Hepatitis B_s antibody in alcoholic cirrhosis

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SUMMARY Sera from patients with chronic liver disease were tested for antibody against hepatitis B surface antigen by radioimmunoassay. The antibody was found in 25% of patients with alcoholic cirrhosis and in 52% when alcoholic cirrhosis was associated with portal hypertension, these results being significantly higher than in a matched control population. Other forms of chronic liver disease did not differ from the control population. Hepatitis B virus infection might be a factor in determining which alcoholic patients go on to develop chronic liver disease and cirrhosis.

The hepatitis B virus appears to cause widespread subclinical infection as judged by the frequency with which serum surface antibody (anti-HB_s) to the hepatitis B surface antigen (HB_sAg) is found in the community (Cherubin *et al.*, 1972; Szmuness *et al.*, 1973). The development of sensitive radioimmunoassay and passive haemagglutination tests have markedly increased the yield of antibody positive sera (Walsh *et al.*, 1970; Lander *et al.*, 1971). Experience has shown that anti-HB_s first appears in the early convalescent stage after clinical hepatitis and persists for a number of years as an indicator of previous infection (Barker *et al.*, 1973).

There is surprisingly little information about anti-HB_s in chronic liver disease (Chiaramonte *et al.*, 1977). Most studies have concentrated on HB_sAg, which was found almost exclusively among patients with viral or chronic hepatitis (Fox *et al.*, 1969; Wright *et al.*, 1969; Reinicke and Nordenfeldt, 1970). However, for epidemiological work the virologist's natural inclination would be to examine for serological evidence of exposure to a virus by antibody measurement.

We report the results of a prospective survey of anti-HB_s in patients with various types of chronic liver disease.

Survey details

One hundred and sixty patients with chronic liver disease were seen clinically by one doctor (PM) over a period of one year at the Western Infirmary. All patients were British and Caucasian. Particular attention was paid to previous history of hepatitis

or possible exposure to the virus by means of blood transfusion, tattoos, or recent injections. Known cases of HB_sAg positive liver disease were excluded. The liver disease was classified using current clinical, biochemical, serological, and histological criteria. All patients had their liver disease confirmed histologically. In addition to routine stains all liver sections were examined using Shikata's orcein stain, and many liver biopsies were submitted for examination by electron microscopy. The diagnosis of portal hypertension depended on the endoscopic or radiological demonstration of oesophageal varices or palpable splenomegaly and hypersplenism on blood film.

Control groups consisted of hospital inpatients without liver disease; blood donors; renal unit patients and staff; and patients with a previous history of jaundice compatible with virus hepatitis. A group of 28 chronic alcoholics without clinical or biochemical evidence of liver disease undergoing treatment in a psychiatric hospital were also examined. If there was any doubt about the liver being involved (9 cases) a biopsy was performed and confirmed that liver histology was normal.

Statistical analysis was by Chi-squared test and, where appropriate, by Fisher and Yates' exact test.

Serology

All sera were coded and reported without knowledge of their origin. HB_sAg and anti-HB_s were measured by solid phase radioimmunoassay using the Ausria and Ausab kits respectively (Abbott Laboratories, Chicago, Ill, USA). Both were reported as positive or negative compared with standard controls. All positive sera were retested and confirmed to be

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repeatedly reactive. The anti-HBs result is expressed in counts per minute (cpm), and the manufacturer recommends that specimens with a cpm rate of ≥ 2.1 times the normal control mean (NC $\bar{x}$) should be regarded as positive. However, we have accepted only those results $> 5 \times$ NC $\bar{x}$ as positive, thus excluding any borderline cases.

Results

The results of the survey are summarised in Tables 1 to 4 and in the Figure. All control and patients'

Table 1 Hepatitis B_s antibody

Controls	Number	Antibody positive (%)	
Hospital patients without liver disease	161	14	(8.7)
Blood donors	55	5	(9.1)
Renal unit patients	111	9	(8.1)
Renal unit staff	62	5	(8.0)
History of hepatitis	73	13	(17.8)

sera were negative for HBsAg. The antibody frequency in the 389 control patients (Table 1) was 8.9% with an increase in the 73 patients with a previous history of hepatitis to 17.8%.

The 160 patients with chronic liver disease divided into five groups, the majority (58%) having alcoholic liver disease (Figure). The control population of 161 hospital patients without liver disease was selected to match the alcoholic cirrhosis patients for age and sex. Significant differences between

Table 3 Social class

Class	Alcoholic cirrhosis	Chronic alcoholism	Result
1	2	2	Compare totals social class 1-3 with 4-5. Significant difference $P < 0.001$ χ^2 test
2	10 (36)	2 (8)	
3	24	4	
4	4 (9)	10 (18)	
5	5	8	
Unknown	6	2	
Totals	51	28	

controls and patient groups were found only in alcoholic liver disease. The antibody frequency was 25% in 28 patients with alcoholic cirrhosis, and this was significantly different from the controls ($P < 0.05$). In 23 alcoholic cirrhosis patients with portal hypertension (oesophageal varices in 19, palpable splenomegaly and hypersplenism in 4) the antibody carrier rate was significantly higher at 52% ($P < 0.001$).

In an assessment of previous possible exposure to the virus (Table 2), presumed reliable data were obtained in 155 patients with chronic liver disease. On analysis there was no significant increase in the antibody frequency following a history of clinical exposure. The number of patients in the higher social classes, I-III, was significantly greater in the alcoholic cirrhosis group than in those with alcoholism alone (Table 3).

Results of positive anti-HBs counts are shown in Table 4 expressed as a ratio of the result compared to the NC $\bar{x}$ in cpm. While we have accepted $> 5 \times$

Table 2 History of previous exposure to hepatitis

	Total	Exposure			Sum of		Analysis	
		BT	J	T	BT + J	BT + T	Exposed	Not exposed
Chronic liver disease								
Number	155	21	17	7	4	5	54 (20%)	101 (24%)
Positive antibody	35	5	2	3	0	1	11	24
Alcoholic cirrhosis and portal hypertension								
Number	21	7	2	1	1	—	11 (36%)	10 (70%)
Positive antibody	11	3	0	1	0	—	4	7

BT = blood transfusion; J = jaundice; T = tattoo; NS = not significant

Table 4 Ratio of positive anti-HBs results to normal control means in counts per minute

Subjects	Positive result: NC $\bar{x}$ (cpm)					
	5-10	10-20	20-50	50-100	100-150	> 150
Hospital inpatients without liver disease (14/161 + ve)	4	2	1	3	2	2
Alcoholic cirrhosis (18/51 + ve)	3	4	3	3	2	3

Figure Hepatitis B_s antibody.

NCx as positive, the majority of positive sera had much higher counts. Patients with alcoholic cirrhosis had counts of similar magnitude to the matched control population.

No HB_s-containing hepatocytes were demonstrated on any of the biopsy specimens using the Shikata (orcein) stain. Similarly, in the 21 alcoholic liver disease specimens examined electron microscopically no demonstrable cytoplasmic or nuclear viral particles were found.

Discussion

The results of this study indicate that sera from patients with alcoholic cirrhosis, especially if associated with portal hypertension, more commonly contained anti-HB_s than would have been expected. The high frequency of anti-HB_s could be caused by an increased incidence of past infection or an abnormally prolonged or intense antibody response

to past infection with the hepatitis B virus. Current infection without detectable serum HB_sAg is an unlikely third possibility.

Anti-HB_s generally increases with age and is more common in males, Negroes, and the lower social classes (Szmunes *et al.*, 1973; Burrell *et al.*, 1977). Our patients were matched for age, sex, and race with the control population, and social class has been demonstrated not to have influenced our results. The high antibody frequency in alcoholic cirrhosis was not associated with a clinical history of previous exposure to the hepatitis B virus. The infection must have been subclinical, and the mode of transmission remains unknown. The way of life of chronic alcoholics without liver disease also did not seem to predispose them to hepatitis B virus infection. Sexual histories were not taken from our patients but homosexual contact would be an unlikely mode of transmission of hepatitis to our alcoholic cirrhosis patients as the remaining alco-

holic patients, whose behaviour would be unlikely to be different, were unaffected.

The 28 patients with primary biliary cirrhosis should strictly be re-examined statistically against a predominantly female control population before an antibody frequency of 21% is dismissed as insignificant. However, a previous study of anti-HB_s in the sera of 64 patients with primary biliary cirrhosis compared with age and sex-matched controls showed no significant difference (MacSween *et al.*, 1973).

Anti-HB_s has previously been examined in a British study of 411 patients with chronic liver disease using a passive haemagglutination test (Chiaromonte *et al.*, 1977). Antibody was found in 6.3% of these patients, but when 156 foreign patients were excluded the carrier rate for UK patients became 2.3%. However, it is notable that, of 43 UK patients with alcoholic liver disease of unspecified type, the antibody was found in 9.3%.

In an as yet unconfirmed study (Pettigrew *et al.*, 1972) using a lymphocyte transformation technique, sensitisation to a HB_sAg serum was demonstrated in all of 11 patients with alcoholic liver disease, suggesting cell-mediated reactivity to the virus in these patients.

Hyperglobulinaemia is a frequent finding in all forms of liver disease (Triger and Wright, 1973). There is an increase in gammaglobulin synthesis due to either a hyperreactive immune state or increased antigenic stimulus, the latter being thought to be predominant. Antibody titres to gut-associated bacteria and dietary proteins are high in cirrhosis (Triger *et al.*, 1972), probably due to the contents of portal venous blood gaining access to the systemic circulation. The gut antigens may avoid sequestration in the liver because of direct Kupffer cell damage or because they bypass the Kupffer cell filter by way of collateral circulation. Antibodies to *Escherichia coli* in patients with cirrhosis correlated well with the degree of architectural destruction and fibrosis on liver biopsy (Prytz *et al.*, 1977) but were not raised in the setting of portocaval shunting and inactive cirrhosis (Simjee *et al.*, 1975). Therefore, hepatic Kupffer cell damage appears to be the important factor in the increased response to gut antigens. This is a specific response to excess antigenic stimulation because antibody titres to non-gut-associated bacteria such as *Haemophilus influenzae* are not increased (Triger *et al.*, 1972).

The role of portal hypertension in selecting out that subgroup of patients with alcoholic cirrhosis who had the highest antibody frequency is difficult to understand. While portal hypertension in our alcoholic cirrhotic patients may merely be an indicator of advanced liver disease, it is tempting

to speculate that the resulting portosystemic collateral circulation may allow small quantities of antigenic material from enteric hepatitis B virus to enter the systemic circulation. Certainly there is evidence that the hepatitis B virus may be transmitted by infected blood given orally (Krugman *et al.*, 1967; Krugman and Giles, 1970), but there is no evidence that a large proportion of the population harbour the virus in the gut (Piazza *et al.*, 1975).

Viral antibody titres have been investigated in chronic liver disease (Triger *et al.*, 1974). Patients with alcoholic cirrhosis were found to have increased antibody titres against cytomegalovirus but not against a spectrum of other viruses, including measles, rubella, herpes simplex, varicella/zoster, and parainfluenza. Again this is a specific response rather than generalised immune hyperreactivity.

The HB_sAg is commonly found in sera from patients with lymphocytic leukaemia, uraemia, lepromatous leprosy, and systemic lupus erythematosus as a reflection of impaired immune status (Blumberg *et al.*, 1970; Alarcón-Segovia *et al.*, 1972). Alcoholic cirrhosis is often associated with impaired cell-mediated immunity perhaps predisposing to viral infections (Berenyi *et al.*, 1975). However, none of our patients was a chronic carrier of the HB_sAg, and all mounted a good antibody response clearing the antigen from serum, thus indicating a normal immune response to this virus.

We therefore have no adequate explanation for our findings of previous infection with the hepatitis B virus in patients with alcoholic cirrhosis, especially when the cirrhosis is advanced and associated with portal hypertension. The evidence discussed (Triger and Wright, 1973; Triger *et al.*, 1974) suggests that antibody responses are normal in cirrhosis, indicating that there probably is a true increase in the incidence of past infection with the hepatitis B virus in these patients. It is possible that the hepatitis infection might be a factor in selecting out which alcoholic patients go on to develop chronic liver disease.

These results require confirmation in another centre but are currently being investigated further by the examination of sera for antibody to hepatitis B core antigen and liver biopsies for evidence of surface and core antigen using the immunoperoxidase technique.

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