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Abstract

Antibody to hepatitis B core antigen (anti-HBc) was measured by radioimmunoassay using CORAB (Abbott Laboratories) in 10 cases of chronic persistent hepatitis (CPH), 46 cases of chronic aggressive hepatitis (CAH), 33 cases of liver cirrhosis (LC) and 53 cases of hepatocellular carcinoma (HCC) in relation to hepatitis B surface antigen (HBsAg) and its antibody (anti-HBs). Ninety-eight point four percent of patients with HBsAg and 93.8% of patients with anti-HBs were positive for anti-HBc and the titers of anti-HBc in patients with HBsAg were significantly higher than those with anti-HBs. Thirty-five point five percent of patients negative for either HBsAg or anti-HBs were positive for anti-HBc. The titers of anti-HBc in patients with CPH, CAH and LC were relatively low, whereas 7 (46.8%) of the HCC patients negative for either HBsAg or anti-HBc had high titers of anti-HBc. The significance of the presence of anti-HBc alone is discussed.

KEYWORDS: anti-HBc, HBsAg, anti-HBs, radioimmunoassay, chronic liver disease.

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DETERMINATION OF ANTIBODY TO HEPATITIS B CORE ANTIGEN BY RADIOIMMUNOASSAY IN CHRONIC LIVER DISEASE

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Abstract. Antibody to hepatitis B core antigen (anti-HBc) was measured by radioimmunoassay using CORAB (Abbott Laboratories) in 10 cases of chronic persistent hepatitis (CPH), 46 cases of chronic aggressive hepatitis (CAH), 33 cases of liver cirrhosis (LC) and 53 cases of hepatocellular carcinoma (HCC) in relation to hepatitis B surface antigen (HBsAg) and its antibody (anti-HBs). Ninety-eight point four percent of patients with HBsAg and 93.8% of patients with anti-HBs were positive for anti-HBc and the titers of anti-HBc in patients with HBsAg were significantly higher than those with anti-HBs. Thirty-five point five percent of patients negative for either HBsAg or anti-HBs were positive for anti-HBc. The titers of anti-HBc in patients with CPH, CAH and LC were relatively low, whereas 7 (46.8%) of the HCC patients negative for either HBsAg or anti-HBs had high titers of anti-HBc. The significance of the presence of anti-HBc alone is discussed.

Key words: anti-HBc, HBsAg, anti-HBs, radioimmunoassay, chronic liver disease.

Several lines of evidence indicate that the hepatitis B virus (HBV) possesses two distinct antigen-antibody systems: one associated with the surface of the virus particle-hepatitis B surface antigen (HBsAg) and its antibody (anti-HBs), and the other associated with the core of the particle-hepatitis B core antigen (HBcAg) and its antibody (anti-HBc) (1-4). Almeida *et al.* first demonstrated the presence of this second antigen-antibody system (HBcAg-anti-HBc) on the basis of immune-electron microscopy (1). Their findings were in accord with the results of the fluorescent antibody technique in which indirect staining of liver tissue from patients with viral hepatitis, type B, revealed two patterns of staining, one cytoplasmic (anti-HBs) and one nuclear (anti-HBc) (2). Hoofnagle *et al.* purified HBcAg and measured anti-HBc in human sera by a complement

fixation test (3, 4). The development of this complement fixation technique and other laboratory assays for the detection of anti-HBc has provided better understanding of the natural history of HBV infection and of the significance of the presence of anti-HBc as a sensitive indicator of HBV infection (3–11).

Recently a sensitive assay method for anti-HBc utilizing radioimmunoassay (RIA) has been developed for practical use. This report presents the results of the frequency, distribution, and titers of anti-HBc measured by RIA in chronic persistent hepatitis (CPH), chronic aggressive hepatitis (CAH), liver cirrhosis (LC) and hepatocellular carcinoma (HCC) related to the presence of HBsAg and anti-HBs.

MATERIALS AND METHODS

Sera were obtained from 142 cases of chronic liver diseases seen at the First Department of Internal Medicine, Okayama University Medical School. The cases consisted of CPH (10), CAH (46), LC (33) and HCC (53). Cases of CPH, CAH and LC were all histologically proven. The diagnosis of HCC was based on histological findings or positive liver scans, typical celiac angiograms and other physical and biochemical findings.

HBsAg was measured by reversed passive hemagglutination (Serodia-HBs, Fujizoki Pharmaceutical Co., Ltd.) or RIA (Ausria II, Abbott Laboratories) and anti-HBs was measured by passive hemagglutination (Serodia-Anti-HBs, Fujizoki Pharmaceutical Co., Ltd.) or RIA (Ausab, Abbott Laboratories). Anti-HBc was measured by competitive binding radioimmunoassay (CORAB, kindly supplied by Abbott Laboratories). Anti-HBc was measured in original sera and 200-fold dilutions and the result was expressed as inhibition % = (the count per minute (cpm) of the negative control-cpm of the sample/cpm of the negative control-cpm of the positive control) $\times$ 100. The sample was considered positive for anti-HBc if the inhibition was more than 80%.

RESULTS

Of the 142 patients, 64 were positive for HBsAg, 16 were positive for anti-HBs, and 62 were negative for either HBsAg or anti-HBs. Of the 64 patients with HBsAg, in 63 (98.4%) inhibition of anti-HBc was more than 80% with original sera and in 54 (84.4%) with 200-fold diluted sera. Of the 16 patients with anti-HBs, comparable figures were 15 (93.8%) and 1 (6.3%) respectively and this difference in the titers of anti-HBc was significant ($P<0.01$). More than 30% of patients negative for either HBsAg or anti-HBs had anti-HBc and 7 of them had high titers of anti-HBc (Table 1).

Table 2 represents a comparison of the positivity rate for the three serological parameters among the groups of CPH, CAH, LC and HCC. The figures show that 50.0% of the CPH patients, 67.4% of the CAH patients, 66.7% of the LC patients and 79.2% of the HCC patients had evidence of HBV infection,

TABLE 1. TITER OF ANTI-HBC IN RELATION TO HBSAG AND ANTI-HBS

HBsAg	Anti-HBs	Number of patients	Inhibition % of anti-HBc (80%≦)	
			×1	×200
+	−	64	63(98.4%)	54(84.4%)[a]
−	+	16	15(93.8%)	1(6.3%)
−	−	62	22(35.5%)	7(11.3%)

a $P<0.01$

TABLE 2. FREQUENCY OF POSITIVE TESTS FOR HBSAG, ANTI-HBS, AND ANTI-HBC IN PATIENTS WITH CPH, CAH, LC AND HCC

	Number of patients	HBsAg(+)	Anti-HBs(+)	Inhibition % of anti-HBc (80%≦, ×1)
CPH	10	2(20.0%)	1(10.0%)	5(50.0%)
CAH	46	22(47.8%)	4(8.7%)	31(67.4%)
LC	33	13(39.4%)	4(12.1%)	22(66.7%)
HCC	53	27(50.9%)	7(13.2%)	42(79.2%)

CPH, chronic persistent hepatitis; CAH, chronic aggressive hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma.

past or present. Each figure was approximately 15% greater than the positivity rates for HBsAg and anti-HBs which are conventional parameters for HBV infection.

Of the patients negative for either HBsAg or anti-HBs, a comparison of titers of anti-HBc in 4 kinds of diseases is presented in Table 3. The titers of anti-HBc were relatively low in patients with CPH, CAH and LC. Of the 19 patients with HCC, 7 (46.8%) had high titers of anti-HBc as expected in patients carrying HBsAg.

TABLE 3. TITER OF ANTI-HBC IN SUBJECTS NEGATIVE FOR EITHER HBSAG OR ANTI-HBS; COMPARISON OF PATIENTS WITH CPH, CAH, LC AND HCC

	Number of patients	Inhibition% of anti-HBc (80%≦)	
		×1	×200
CPH	7	2(28.6%)	0
CAH	20	5(25.0%)	0
LC	16	4(31.3%)	0
HCC	19	10(52.6%)	7(46.8%)[a]

a $P<0.05$

DISCUSSION

Anti-HBc can exist in combination with HBsAg or anti-HBs or alone in an individual serum sample (3). Anti-HBc with HBsAg is found in the course of acute viral hepatitis, type B, and also in cases of the chronic HBsAg carrier state. The highest titers of anti-HBc have been found in the chronic HBsAg carrier state (3, 4). In our study, anti-HBc was detected in 98.4% of chronic HBsAg carriers and the titers were significantly higher than those of patients with anti-HBs. This finding confirms earlier observations that detection of anti-HBc with high titers indicates persistent HBV replication (4, 5).

Sera positive for both anti-HBc and anti-HBs are considered to indicate recovery from acute HBV infection and resolution of the chronic carrier state (3, 4). These two antibodies can persist for many years after HBV infection although the titers of anti-HBc in most subjects are relatively low (5, 11). In our study on patients with chronic liver diseases as shown in Table 1, 93.8% of sera with anti-HBs had anti-HBc. The titers were significantly lower than those with HBsAg in accord with the reports mentioned above. In these patients, the existence of anti-HBc may not indicate persistent HBV replication but the memory of HBV infection probably years earlier.

The third situation is the presence of anti-HBc alone. Previous studies of HBV infection revealed that during the time between the disappearance of HBsAg and the appearance of anti-HBs, anti-HBc is detectable alone. This period of positivity for anti-HBc alone appears to be relatively short after acute HBV infection but may be quite long after the resolution of the chronic carrier state since the longer the HBsAg circulates before disappearance, the longer it may take for the production of anti-HBs (3, 4, 10). In these cases titers of anti-HBc may be relatively low since the titers decline with the clearance of HBsAg (3). In cases of low titers of anti-HBc alone another possibility is that anti-HBs coexists but that the amount is not detectable by passive hemagglutination or RIA (7). The advent of sensitive assays for anti-HBc has revealed that the prevalence of anti-HBc is greater than that of anti-HBs and that anti-HBc is a long-lived marker of HBV infection as Hoofnagle *et al.* predicted (3, 9). It remains open to further investigation whether low titers of anti-HBc alone indicates the period between the disappearance of HBsAg and the appearance of anti-HBs, or the time after the disappearance of anti-HBs. Of 62 patients negative for either HBsAg or anti-HBs, 15 (24.2%) with low titers of anti-HBc alone (except 7 cases with high titers of anti-HBc alone) probably had HBV infection in the past.

It has been suggested that some individuals with high titers of anti-HBc alone may carry HBsAg in subdetectable concentrations (3, 4). High titers of

anti-HBc alone were observed in patients with significant HCC as shown in Table 3. It has been reported that in HCC patients anti-HBc is present in high titers, whereas HBsAg is present only at low concentrations (8). Therefore it is reasonable to consider that in our 7 cases of patients with HCC and high titers of anti-HBc alone, viral replication still persisted and that subdetectable amounts of HBsAg were present. The prevalence of anti-HBc in HCC by RIA in our study agrees with the results of immune adherence hemagglutination technique (11).

The high rate of positive anti-HBc in patients with chronic liver disease indicates that many patients with chronic liver diseases in Japan have some kind of relation to HBV infection, therefore, anti-HBc determination is a useful tool for etiological appreciation of HBV infection in these patients. The high rate and high titers of positive anti-HBc alone in some individuals with HCC suggest that anti-HBc measurement is needed to check for subdetectable amounts of HBsAg. Anti-HBc is a potent marker defining the true prevalence of HBV infection in the liver disease more accurately.

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