SEROPREVALENCE OF HEPATITIS B AND C VIRAL MARKERS IN PATIENTS WITH PRIMARY HEPATOCELLULAR CARCINOMA IN SINGAPORE

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ABSTRACT

The prevalence of hepatitis B and C serological markers were studied in 55 patients (45 males and 10 females) with primary hepatocellular carcinoma (PHC). Their ages ranged from 28 years to 79 years (mean age: 56 years). Fifty-five other patients with non-hepatic diseases were used as age and sex matched controls. Forty-one PHC patients (74%) had chronic hepatitis B infection alone, 5 patients (9%) had chronic hepatitis C infection alone, 6 patients (11%) had chronic hepatitis B and C co-infection, 2 patients (4%) had evidence of previous exposure to HBV and one patient (2%) had no hepatitis B and C serological markers. Among those patients with chronic HBV infection alone, the commonest serological pattern was HBsAg and anti-HBe positive (66%;27/41) followed by HBsAg and HBeAg positive (i.e. highly HBV infectious group) (24%;10/41). All the positivity rate for HBsAg (including co-infection with HBV and HCV) was 85% and all the positivity rate for anti-HCV (including co-infection with HBV and HCV) was 20%. In the control group, positivity rate for HBsAg was 13% (7/55). None of the control sera was positive for anti-HCV. Positivity rates for HBsAg and anti-HCV were significantly higher in the 55 PHC cases than in controls. The odds ratio for HBsAg was 40.3 (p value: <0.001) (95% CI limits: 12.1 to 143.3) and for anti-HCV was indeterminate.

Keywords: seroprevalence, hepatitis B virus infection, hepatitis C virus infection, HBsAg, anti-HCV, primary hepatocellular carcinoma

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INTRODUCTION

Primary hepatocellular carcinoma (PHC) is one of the most common malignant neoplasm throughout the world, although its incidence shows great geographic variations, being common in Africa and SE Asia but rare in temperate region. It is the third commonest cancer for males in Singapore and its incidence is highest among Chinese males above 40 years of age [1]. Epidemiological studies have revealed a very strong correlation between the geographical frequency of the HBsAg carrier state and prevalence of PHC. PHC is common in regions where HBV is endemic. Serological evidence of HBV infection is detected in about 70% of PHC patients in Africa [2], 98% in China [2], 81% in Japan [2], 100% in Taiwan [2], and 78% in Singapore [3]. It has been suggested that asymptomatic HBsAg carrier state, chronic hepatitis, liver cirrhosis, and PHC are successive sequelae of chronic hepatitis B virus infection.

The recent identification and characterisation of HCV has permitted detailed epidemiological investigations of HCV infection associated with PHC development. Several retrospective studies have already demonstrated that 50% to 80% of the patients previously thought to have chronic non-A, non-B (NANB) hepatitis (on the basis of exclusion criteria) were, in fact, positive for anti-HCV.

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The aim of this study was to determine the seroprevalence of hepatitis B and C serological markers in PHC patients admitted to the Department of Medicine, National University Hospital.

MATERIALS AND METHODS

Patients

Sera from 55 consecutive primary hepatocellular carcinoma patients (from 1990 to 1993) were studied. Hospital records were reviewed to obtain CT scan, hepatic ultrasound, hepatic angiogram and histological findings of liver biopsies. The diagnosis of PHC was made on either histological evidence of liver biopsy or at least two of the following:

1. Computerised tomography (CT scan) or abdominal ultrasound findings.
2. Hepatic angiogram.
3. Raised serum alpha feto-protein level (AFP) (Expected range 0.5 to 15 ng/mL)

Sera from 55 inpatients without any liver diseases were used as age and sex matched controls.

Serological assays

Serum samples, including control sera, were tested for HBsAg, HBeAg, anti-HBe, anti-HBc (corzyme) (total antibody) by commercially available enzyme linked immunoassay kits. [Auzyme II for HBsAg, EIA for HBeAg, anti-HBe, and anti-HBc total antibody (corzyme), Abbott Laboratories North Chicago Ill]. Serum anti-HCV was measured by using ABBOTT HCV EIA 2nd Generation, Qualitative Enzyme Immunoassay. Serum anti-HBs was measured by the IMX AUSAB assay using the IMX machine. The IMX AUSAB assay is a microparticle Enzyme Immunoassay (MEIA) for the qualitative and quantitative determination of antibody to HBsAg (anti-HBs).

AFP was measured by IMX AFP assay procedure by using the IMX machine. The IMX AFP assay is a MEIA for the quantitative determination of AFP in human serum.

Statistical analysis

Exposure - response association was calculated by chi-squared

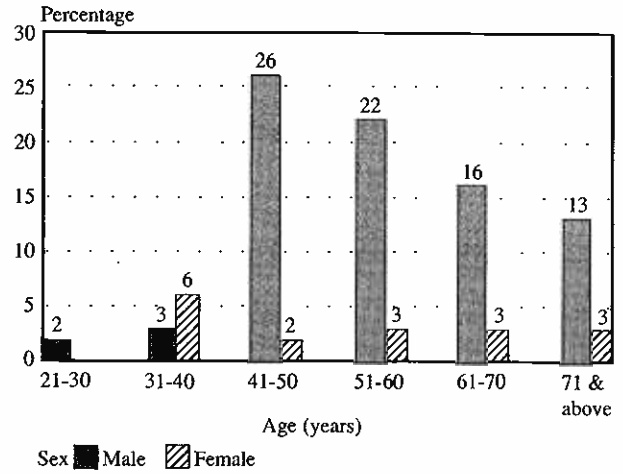
test and Fisher's exact probability test by using Epistat software programme.

RESULTS

Patient characteristics

There were 45 males (82%) and 10 females (18%). Their ages ranged from 28 years to 79 years (mean age: 56 years). The peak age group for all 55 patients was between the 4th and 5th decade. Males predominate with a male to female (M:F) ratio of 4.5:1. The mean age was similar for both male and female patients (56 years). All the patients studied were Chinese. The incidence of PHC according to age groups and sex is shown in Fig 1.

Fig 1 – Incidence of PHC according to age group and sex



Serological characteristics

HBsAg was positive in 85% (47/55) of all patients. Anti-HCV was positive in 20% (11/55) of all patients. Anti-HBc was detected in 96% (53/55) of patients. One 55-year-old male patient (2%;1/55) was negative for all HBV and HCV serological markers and this patient had no history of previous exposure to viral hepatitis, alcoholic intake and smoking.

1. PHC patients associated with chronic hepatitis B infection alone

There were 41 patients (74%) (33 males and 8 females) in this group. Their ages ranged from 28 years to 79 years (mean age 54 years). Twenty-seven patients (66%) were HBsAg/anti-HBe positive (mean age 53) and 10 patients (24%) were HBsAg/HBeAg positive (mean age 57). Four patients (10%) had no 'e' markers.

Among these patients, 9 patients (8 males and 1 female) had co-detection of HBsAg and anti-HBs with anti-HBs titres higher than 10 mIU/mL in 6 patients (mean age 54 years). Two of them were also positive for HBeAg and another 5 were anti-HBe positive. The remaining 2 patients did not have any "e" markers.

. Patients with co-detection of HBsAg and anti-HBs with different antibody levels and their HBeAg and anti-HBe status were shown in Table I.

2. PHC associated with chronic hepatitis C infection alone

There were 5 male patients in this group (9%). Their ages ranged from 63 years to 77 years (mean age 70 years). Four of them were positive for anti-HBc, indicating a previous exposure to HBV infection. The remaining 77-year-old patient did not have any serological evidence of previous exposure to HBV infection.

Table I – Patients with co-detection of HBsAg & anti-HBs with different antibody levels and their HBeAg & anti-HBe status

Anti-HBs titre	No. of patients			
	Anti-HBs (positive)	HBeAg (pos:) Anti-HBe (neg:)	HBeAg (neg:) Anti-HBe (neg:)	HBeAg (neg:) Anti-HBe (pos:)
< 10 mIU/mL	3	1		2
10-20 mIU/mL	2	1		1
20-50 mIU/mL	1		1	
50-100 mIU/mL	1			1
>100 mIU/mL	2		1	1

3. PHC associated with chronic co-infection with hepatitis B and C viruses

There were 6 patients (11%) (4 males and 2 females) in this group. All of them were positive for HBsAg, anti-HBc and anti-HCV. Four patients (3 males and 1 female) (67%) were positive for anti-HBe. The remaining 2 (33%) were HBeAg positive.

Among these 55 PHC patients, 85% positivity rate for HBsAg was considerably higher than the 20% positivity rate for anti-HCV.

4. HBsAg negative PHC with serological evidence of previous exposure to HBV infection alone

There were 2 male patients (4%) in this group. Their ages were 42 years and 61 years. Both were negative for HBeAg and anti-HBe but positive for anti-HBs (above protective levels) and anti-HBc.

Distribution of PHC associated with chronic HBV infection alone, chronic HCV infection alone, chronic co-infection with HBV and HCV and HBsAg negative PHC cases by different age group are shown in Fig 2.

In summary, 74% (41/55) of PHC patients were associated with chronic HBV infection alone. Nine percent (5/55) was associated with chronic hepatitis C infection alone and 11% (6/55) were associated with chronic co-infection with HBV and HCV. HBsAg negative PHC with serological evidence of previous exposure to HBV infection alone was found in 4% (2/55) of cases. Only 2% (1/55) was negative for all HBV and HCV serological markers (Fig 3).

Fig 2 – Distribution of PHC associated with chronic HBV infection, chronic co-infection with HBV & HCV, chronic infection & HBsAg negative PHC by age group

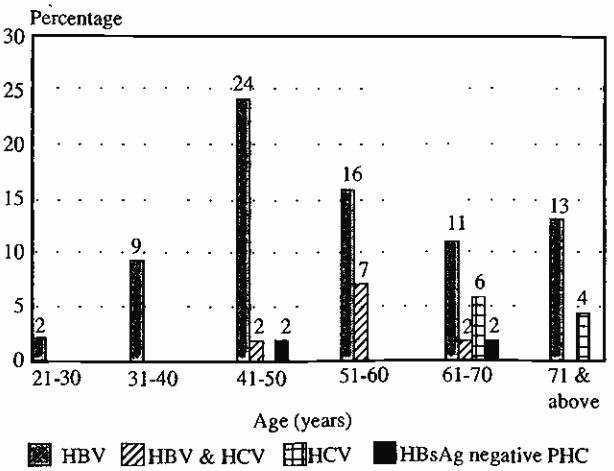
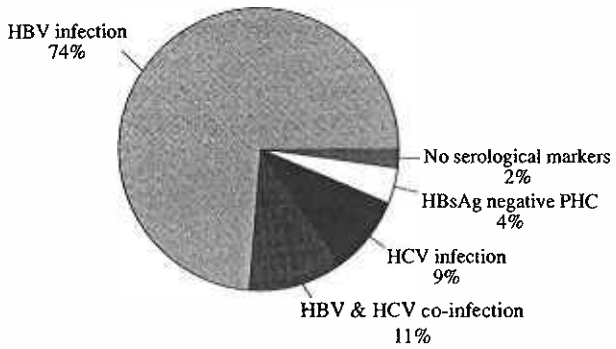


Fig 3 – Distribution of PHC associated with HBV, HBV & HCV co-infection, HCV infection, HBsAg negative PHC & no serological markers



Serological characteristics in age and sex matched controls

There were 55 age-and sex-matched controls.

Hepatitis B and C serological status in PHC cases and their age-and sex-matched controls are shown in Table II.

Twenty-three of them (42%) did not have any HBV and HCV related serological markers. Three subjects had anti-HBs only (5%) (antibody levels > 10 mIU/mL). Nineteen (35%) were HBsAg negative with anti-HBc positive and 12 of them were also positive for anti-HBs (antibody levels > 10 mIU/mL). Three (5%) were negative for HBsAg but positive for anti-HBe and anti-HBc. Five (9%) were positive for HBsAg and anti-HBc with no "e" markers. Two (4%) were positive for HBsAg and anti-HBs (antibody levels > 10 mIU/mL) with anti-HBc negative. The HBsAg seropositivity rate in the control group was 13%, anti-HBs was 31% and anti-HBc was 49%.

A comparison of Hepatitis B and C serological status between PHC cases and their controls is shown in Table III.

HBsAg was present in 85% of PHC patients compared to only 13% of controls (odds ratio: 40.29; p value < 0.001; 95% CI limit: 12.13 to 143.26). Anti-HBc was positive in 96% of PHC patients and in only 49% in controls (odds ratio: 27.48; p value < 0.001; 95% CI limit: 5.69 to 92.48). The positivity rates for HBsAg and anti-HBc were significantly higher in PHC cases than in controls. The positivity rate for anti-HBc was 1.1 times that for HBsAg in PHC patients, whereas this ratio was 3.8 greater in controls.

Anti-HBs positivity rates for PHC patients and control group were 29% and 31% respectively. The difference between these 2 groups was not significant. (Odds ratio: 0.92; 95% CI limit: 0.37 to 2.2) and exposure-response association was inverse between cases and controls. HBeAg was 22% (12/55) positive in cases but none of the controls were positive for HBeAg.

In Singapore, the positivity rate for anti-HCV is 1.7% in normal healthy population and 3% in chronic hepatitis B carrier [4]. All our control sera were negative for anti-HCV but the positivity rate for anti-HCV was 20% in our PHC cases.

DISCUSSION

Aetiological and seroepidemiological association between PHC and chronic HBV infection is firmly established by extensive prospective and retrospective studies in many parts of the world. Globally, HBV is probably the aetiological agent responsible for 75% - 90% of PHC [5]. Hepatitis C virus (HCV) was discovered in 1989 [6], and is also known to cause PHC. The incidence of PHC is considerably higher in males than in females throughout the world. Our study confirms this trend.

The 86% positivity rate for HBsAg in PHC cases is significantly higher than the 13% positivity in controls. This result is similar to those found in Japan [7], Taiwan [8] and Hong Kong [9]. A marked increase risk of PHC has also been shown among HBsAg carriers compared with non-carriers (risk factor up to 200 has been reported in different ethnic or social groups) [7,10].

The potential oncogenic role of HBV in PHC is unclear. Some hypothesised that non-specific mechanism triggered by the immune response against infected hepatocytes results in chronic inflammation of the liver, continuous cell death and

Table II – Hepatitis B & C serological markers in PHC cases and their age and sex matched controls

HBsAg	HBeAg	anti-HBe	anti-HBs	anti-HBc	anti-HCV	PHC cases (n=55)	Controls (n=55)
+	+	—	—	+	—	8	0
+	+	—	+	+	—	2	0
+	—	+	—	+	—	22	0
+	—	+	+	+	—	5	0
+	—	—	—	+	—	2	5
+	—	—	+	+	—	2	0
+	—	—	+	—	—	0	2
+	—	—	+	+	+	4	0
+	+	+	—	+	+	1	0
+	+	—	—	+	+	1	0
—	—	—	—	+	+	1	0
—	—	+	—	+	+	2	0
—	—	+	+	+	+	1	0
—	—	+	—	—	+	1	0
—	—	—	+	+	—	2	12*
—	—	+	—	+	—	0	3
—	—	—	—	+	—	0	7
—	—	—	+	—	—	0	3
—	—	—	—	—	—	1	23*

* p=<0.05

Table III – Comparative study of hepatitis B and C serological status between PHC cases and their controls

Serological markers	No. of subjects (%) PHC cases	Controls	Remarks
HBsAg			
Positive	47/55 (85)	7/55 (13)	Odds Ratio: 40.3
Negative	8/55 (15)	48/55 (87)	95% CI : 12.1 to 143.2 ($p<0.001$)
Anti-HCV			
Positive	11/55 (20)	0/55	Odds ratio:
Negative	44/55 (80)	55/55 (100)	(Indeterminate)
Anti-HBc			
Positive	53/55 (96)	27/55 (49)	Odds ratio: 27.48
Negative	2/55 (4)	28/55 (51)	95% CI: 5.69 to 92.48 ($p < 0.001$)
Anti-HBs			
Positive	16/55 (29)	17/55 (31)	Odds ratio: 0.92
Negative	39/55 (71)	38/55 (69)	95% CI: 0.4 to 2.2 ($p > 0.05$)

consequent cell proliferation might increase the risk factors for carcinoma of the liver [9]. It has also been postulated that the virus might play a direct role as an insertional mutagen: integration of viral DNA into the host genome or direct activation of proto-oncogenes or, most likely in the inactivation of tumour suppressing genes [11,12]. The HBV X gene has also come under suspicion due to its trans-activating activity [13].

Our figure of 49% positivity rate for anti-HBc in the control group is quite high and possibly represents the high incidence of HBV infection in Singapore. (In Singapore, about 6% of the population are HBV carriers) [14]. It was also noted that anti-HBS positivity rate was less frequent than HBsAg positivity rate in PHC cases and vice versa in controls. This emphasises the importance of continuous antigenemia in the pathogenesis of PHC cases [15] in endemic areas.

The commonest serological pattern found in patients with PHC is positivity for both HBsAg and anti-HBe. This type of serological pattern is most prevalent in the Mediterranean and East Asian countries [16,17]. The HBeAg/anti-HBe serological status is determined not only by the extent of virus replication and heterogeneity of HBV but also by integration of HBV DNA into the host genome of infected hepatocyte which may be responsible for the persistence of viral replication and development of PHC [18].

Five PHC patients (9%) were positive for HBsAg, anti-HBs and anti-HBe. This pattern may be due to the rearrangement in the PreS/S and PreC/C genes leading to changes in the immmunogenicity of virus particles that effect the clearance of the virus by the host immune system. Integration of HBV genome into the host hepatocyte genome might result in development of PHC [19].

Some studies also showed that co-detection of HBsAg and anti-HBs in progressive liver disease and PHC, could be caused by an HBV "escape mutant". This point mutation in the S region can produce a HBV variant that is able to escape the circulating antibody to hepatitis B surface antigen. This co-detection of antigen and antibody is an indication that the immune response to viral envelope is being activated and is thought to be an indirect evidence of a greater degree of inflammatory activity in such patients [20]. It has also been postulated that co-existing antibodies are usually directed at epitopes of a strain of HBV other than the one that is infecting the patients [21,22]. The origin of these antibodies is unknown, but their presence is believed by some to correlate with viral replication [20]. Nine patients had HBsAg and anti-HBs with different antibody levels (range < 10 mIU/mL to > 700 mIU/mL) (Table I).

A number of epidemiological studies previously showed presence of anti-HBs and anti-HBc in HBsAg negative PHC cases; 40% - 50% in France [23,24], 2/5 cases in Taiwan [25]. Two PHC patients (4%) in our study were positive for anti-HBs and anti-HBc in HBsAg negative PHC patients (in the absence of anti-HCV).

These findings suggest that serological recovery from HBV infection does not always indicate complete resolution of HBV associated clinical diseases. HBV may still be present in a "latent" form in this type of HBsAg negative PHC cases [26].

According to our results, 74% of PHC associated with chronic HBV infection alone is considerably higher than the 9% of PHC associated with chronic HCV infection alone. Eleven percent of PHC associated with chronic co-infection with HBV and HCV is slightly higher than the 9% of PHC associated with chronic HCV infection alone. There is also a significant difference between the HBsAg positivity rate (85%) and anti-HCV positivity rate (20%) within our PHC cases.

PHC associated with chronic hepatitis B infection alone is common in younger age group (peak age group between 4th and 5th decade and mean age 54 years) whereas PHC associated with chronic hepatitis C infection alone is common in older age group (peak age group between 6th & 7th decade and mean age 70 years) (Fig 2). The mean age of 54 years of PHC patients associated with chronic hepatitis B infection alone is slightly younger than that of the PHC patients associated with chronic co-infection with HBV and HCV (mean age 58 years).

These findings may be due to the fact that the hepatocarcinogenic role of HCV infection is perhaps a late event compared with HBV related PHC and HCV infection generally follows a prolonged, silent clinical course [27].

There were 47 HBsAg positive PHC patients (85%) and 7 (13%) HBsAg negative PHC patients. HBsAg positivity is therefore 6.7 times greater than HBsAg negativity in PHC patients. Of the 47 HBsAg positive PHC patients, 6 patients were positive for anti-HCV (13%). These are similar to results obtained in other hepatitis B endemic countries: 16% in Japan [28] and 17% in Taiwan [29].

Of the 7 HBsAg negative PHC patients, 5 (71%) were positive for anti-HCV in this study. In Japan, 76% of HBsAg negative PHC cases were anti-HCV positive [28]. In Taiwan, the figure was 63% [29].

The exact mechanism whereby HCV causes hepatocellular carcinoma is not known. Some researchers suggested that HCV might be directly involved in the pathogenesis of PHC. HCV infection is a major pathogenic agent of HBsAg negative PHC cases.

Fifty-seven percent (4/7) of HBsAg negative PHC patients had concomitant presence of anti-HCV and anti-HBc in this study. The frequency of co-positivity for anti-HBc and anti-HCV in HBsAg negative PHC patients is 23% in Japan [30] and 54% in Italy [31].

Colombo et al (1989) [31] postulated the more frequent co-existence of anti-HBc and anti-HCV in patients with PHC than in patients with chronic non-B hepatitis, suggesting an indirect role for past HBV infection.

Some authors have also speculated upon the possibility of interaction between HBV and HCV that might alter the natural course of each separate viral disease [32]. Yu et al suggested a possible synergistic effect of past or current HBV and HCV infection on PHC risk [33]. The possible pathogenic implication of co-infection by the two viruses remains to be established by future studies.

These data emphasise the importance of effective planning strategies for hepatitis B immunisation, development of modified formulation in Pre S HBV vaccine for vaccines; development of HCV vaccine, effective antiviral treatment, and adequate screening programmes for the early diagnosis of PHC in patients with chronic hepatitis and cirrhosis.

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