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Sera from black patients with primary hepatocellular carcinoma and Hepatitis B surface antigenemia were tested for immunoglobulin M antibody against hepatitis B core, hepatitis B e antigen, and hepatitis B viral DNA. Their prevalences were compared to those of a control group of age and sex matched black HBV carriers without tumor. IgM anti-HBC was present in 58.6%; HBeAg in 32.3%; and HBV-DNA in 26.7% of the patients, compared to 45%. 3.2% and 25.8% respectively, in the control group. We conclude that IgM anti-HBc is present appreciably more often than either HBeAg or HBV-DNA in patients with PHC HBeAg or IgM anti-HBc in serum of HBsAg positive carriers may predict an added risk of PHC developement in South African blacks.

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HEPATITIS B VIRUS INFECTION AND HEPATOCELLULAR CARCINOMA: CORRELATION BETWEEN IgM ANTIBODY TO HEPATITIS B CORE ANTIGEN, HEPATITIS B e ANTIGEN, AND HEPATITIS B DNA

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Abstract. Sera from 102 black patients with primary hepatocellular carcinoma (PHC) and hepatitis B surface antigenemia were tested for immunoglobulin M antibody against hepatitis B core (IgM anti-HBc), hepatitis B e antigen (HBeAg), and hepatitis B viral (HBV) DNA. Their prevalences were compared to those of a control group of 124 age and sex matched black HBV carriers without tumor. IgM anti-HBc was present in 68.6%, HBeAg in 32.3%, and HBV-DNA in 26.7% of the patients. In the control population, IgM anti-HBc was present in 45%, HBeAg was detected in 3.2%, and HBV-DNA in 25.8%. We conclude that IgM anti-HBc is present appreciably more often than either HBeAg or HBV-DNA in patients with PHC. HBeAg or IgM anti-HBc in serum of HBsAg positive carriers may predict an added risk of PHC development in South African blacks.

Commercially available tests designed to detect immunoglobulin M antibody to the hepatitis B virus core antigen (IgM anti-HBc) in serum enables this antibody to be detected in patients with acute hepatitis B, and in some patients with chronic hepatitis B virus infection.[1,2] Recently, sensitive radioimmunoassays capable of detecting much lower titers of IgM anti-HBc in patients with chronic hepatitis B have been developed.[2,3] IgM anti-HBc is demonstrable by sensitive radioimmunoassay in the majority of hepatitis B e antigen (HBeAg) positive carriers with active hepatic disease. However, the relation between serum IgM anti-HBc, markers of HBV replication in serum, such as HBeAg and HBV-DNA, and disease activity is not a simple one, and requires further elucidation.

IgM anti-HBc has recently been shown to be present in the serum of patients with HBV related primary hepatocellular carcinoma (PHC) appreciably more often than in a control HBsAg positive population.[4,5] In a previous study, we found HBeAg to be the most sensitive and HBV-DNA polymerase the least sensitive marker of vial replication in South African blacks with PHC.[6] The purpose of the present study was to determine and correlate the prevalence of IgM anti-HBc, HBV-DNA, and HBeAg in patients with HBsAg positive PHC, and to compare the frequency of these markers in HBV carriers with PHC to HBV carriers without the tumor.

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MATERIALS AND METHODS

One hundred two hepatitis-B surface antigen (HBsAg) positive South African blacks with histologically proven PHC were studied. Their ages were 16–70 years with a mean of 39.3 years; there were 94 men and 8 women.

IgM anti HBc was measured in the patient's sera using a solid-phase antibody-capture radioimmunoassay, as previously described.[2] Results were expressed as a ratio of cpm in wells containing test sera (P) to the mean cpm of wells containing negative sera (N). A P/N ratio of ≥ 2.1 was considered positive.

HBV-DNA was measured in all serum samples by the following method: 300 μl of serum was incubated for 2 hr at 70°C in 0.1 M sodium acetate buffer, 2% SDS, and 50 μg/ml of yeast RNA to which was added 1 mg of proteinase K (Merck Sharp & Dohme, Rahway, NJ). The DNA was extracted with 2 phenol-chloroform-isoamyl alcohol extractions. The aqueous phase was then denatured with 1.0 M NaOH, neutralized, and 50 μl immediately spotted onto a nitrocellulose filter. After washing with 6 × SSC, the dried

nitrocellulose filters were baked at 80°C for 1 hr and prehybridized according to the method of Wahl and others.[7] The filters were hybridized at 42°C for 16 hr to repurified, nick translated ^{32}P labeled cloned HBV-DNA insert (AM 12; a gift from the laboratory of J. Gerin, Georgetown University, Washington, DC). The hybridization mixture contained 50% formamide, 5 × SSC, 1 × Denhardt's solution, 50 mM $NaPO_4$ (pH 6.5), 10% sodium dextran sulfate (Pharmacia Fine Chemicals, Piscataway, NJ), and 100 μg of calf thymus DNA/ml. At least 5 × 10^7 cpm were routinely added to each filter. After repeated washing in 2 × SSC, 0.1% SDS at room temperature, and then 0.1 × SSC and 0.1% SDS at 65°C, the dried filters were autoradiographed at −70°C to Kodak XAR-5 X-ray film by using a Cronex DuPont (Wilmington, DE) intensifying screen. Cloned HBV-DNA and sera positive for HBsAg, HBeAg, and DNAp were included as positive controls. Less than 1 pg of cloned HBV-DNA could be detected by this method. Uninfected sera that were negative for HBsAg were included as negative controls. The results were assessed semi-quantitatively as 0–5+ positive. HBsAg, HBeAg, antibody to HBeAg (anti-HBe), antibody to HBsAg (anti-HBs), and anti-HBc were measured using commercially available radioimmunoassays (Abbott Laboratories, North Chicago, IL).

The prevalence of these markers was compared to a control group of 124 consecutively referred South African blacks with chronic hepatitis B without PHC. These patients were referrals to a screening program to detect small PHC in asymptomatic carriers. There were 116 men and 8 women, 21–60 years of age (mean age 36 years). Seventy-six percent were rural adult blacks in whom the disease is known to be predominantly acquired in childhood.[8] Because liver biopsies were not performed for ethical reasons in the control group, a comparison of the liver disease in the patient and control group was not possible.

Possible differences in the prevalences of the markers were analyzed statistically using the Chi square test with Yates' modification for small numbers, and Fisher's exact test.

RESULTS

Detectable serum levels of IgM anti-HBc were present in 68.6% (70/102) of the PHC patients.

TABLE 1

The inter-relation between IgM anti-HBc, HBeAg, and HBV-DNA in 101 South African blacks with HBsAg positive hepatocellular carcinoma

IgM anti-HBc	HBeAg	HBV-DNA	Number	Percentage
+	+	+	14	13.9
+	+	−	12	11.9
+	−	+	8	7.9
+	−	−	35	34.6
−	+	+	4	4.0
−	−	+	1	1.0
−	+	−	3	3.0
−	−	−	24	23.8

Values for the P/N levels were 2.3–14.5 (mean P/N: 6.03). HBeAg was present in the serum of 32.3% (33/102) and HBV-DNA in 26.7% (27/101) of the PHC patients. Each one of the latter figures is appreciably lower than the prevalence of IgM anti-HBc ($P < 0.001$ in each instance). Of the 27 patients with detectable HBV-DNA in the serum, 15 were judged to be 1+ positive; 5, 2+; and 7, 3+. Twenty-two (81.5%) of these 27 patients had detectable IgM anti-HBc.

IgM anti-HBc was detected in 26/33 HBeAg positive patients (78.8%) and in 43/68 anti-HBe positive ones (63%), an insignificant difference. Only 18 of 101 (17.8%) patients had both HBeAg and HBV-DNA in serum.

The interrelation between IgM and HBc, HBcAg, and HBV-DNA is shown in Table 1. One or another of the 3 markers of HBV replication was present in 76.2% of the patients. The IgM antiHBc serum levels were not age related. Patients ≤30 years of age had a P/N mean concentration of 4.1, patients aged ≥50 years of age had a mean P/N concentration of 5.0 ($P > 0.05$). The prevalence of HBeAg, HBV-DNA, and IgM anti-HBc in the control population are shown in comparison with those in the PHC patients in Table 2. Of 116 anti-HBe positive carriers, 50 (43.1%) were IgM anti-HBc positive.

DISCUSSION

The findings in this study are consistent with those of previous investigations which showed that the majority of patients with HBV associated PHC have low levels of viral replication. Most of our patients were HBeAg negative, and less than 20% were positive for both HBeAg and HBV-DNA. Although most were negative for

TABLE 2

Comparison of the frequency of HBeAg, HBV-DNA, and IgM anti-HBc in HBsAg positive carriers with and without hepatocellular carcinoma

	PHC n = 102	Chronic HBV n = 124	
HBeAg positive	33 (32%)	4 (3.2%)	$P < 0.001$
HBV-DNA positive	27 (26%)	32 (25%)	NS
IgM anti-HBc positive	70 (68%)	56 (45%)	$P < 0.001$

NS = not significant.

HBeAg, a higher proportion were HBeAg positive than the asymptomatic HBV carrier without PHC.

Because it is frequently difficult to ascertain the disease activity or presence of cirrhosis in the surrounding non-neoplastic tissue in percutaneous liver biopsy sections from patients with PHC, and because liver biopsies were not performed in the control group, a comparison of liver disease in the patient and control groups was not possible.

The uniquely low prevalence of HBeAg in asymptomatic black South African adult carriers is noteworthy, and reflects the duration of the carrier state in long-standing chronic hepatitis B carriers acquiring the disease in childhood, and a HBeAg-anti-HBe seroconversion rate of 19.6% per annum.[9,10] Although the low prevalence of HBeAg differs from that reported in Western and Chinese carriers, we believe that the asymptomatic, age, sex, and ethnically matched HBV carriers are valid and appropriate controls to compare the serological status of HBV infected patients to HBV infected patients with tumor, PHC is prevalent in black South African carriers, yet virtually all black South African patients with PHC do not recall a previous history of acute or chronic hepatitis. Also, PHC together with coexistent chronic active hepatitis or cirrhosis has been detected in asymptomatic carriers by alpha-fetoprotein screening.[11] Using a radioimmunoassay designed to yield maximum sensitivity, we found that most black patients with HBsAg associated PHC are positive for IgM anti-HBc. Our data confirm a similar high prevalence of IgM anti-HBc recently reported in Koreans with PHC.[5] The lower prevalence of 48% found an earlier study of black patients probably reflects a different, presumably less sensitive assay used at that time.[4]

The explanation for the raised serum IgM anti-HBc levels in patients with PHC is uncertain. There is some evidence that IgM anti-HBc may be a sensitive index of HBcAg production. Thus the majority of HBeAg-negative, HBV-DNA polymerase positive carriers with replicative chronic hepatitis B are also positive for IgM anti-HBc in serum;[12] IgM anti-HBc in serum correlates with infectivity and sexual transmission of HBV;[13] fewer HBV carriers with superimposed hepatitis D (delta) virus infection (which is known to suppress HBcAg production) are IgM anti-HBc positive;[12] and IgM anti-HBc titers decline after seroconversion from HBeAg to anti-HBe and increase after reactivation.[12] Although IgM anti-HBc is also detectable in a proportion of anti-HBe positive patients, the absence of HBeAg from serum does not negate ongoing HBV replication.[14] A positive IgM anti-HBc may therefore be a sensitive, albeit indirect, indicator of HBV replication. The presence of IgM anti-HBc in the majority of our patients may indicate low grade HBV replication, or at least a relatively recent phase of HBV replication.

The relation between HBV replication, HBeAg and HBV-DNA in serum, disease activity, and IgM anti-HBc is complex. Because certain patients with HBeAg in serum are IgM anti-HBc negative, the latter is not always a direct marker of HBV replication and HBcAg in liver, but rather may reflect the histological and biochemical activity of the disease and the complex host response to the viral core antigen. It is primarily seen in patients with abnormal serum ALT values and active hepatic disease, while otherwise healthy HBsAg carriers are usually seronegative for antibody.[10] It could therefore be argued that IgM anti-HBc better indicates an active host response to ongoing HBV replication. The presence of IgM anti-HBc in the majority of patients with PHC would imply recent or possibly continued production of HBcAg and active hepatic disease in these patients.

There are no certain clinical, histological, or molecular biological criteria which will indicate whether a particular HBsAg positive carrier will

develop PHC. Therefore many factors, including age, infection in infancy, sex, race, history of hepatitis, and the presence of cirrhosis, have to be taken into account in assessing individual risk.[15] We found IgM anti-HBc and HBeAg to be present in serum in a significantly greater proportion of patients with HBsAg associated PHC than in comparable carriers without malignancy. Although the majority of patients with HBsAg positive PHC are anti-HBe positive, HBV carriers with either HBeAg or IgM anti-HBc may be at relatively greater risk for the development of PHC. This conclusion is supported by similar findings in a prospective evaluation of a large cohort of HBsAg positive Taiwanese males,[15] and by a statistically significant increase in IgM anti-HBc in Korean carriers with PHC compared to a group with chronic HBV only.[5] IgM anti-HBc and HBeAg may therefore need to be added to the list of factors presaging a greater probability of developing hepatocellular carcinoma.

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