HEPATITIS B VIRUS AND ALPHAFETOPROTEIN IN LIVER DISEASES IN SINGAPORE

Study Group On
Liver Diseases
of Singapore

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SYNOPSIS

HBsAg, anti HBcAg and AFP markers were investigated in patients with liver diseases. The frequency of HBsAg and anti HBcAg were significantly higher among patients with hepatocellular carcinoma, chronic active hepatitis, non alcoholic cirrhosis and acute viral hepatitis than normal subjects. Seventeen of 18 (94.4%) HCC patients were positive to anti HBcAg compared to 14/52 (26.9%) normals ($p < .0001$; relative risk = 46). The anti HBcAg assay appears to be more sensitive than HBsAg assays for detecting exposure to HBV.

INTRODUCTION

Hepatitis B virus (HBV) is the etiological agent for hepatitis B infection (Woolf and Williams, 1977). It has also been implicated in other liver diseases, eg. hepatocellular carcinoma (HCC) and Chronic Active Hepatitis (CAH) (Szmuness, 1978); Trichopoulous et al., 1978; Prince et al., 1975). In 1975 a systematic study of liver diseases in Singapore was conducted involving clinical, biochemical, histopathological, immunopathological and immunological parameters. Reported here are the HBV and alphafetoprotein (AFP) data of this study. Details of other studies on this project will be reported elsewhere.

PATIENTS AND METHODS

The study included patients with liver diseases admitted to Medical Units I, II and III of Singapore General Hospital between January 1975 and March 1976. All patients had liver biopsy and biochemical tests on admission and some also had repeated tests at 6 and 12 months. Diagnosis was based on clinical and biochemical grounds and confirmed by histopathology. Patients studied included hepatocellular carcinoma (19), chronic active hepatitis (15), non-alcoholic (18) and alcoholic (10), cirrhosis, acute viral hepatitis (74) and miscellaneous liver diseases (27). Control subjects consisted of healthy blood donors.

Sera from these patients were collected at the time of diagnosis and stored at -20°C until tested in batches. Hepatitis B surface antigen (HBsAg) was tested by counterimmunoelectrophoresis (CIE) and reverse passive haemagglutination (rPHA, Wellcome Laboratory), antibody to hepatitis core antigen (anti HBcAg) by radioimmunoassay (Core Ab, Abbott Laboratory) and AFP by immunodiffusion (ID) and CIE.

RESULTS

Table 1 summarises the frequency of HBsAg, anti-HBcAg and AFP among the various groups of liver diseased patients. The frequency of HBsAg and anti HBcAg were significantly higher among patients with HCC, CAH and AVH when compared to control subjects indicating that these patients had higher exposure to HBV. It is interesting to note that patients with non-alcoholic cirrhosis also had significantly higher frequency of HBsAg but not in patients with cirrhosis associated with alcohol. The relative risks associated with HBsAg and anti-HBcAg in these liver diseases are shown in Table II.

TABLE I

FREQUENCY OF HEPATITIS B SURFACE ANTIGENEMIA, HEPATITIS CORE ANTIBODY AND ALPHAFETOPROTEIN IN LIVER DISEASES

	HBsAg		Anti HBcAg	AFP	
	CIE	rPHA	RIA	I.D.	CIE
Hepatocellular Carcinoma	*7/19 (36.8) xxx	9/16 (56.3) xxx	17/18 (94.4) xxx	11/19 (57.9) xxx	14/19 (73.7) xxx
Chronic Active Hepatitis	7/15 (46.7) xxx	5/ 8 (62.5) xx	13/15 (86.7) xxx	0/15 NS	0/15 NS
Non Alcoholic Cirrhosis	5/18 (27.8) xxx	5/11 (45.5) x	ND	1/18 (5.6) NS	1/18 (5.6) NS
Alcoholic Cirrhosis	1/10 (10.0) NS	1/ 8 (12.5) NS	ND	0/10 NS	0/10 NS
Acute Viral Hepatitis	28/74 (37.8) xxx	34/56 (60.7) xxx	56/67 (83.6) xxx	0/74 NS	1/50 (2.0) NS
Miscellaneous	2/27 (7.4) NS	3/16 (18.8) NS	ND	0/17 NS	0/17 NS
Normal	42/1000 (4.2)	32/245 (13.1)	14/52 (26.9)	0/56	0/30

* Number positive/Number tested (%)
ND Not done

Comparison of patient groups and normals

xxx $p < .0001$
xxx $p < .001$
xxx $p < .005$
NS Not significant

TABLE II

RELATIVE RISK ASSOCIATED WITH HBsAg AND ANTI HBcAg IN LIVER DISEASES

	HBsAg		ANTI HBcAg
	CIE	rPHA	RIA
Hepatocellular carcinoma	13.3	8.6	46.1
Chronic active hepatitis	20.0	11.1	17.6
Cirrhosis	8.8	5.5	ND
Alcoholic cirrhosis	2.5	0.95	ND
Acute viral hepatitis	13.9	10.3	38.8
Miscellaneous	1.8	1.5	ND

As expected, for the detection of HBsAg the rPHA assay was more sensitive than the CIE assay. In all patient groups, the frequency of HBsAg was higher in the rPHA assay than the CIE assay (Table 1). However, the relative risks associated with HBsAg in these diseases were higher with the CIE assay than the rPHA assay (Table 2). The frequency of anti-HBcAg was higher than that of HBsAg (rPHA) in all patient groups tested, suggesting that the former assay may be a more sensitive indicator of HBV exposure. Seventeen of 18 (94.4%) HCC patients were positive to anti-HBcAg compared to 14/52 (26.9%) normals ($p < .0001$ relative risks = 46.1). The relative risk in HCC associated with anti-HBcAg was higher than that associated with HBsAg. Similarly the frequency of anti-HBcAg was also very high among patients with CAH and AVH with relative risks of 17.6 and 38.8 respectively. The frequency of AFP detected either by ID or CIE was significantly increased only in HCC patients.

DISCUSSION

In agreement with other reports, the present study showed that HBV infection is strongly associated with HCC, CAH and AVH as indicated by the presence of HBsAg and anti-HBcAg. A substantial proportion of AVH in Singapore is caused by HBV; a more concise report of the relationship of hepatitis A and B viruses and AVH will be given elsewhere.

To answer the question as to whether HBV infection preceeded HCC or is a consequence of the disease will have to await for prospective studies. However the high frequency of HBsAg in non-alcoholic cirrhosis, a pre-malignant condition, support the hypothesis that HBV infection probably occurs before the onset of HCC. This group of cirrhosis patients will be followed up in a prospective study for HCC development. The strong association of HBV with CAH in Singapore is consistent with the hypothesis that the strength of this association is related to the prevalence of HBV infection in the community. In countries where HBV infection is low, there is a corresponding lower proportion of patients with CAH with positive HBV markers.

With regard to sensitivities of assays, rPHA is more sensitive than CIE in detecting HBsAg. However, it appears that CIE is a better assay in discriminating patients from controls. Positivity to anti-HBcAg appears to be more sensitive marker for exposure to HBV than the presence of HBsAg as detected by rPHA. AFP as detected by ID or CIE appears to be a good discriminative assay for HCC.

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REFERENCES

1. Prince, A.M., Szmuness, W., Michon, J., Demaille, J., Diebolt, G., Linhard, J., Quenum, C. and Sankale, M: A case/control study of the association between primary liver cancer and hepatitis B infection in Senegal. International J. Cancer 16, 376 — 383, 1975.
2. Szmuness, W: Hepatocellular carcinoma and Hepatitis B. virus. Evidence for a causal association. Prog. Med. Virol. 24, 40 — 69, 1978.
3. Trichopoulos, D., Gerety, R.J., Sparros, L., Tabor, E., Xirouchaki, E., Munoz, N. and Linsell, C.A.: Hepatitis B and Primary Hepatocellular carcinoma in a European population. The Lancet ii, 1217 — 1218, 1978.
4. Woolf, I. and Williams, R: Acute viral hepatitis in Diseases of the Liver. Brit. J. of Hospt. Medicine. No. 2, 117 — 124, 1977.