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Hepatitis B virus infection in northern India

Prevalence, subtypes, and seasonal variation *

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Previous reports from India have been limited to the incidences of hepatitis B antigen (HB Ag) in health and disease. This paper reports on a study undertaken over the last two years to determine the incidence of hepatitis B virus infection, the serotypes prevalent among apparently healthy individuals and patients with liver diseases, and the seasonal incidence of sporadic acute hepatitis in this geographic area. The incidences of infection in health and disease show an endemic pattern. The Y *subtype predominates among healthy carriers and patients with chronic liver diseases, whereas the* D *subtype predominates in patients with acute hepatitis. The similarity of subtypes of HB Ag among the majority of healthy carriers and patients with chronic liver diseases suggests that the majority of asymptomatic carriers (subclinically infected) would develop chronic liver disease. The proportion of HB Ag-positive cases of acute hepatitis was significantly higher during the two summer seasons than during the two winter seasons; this could be due to more frequent transmission of the virus by the faecal/urinary–oral route and its activation during the summer months, especially in areas with poor hygienic conditions. The urinary/faecal excretion of virus could thus maintain the natural transmission of the virus in such an environment.*

Hepatitis B virus infection is ubiquitous in nature. The incidences of hepatitis B antigen (HB Ag) in healthy persons and in those with liver diseases vary considerably in different parts of the world (3–5, 8, 14, 22). Reports from India (6, 10, 15, 19) have been limited to only the incidences of HB Ag in health and disease.

Our study was undertaken during the period March 1972–February 1974 to determine the incidence of hepatitis B virus infection, the serotypes prevalent among apparently healthy individuals and patients with liver diseases, and the seasonal incidence of sporadic acute hepatitis in northern India.

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MATERIALS AND METHODS

Control subjects

A total of 1 966 serum samples (Table 1) were collected from apparently healthy, adult male voluntary blood donors, healthy women with or without a previous pregnancy, children (aged 6 months–14 years) attending the outpatient department for minor ailments without any evidence of liver diseases, newborn babies (sera from matched cord blood), and patients with diseases not connected with the liver who had a long stay in hospital.

Patients with liver diseases

A total of 383 serum samples (Table 1) were collected from patients with liver diseases, who attended the hospital from provincial areas of Punjab, Haryana, and Himachal Pradesh; 265 of these serum samples were obtained from sporadic cases of hepatitis.

Table 1. Incidence of HB Ag among apparently healthy individuals and patients with hepatic and nonhepatic diseases in northern India

Source of serum samples	Total no. of sera tested	No. positive [a]
Control subjects:		
adult male voluntary blood donors	1040	24 (2.3)
healthy adult females (previously pregnant & nonpregnant)	460	2 (0.43)
cord blood	88	0
all children (6 months–14 years):	238	3 (1.3)
6 months–4 years	109	0
5–9 years	56	2
10–14 years	73	1
hospitalized adult patients with nonhepatic diseases	140	2 (1.4)
Patients with liver diseases:		
sporadic acute hepatitis	265	41 (15)
chronic liver diseases:	78	15 (19)
chronic aggressive hepatitis	5	3
postnecrotic cirrhosis	51	7 (14)
noncirrhotic portal fibrosis	15	2 (13)
malignancy in the liver	7	3
amoebic liver abscess	18	1
alcoholic liver disease	8	0
extrahepatic portal obstruction	14	1

[a] Figures in parentheses are percentages.

Immunoelectro-osmophoresis method (IEOP)

HB Ag was detected in undiluted serum samples by the IEOP method (16). The gel consisted of Noble Agar (Difco) (8.5 g/l) and a constant potential of 180 V (24 V/cm) was applied, which under these conditions gave 3–4 mA/cm. Antisera, kindly supplied by Professor F. G. Wewalka and Professor S. Sherlock, were used in the detection of HB Ag in the serum samples. The results were confirmed against a reference guinea pig antiserum (V801-501-058); [a] some of the positive sera were also confirmed by discontinuous immunoelectrophoresis (21) with a reference caprine antiserum obtained from the World Health Organization, Geneva, in a recently undertaken WHO collaborative study scheme.

[a] Obtained from the Research Reference Reagents Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md., USA.

Preparation of monospecific serum and typing of HB Ag

Monospecific anti-*d* and anti-*y* sera were prepared according to the method of Holland et al. (11) from anti-*ad* guinea pig serum (V801-502-558) and anti-*ay* guinea pig serum (V802-501-558), respectively, by the use of cross absorption with HB Ag containing the *ay* antigenic determinant (V-802-001-027) and HB Ag containing the *ad* antigenic determinant (V-801-001-027). Monospecific anti-*a* serum was kindly supplied by Dr P. V. Holland. Subtyping of HB Ag in the available sera was carried out by the IEOP method.

Because of the limited quantity of monospecific anti-*y* serum, only 30 randomly chosen d^- sera were tested with it. HB Ag that was d^- and y^- was further tested with anti-*a* serum.

RESULTS

The incidence of HB Ag among healthy subjects and patients with liver diseases is shown in Table 1. The clinical diagnosis of liver disease was supplemented by biochemical and histological examinations of liver specimens obtained by biopsy or at autopsy. Two of the hospitalized patients with nonhepatic diseases (rheumatoid arthritis and carcinoma of the stomach) were shown to be carriers of HB Ag. The ages of patients at the onset of apparent or inapparent hepatitis B virus infections in this community were investigated after classifying the serum samples from the apparently healthy children in 3 age groups. No evidence of infection was noted in those aged 6 months to 4 years, whereas 2 of 56 children in the 5–9-year age group and 1 of 73 individuals in the 10–14-year age group were shown to be carrying HB Ag in their blood.

One antigen-positive patient with chronic aggressive hepatitis was shown on histological evidence to have active cirrhosis. Liver biopsies from an antigen-positive fatal case revealed initially persistent hepatitis, which progressed to cirrhosis with hepatoma during a follow-up period of 6 months. One patient with an amoebic liver abscess and another with extrahepatic portal obstruction were shown to be carrying HB Ag. There was no evidence of cirrhosis in the liver biopsy specimen of the latter case.

Table 2. Seasonal incidence of HB Ag-positive cases of acute hepatitis and tests for significance [a]

Groups	Period of observation	Total no. of sera tested	No. positive	Percentage positive
A	March–August 1972 (summer)	71	15	21
B	September 1972–February 1973 (winter)	57	5	9
C	March–August 1973 (summer)	71	14	20
D	September 1973–February 1974 (winter)	66	7	11
E	summer, 1972 and 1973	142	29	20
F	winter, 1972 and 1973	123	12	10

[a] Group A vs B: χ^2 3.7, $p > 0.05$; Group B vs C: χ^2 3.1, $p > 0.05$; Group C vs D: χ^2 2.2, $p > 0.05$; Group E vs F: χ^2 5.7, $p < 0.05$.

Seasonal incidence of HB Ag-positive acute hepatitis

When the HB Ag-positive cases were considered according to seasons during the two years of investigation (Table 2), it was noted that the proportion of positive cases during the 2 summer seasons (Group E) differed significantly at the 5% level from that during the 2 winter seasons (Group F). However, the proportions for individual seasons were not significantly different from each other (Table 2) and this might be due to an error in sampling.

HB Ag subtypes

Seventy-three from a total of 89 HB Ag-positive sera in the different groups were tested with monospecific anti-*d* serum (Table 3). In the group of healthy carriers, the HB Ag detected in 19 of 29 sera (66%) did not possess the *d* antigenic determinants. Twelve d^- sera tested in this group, however, contained HB Ag of *Y* subtype. In contrast, HB Ag in 16 of 26 sera (62%) in the acute hepatitis group were of *D* subtype, whereas 10 of 15 sera (67%) in the chronic liver diseases group contained

Table 3. Subtypes of HB Ag among healthy carriers and patients with hepatic and nonhepatic diseases in northern India

Source of serum samples	No. of sera typed	Subtypes			
		D		*Y*	*A*
		d^+	d^-	y^+	d^-, y^-
healthy carriers	29	10	19 (12)[a]	12	–
acute hepatitis	26	16	10 (8)	8	–
chronic liver diseases:	15	5	10 (10)	9	1
chronic aggressive hepatitis	3	1	2 (2)	2	–
cirrhosis	7	1	6 (6)	6	–
noncirrhotic portal fibrosis	2	2	–	–	–
malignancy in the liver	3	1	2 (2)	1	1
other diseases [b]	3	2	1	–	–
totals	73	33	40 (30)	29	1

[a] Figures in parentheses represent random samples tested with monospecific anti-*y* serum.
[b] These were amoebic liver abscess, extrahepatic portal obstruction, and rheumatoid arthritis.

HB Ag of *Y* subtype. Only one serum with HB Ag of *A* subtype was detected; this was from a patient with a hepatoma. The serum from a patient in a dialysis unit with acute hepatitis and renal failure contained HB Ag of *Y* subtype. A healthy brother of this patient living in the same family carried HB Ag of the same serotype, whereas another healthy brother in the same family had HB Ag of *D* subtype in his serum.

DISCUSSION

The 2.3% incidence of healthy carriers of HB Ag amongst adult male voluntary blood donors in this geographic area is higher than that reported for American, Danish, German, English, French, and Swiss populations. The 0–0.1% carrier rate previously reported in India (10, 19) may have been due to the smaller number of samples tested or the techniques used. However, the results for the groups of children (aged 6 months–14 years) indicated that hepatitis B virus infections in this community appeared only after the age of 4 years. Children under the age of 4 years seem to be free from this infection.

The association of hepatitis B virus infection with acute hepatitis is less frequent in the present study and in other reports from India (10, 19) than in reports from the USA, England, and Sweden (5, 9, 13, 22). The endemicity of hepatitis B virus infection may be responsible for this lower frequency of HB Ag in acute hepatitis in tropical countries, such as India, where drug addiction is less common and hepatitis caused by the hepatitis A virus or other agents is probably more common. It is of interest to note that the incidence of sporadic cases of viral hepatitis type B at the present time is about the same (12%) as it was in 1955–56 during the water-borne Delhi epidemic of viral hepatitis type A (15).

The significant increase in the incidence of HB Ag-positive cases of acute hepatitis during the 2 summer periods of the present study [a] offers some epidemiological evidence that the transmission of hepatitis B virus during the summer months might more frequently be by the urinary/faecal–oral route (1, 7). In countries with poor hygienic conditions, the activation and propagation of enteric organisms are increased during the summer; in these environments, therefore, the urinary/faecal–oral route of transmission may be more important than the parenteral one. The urinary/faecal routes of excretion of virus could thus maintain a natural circulation of hepatitis B virus in this community.

A relatively high proportion of patients with post-necrotic cirrhosis (14%) and noncirrhotic portal fibrosis (13%) was shown in this study to be associated with type B viral hepatitis. Progressive changes from viral hepatitis to cirrhosis, with or without hepatoma, have been well documented, especially in patients in whom the antigen persisted (12, 20, 22). In the present study, these changes were observed in two cases. The demonstration of HB Ag in patients with rheumatoid arthritis, carcinoma of the stomach, amoebic liver abscess, and extra-hepatic portal obstruction suggests that the pathological state of chronic carriers of HB Ag might be different.

A high incidence of HB Ag of *Y* subtype among healthy persons in northern India and among patients with chronic liver diseases was noted; in sporadic acute hepatitis, however, the *D* subtype predominated.

A persistence of antigen occurred more commonly after mild or subclinical infection rather than after the acute disease (2), and asymptomatic carriers, even with apparently normal liver function, were shown histologically to have signs of chronic liver disease (17). This evidence would explain how the higher frequency of association between hepatitis B virus infection and cirrhosis might also be due to the higher incidence of subclinical infections or carrier states in this area, and why asymptomatic carriers may frequently progress to cirrhosis. The similarity of subtypes of HB Ag among the majority of healthy carriers and of patients with chronic liver diseases in the present study and in those by Nielsen & Le Bouvier (13) and Prince et al. (18) might also be a pointer to this view.

[a] Since submitting this paper for publication, we have obtained a 24% incidence of HB Ag-positive cases of acute hepatitis (16 positive of 68 sporadic cases studied) during the period March–August 1974.

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RÉSUMÉ

INFECTION PAR LE VIRUS DE L'HÉPATITE B EN INDE SEPTENTRIONALE : PRÉVALENCE, SOUS-TYPES ET VARIATION SAISONNIÈRE

De mars 1972 à février 1974, on a recherché en Inde septentrionale l'incidence de l'infection par le virus de l'hépatite B et les sérotypes présents chez des porteurs sains et chez des malades atteints d'affections hépatiques, et étudié les variations saisonnières de l'hépatite aiguë dans la région.

Des infections inapparentes par le virus de l'hépatite B sont décelées chez les enfants dès l'âge de 5 ans. La proportion des porteurs de virus est de 2,3% chez les hommes adultes en bonne santé et de 0,43% chez les femmes. Chez des patients atteints d'hépatite aiguë et chez d'autres souffrant d'affections hépatiques chroniques, on compte respectivement 15 et 19% de porteurs de l'antigène de l'hépatite B (Ag HB). Dans le groupe des porteurs sains, 66% des sérums positifs sont d^-, mais la majorité d'entre eux contiennent le déterminant antigénique *y*. Parmi les sérums positifs obtenus chez des malades atteints d'affections hépatiques chroniques, 67% contiennent un Ag HB de sous-type *Y*. Dans les cas d'hépatite aiguë, le sous-type *D* prédomine. La similitude de sous-type antigénique et de fréquence plus élevée du sous-type *Y* parmi les porteurs sains et les patients atteints d'affections hépatiques chroniques semble indiquer que la plupart des cas de cirrhose sont la conséquence d'infections subcliniques. Dans la majorité des cas d'hépatite aiguë positifs, il y a élimination ultérieure de l'antigène du sang.

Parmi les hépatites aiguës, le nombre de cas positifs pour l'Ag HB a été significativement plus élevé pendant les mois d'été 1972 et 1973 que pendant les mois d'hiver. Cela plaide en faveur d'une propagation plus intense du virus par voie fécale et urinaire ou son activation pendant l'été, spécialement dans les pays à niveau d'hygiène médiocre.

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