.09
AVHB	+	+	+	−	1,835.0	1,727.5	.	.	+	M	9	.

Table 9. Anti-Delta in sera of various conditions

Disease	No. of cases	Anti-Delta Positive	Negative
AVHB	3	.	3
CAH	3	.	3
CH	1	.	1
Cirrhosis	7	.	7
Drug H.	1	.	1
Hepatoma	1	.	1
DM	1	.	1
Bil. sys.	1	.	1
Kid. donor	1	.	1
	19	.	19 (100.0%)
Reference (Korea)	143*	2** (1.4%)	

* CPH: 12 cases CAH: 33 CAH c̄ cirrhos: 9 Cirrhosis: 6

** 1 case: multiple transfusion and 1 case c̄ cirrhosis

al., 1983) and he also noted that the failure to detect this antibody was noted in the majority of the HBsAg carrier state. The HBsAg appeared to be not differential among various hepatic diseases. The ALT and AST activities were high mostly in AVHB. However Roggendorf et al. (1981), Chau et al. (1983), Kryger et al. (1981), Akahane et al. (1984) and Yano et al. (1984) also studied follow-up changes in anti-HBc IgM titers which were meaningful mostly by the course of AVHB especially. The positivity of anti-HBc IgM in CLD, cirrhosis, AVHA and non-hepatic disease is under following-up study. However it may be explained as persistence of this antibody as well as low S/N ratio. Also non-specific and false positive situations must be considered.

LDH5 and ALP_{alb} were relatively abnormal in AVHB compared to other hepatic diseases except patients with AVHA.

In age and sex distribution, chronic hepatic diseases existed mostly in the 30-49 and AVH in the 10-20age group.

In relation to other markers, the patient with anti-HBc IgM showed the higher positivity in HBsAg and HBeAg. This result corresponds to the results of (Feinman et al., 1982) in HBeAg.

In relation to the enzyme activities, the patient with anti-HBc IgM showed no difference in ALT and AST activities but the higher rates of abnormal fractions of LDH5 and ALP_{alb} isoenzyme pattern. The dense ALP_{alb} isoenzyme fraction is quite specific to AVH (Kim, 1983; Kim et al., 1984).

The S/N ratio of anti-HBc IgM was found to be relatively specific to AVHB (63.6%). The majority of AVHB cases (14/22) showed S/N ratio greater than 20. The all other conditions showed a range less than 9.9 except for HB in which 20% were in the range of 10-19.9. All anti-HBc IgM negative cases showed the lowest S/N ratio (less than 9.9 in AVHB) whereas most cases were negative in anti-HBc IgM and S/N ratio was 10-19.9 in HB.

The S/N ratio appeared to be higher in higher ALT cases in AVHB. It was significant at the ratio which was more than 20. Chau et al. (1983) noted that 29.0 of the S/N ratio was found in acute hepatitis, 4.5 in convalescence and 2.9 in chronic carrier respecitvely. By Akahane et al. (1984) the S/N ratio was more than 20 in 95.2% of AVHB whereas it was less than 10% in HBsAg carrier. These results are similar to author's results. Perrillo et al. (1983) used ELISA index $\frac{\text{A492 of Sample}}{0.3 \times \text{A492 of PC}}$ instead of above S/N ratio and Yano et al. (1984) reported the use of cut off index $\frac{\text{A of Sample}}{\text{ANC} + \text{Apc} \times 0.25}$ Their results were also similar to author's results and mentioned as above.

Emphasized that only the IgM class of anti-HBc is a reliable indicator of acute hepatitis B.

In the anti-delta test for 19 cases with various hepatic disease. Only 2 cases (1.4%) among totally 70 cases with various HBsAg positive chronic liver disease (CPH, CAH, cirrhosis) were positive (Song et al., 1985). It seems to be extremely rare in Korea as the U.S.A. (Schumacher, 1985). In the results of Govindarajan et al.(1984), he prevalence of delta-markers (delta-Ag, anti-delta and anti-delta IgM) was found to be 33.8% in fulminant AVHB and only small numbers of this patients were presumably infected chronically without detectable anti-HBc IgM. Moestrup et al. (1983) noted that the instances of acute hepatitis in a chronic carrier previously termed hepatitis non-A, non-B, may be actually be episodes of delta infection and in chronic HBsAg carriers the most common clinical manifestation was an episode of acute hepatitis, which in some individuals became severe with a pronounced rise in serum ALT for many months.

An interpretation of hepatitis B virus and hepatitis delta virus serological profiles was described by (Mushahwar et al., 1984). These serological profiles were assesed as a diagnostic and prognostic guides for clinical management of the disease.

Other markers under development are hepatitis B viral DNA (HBV DNA) and its DNA polymerase as references Kaplan et al. (1973), Tong et al. (1977) Brechot et al. (1985) Mutsuyama et al. (1985) noted that HBV DNA market pararells the progress of the disease and there is a dissociation between HBeAg/anti-HBe and HBV DNA positivity.

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