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Etiological spectrum of viral hepatitis and prevalence of markers of hepatitis A and B virus infection in north India*

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The etiological spectrum of viral hepatitis and the prevalence of serological markers of hepatitis A and B virus infection in healthy persons in north India were studied. Hepatitis A virus was found to be the most common cause of acute hepatitis in children (67%). It was a less frequent cause of this disease in adults (14%). Hepatitis A virus was only rarely the cause of acute (12%) and subacute (4%) liver failure. It was recorded as the etiological agent in an epidemic among schoolchildren. Exposure to hepatitis A virus occurs in early childhood, and by the age of 10 years, 90% of healthy persons have serological evidence of hepatitis A virus infection.

Hepatitis non-A non-B virus was the cause of acute hepatitis in 44% of adults and 24% of children with this disease. This virus was also the most important etiological agent in acute liver failure (55%) and subacute hepatic failure (51%). It was the cause of all the hepatitis epidemics in the general population.

Only 9% of hepatitis cases in children were due to hepatitis B virus whereas 42% of cases in adults were attributable to this virus. Hepatitis B virus was the causative agent in 33% of cases of acute hepatic failure and 45% of cases of subacute hepatic failure. The carrier rate for hepatitis B virus was 5% and antibody to hepatitis B surface antigen was found in up to 38% of specific population groups.

The discovery of serological markers has made it possible to distinguish viral hepatitis A, hepatitis B, and non-A non-B hepatitis. The relative frequency of these three forms of hepatitis varies from country to country. Each country has to guide the control and management of viral hepatitis with reference to its etiological spectrum. Very few countries have published data on the relative frequency of hepatitis A, B, and non-A non-B infections in their population (*1*). The present paper reports the results of surveys carried out in India and discusses their significance.

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MATERIALS AND METHODS

Serological studies for hepatitis A and B viruses were carried out on the following groups of patients:

— patients with sporadic acute hepatitis (78 children below 10 years of age and 100 adults);
— 93 patients with fulminant hepatitis;
— 42 patients with subacute hepatic failure;
— 385 patients with epidemic hepatitis.

Diagnosis of sporadic, fulminant and subacute hepatitis was based on accepted clinical, biochemical, and histological criteria (*2-4*). Serum samples from patients with epidemic hepatitis were collected during seven outbreaks. Three of these epidemics were investigated in detail, with 70, 100, and 101 samples, respectively, being tested for hepatitis B surface

antigen and anti-hepatitis A virus IgM. A small number of stool samples were also collected during the early icteric phase of two of these epidemics, and tested for hepatitis A virus antigen and particles. Between 9 and 40 acute-phase sera were collected during the four other outbreaks. Diagnosis of epidemic hepatitis was based on clinical and biochemical criteria.

Blood samples were also collected from 483 healthy volunteers. These included children below the age of 10 years, medical students, hospital doctors, staff nurses, hospital technical staff, office staff, policemen, adults over 60 years of age, and members of a tribal population. Twenty-five samples of cord blood were also taken. Except for the tribal population, which was from the state of Madhya Pradesh, the volunteers represented a highly selective upper middle class group from north India comprising Hindus, Muslims, and Christians.

Techniques

The following techniques were used to study the serological markers for hepatitis A and B virus infection:

Anti-hepatitis A virus (anti-HAV) IgM antibody was detected by the enzyme-linked immunosorbent assay (ELISA) technique of Moller & Mathiesen (*5*). Microtitration plates were precoated with 75 μl of rabbit anti-human IgM specific for μ chains, diluted 1:25 000 in phosphate-buffered saline (PBS), pH 7.4. The plates were incubated for 24 h at 4 °C in a humidified box, and then washed 3 times with PBS containing 0.5 ml/litre Tween 20 (PBS-T). The wells were filled with 1% bovine serum albumin and the plates incubated for a further 24 h at 4 °C. After 3 washings with PBS-T, 25 μl of serum, diluted 1:100 in PBS-T containing 10 ml/litre cord blood, was added and incubated at room temperature for 4 h. The plates were again washed 3 times with PBS-T, and 25 μl of antigen (20% stool filtrate, diluted 3 times in PBS) was added and incubated overnight at 4 °C. Anti-HAV horseradish peroxidase was diluted 1:40 in 50% human serum, and 50 μl was added to the plates as conjugate. The plates were incubated for 2 h and then washed 5 times, and *o*-phenylenediamine was added as substrate.

Anti-hepatitis A virus IgG was detected by radioimmunoassay using a HAVAB kit.[a]

Hepatitis B surface antigen (HBsAg) was detected by ELISA using an AUSZYME kit.[a]

Antibody to hepatitis B surface antigen (anti-HBs) was detected by radioimmunoassay using an AUSAB kit.[a]

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Hepatitis B infection was confirmed if the serum was positive for HBsAg. Hepatitis A was diagnosed if anti-HAV IgM was found in the serum. If neither of these was present, the disease was concluded to be a non-A non-B virus infection. Anti-HBs or anti-HAV IgG antibodies in the blood of healthy persons were taken as indicative of past infection.

RESULTS

Sporadic hepatitis

Acute sporadic hepatitis in adults was due to hepatitis A virus infection in 14% of cases, hepatitis B virus in 42%, and non-A non-B virus in 44% (Table 1). The distribution of A, B, and non-A non-B virus infection in children with sporadic hepatitis was 67%, 9%, and 24%, respectively.

Acute fulminant hepatitis

Acute fulminant hepatitis was due to hepatitis A virus infection in 12% of cases, hepatitis B virus in 33%, and non-A non-B virus in 55%.

Subacute hepatic failure

Of the 42 cases of subacute hepatic failure, 4% were found to be due to hepatitis A virus, 45% to hepatitis B virus, and 51% to non-A non-B infection.

Epidemic hepatitis

The results of studies in patients with epidemic hepatitis are shown in Table 2. Anti-HAV IgM was found in 80% of patients in the Kerala epidemic, in 22% of those in the Haryana epidemic, and in 10% of those in the Vrindavan epidemic. It was not found in the remaining four epidemics. The positivity rate for hepatitis B surface antigen varied from 3% to 18%. By exclusion of hepatitis A and B virus infection, the remaining patients were classified as having a non-A non-B infection. One of the seven epidemics (which was primarily among schoolchildren) was due to hepatitis A virus; the remainder were due to non-A non-B virus. All the epidemics were water-borne.

Healthy persons

Table 3 presents the data on the presence of anti-HAV IgG, HBsAg, and anti-HBs in the blood of healthy persons. Antibody to HAV was found in 68–100% of samples from the different categories. It is perhaps surprising that 94% of the tribal population were positive for anti-HAV IgG antibody, since they live in inland rural and forest regions, obtain drinking-water from wells and fresh streams, and defecate in open fields where there is little chance of

Table 1. Prevalence of hepatitis markers in patients with clinical hepatitis

Diagnosis	Anti-HAV		Anti-HAV IgM		HBsAg		Anti-HBsAg		Non-A non-B (by exclusion) (%)
	No. positive/ No. tested	%	No. positive/ No. tested	%	No. positive/ No. tested	%	No. positive/ No. tested	%	
Acute viral hepatitis:									
Adults	30/30	100	14/100	14	42/100	42	11/50	22	44
Children (below 10 years)	N.D.[a]		18/27	67	7/78	9	N.D.		24
Subacute hepatic failure	N.D.		1/29	4	19/42	45	2/28	7	51
Fulminant hepatitis	15/15	100	9/74	12	31/93	33	2/37	5	55

[a] N.D. = not done.

Table 2. Prevalence of hepatitis markers in patients with epidemic hepatitis

Location of epidemic	Anti-HAV IgM		HBsAg		Non-A non-B (by exclusion) (%)
	No. positive/ No. tested	%	No. positive/ No. tested	%	
Kerala	56/70	80	2/70	3	17
Azamgarh	0/39	0	15/100	15	85
Allahabad	0/9	0	0/9	0	100
Vrindavan	1/10	10	1/10	10	80
Jammu (Central Reserve Police Force)	0/101	0	4/90	5	95
Neemuch (Central Reserve Police Force)	0/13	0	0/13	0	100
Haryana	18/82	22	7/40	18	60

contaminating the drinking-water. The antibody appears to be transferred to the newborn, as shown by its 100% prevalence in cord blood.

The titres of anti-HAV IgG antibody in the healthy population were also studied; the results are shown in Table 4. Altogether 72.5% had titres of less than 1:75, 16.7% had a titre of 1:75, and only 5% and 6% showed titres of 1:150 and 1:300, respectively.

HBsAg was detected in 2–8% of the general population. Among the hospital staff, which included nurses, medical students, resident doctors, and laboratory staff working with blood samples, the prevalence was 8–18%. The prevalence was higher in staff nurses working in gastroenterology and dialysis wards compared with those working in general wards. Among the technical staff, a high prevalence was recorded in those working in renal dialysis, gastroenterology, and blood-bank departments. Recently enrolled medical students had a lower prevalence (5%) than those who had been working in the hospital for more than 3 years (16%).

Anti-HBs positivity varied between 10% and 38% in different groups. The highest rate was recorded among nurses. Of the cord blood samples, 23% were positive for anti-HBS.

DISCUSSION

The present study has established that non-A non-B viruses are the most important etiological agents for hepatitis in India. Six of the seven epidemics, 44% of adult patients with sporadic hepatitis, 55% of

Table 3. Prevalence of hepatitis markers in different categories of healthy people in India

Population group	Anti-HAV IgG		HBsAg		Anti-HBs	
	No. positive/ No. tested	%	No. positive/ No. tested	%	No. positive/ No. tested	%
Newborn infants (cord blood)	25/25	100	3/57	6	5/22	23
Children:						
under 5 years	50/73	68	1/39	2.5	2/10	20
5–10 years	47/52	90	4/33	12	1/10	10
Adults over 60 years	24/25	96	2/25	8	4/20	20
Medical students	19/20	95	1/20	5	5/20	25
Hospital doctors	2/2	100	3/18	16	2/20	10
Staff nurses	36/43	84	4/52	8	20/52	38
Technical staff	N.D.[a]		5/28	18	7/28	25
Clerical staff	N.D.		1/21	5	4/21	19
Policemen	N.D.		6/90	7	N.D.	
Tribal population	64/68	94	2/104	2	8/50	16

[a] N.D. = Not done.

Table 4. Anti-HAV titres in the healthy population

Age group (years)	No. of samples	Titre			
		< 1:75	1:75	1:150	1:300
Newborn infants (cord blood)	10	10	0	0	0
< 5	25	13	7	1	4
5–10	25	19	4	1	1
10–30	25	20	3	2	0
30–60	25	19	4	1	1
> 60	10	6	2	1	1
Total[a]	120 (100)	87 (72.5)	20 (16.7)	6 (5.0)	7 (5.8)

[a] Figure in parentheses gives percentage.

patients with acute fulminant hepatic failure, and 51% of patients with subacute hepatic failure were found to have a non-A non-B virus infection. Such a high infection rate has not been noted in any other country. An epidemic of non-A non-B infection has been reported from only one other country, Japan (*6*).

Sporadic hepatitis has only infrequently been reported as due to non-A non-B virus (*7–10*); the virus was reported as the cause of this disease in 9% of patients in Greece (*12*), 18% in Italy (*13*), and 10% in the USSR (*11*). Alter et al. found a 24% prevalence of non-A non-B virus infection among patients with non-post-transfusion hepatitis (*14*).

In this study, the non-A non-B hepatitis virus was the most important agent in highly fatal conditions such as acute liver failure due to fulminant hepatitis, and subacute hepatic failure due to acute hepatitis with bridging necrosis. This last condition has not often been reported, but in most developed countries, acute liver failure is primarily due to hepatitis B virus (*15–18*). The viral etiology was established in 176 of 188 patients with fulminant hepatitis reported by the Acute Hepatic Failure Study Group; of these, 106 (56%) had a hepatitis B infection (*19*). Rakela et al. in the USA (*18*) and Mathiesen et al. in Copenhagen (*15*) found hepatitis B virus in 40% of their patients with fulminant hepatitis.

The mode of non-A non-B infection in India was found to be different from the parenteral route often reported in the literature. Epidemic non-A non-B hepatitis was found to be due to contamination of drinking-water. In other clinical types of hepatitis, it was difficult to establish the mode of infection, but the parenteral route seemed to be unlikely, since a recent history of injection, transfusion, or surgical treatment was found in only 25.9% of patients.

Non-A non-B viral hepatitis is an important public health problem in India. It is not certain that the non-A non-B virus in India is the same as its counterpart described in other countries as the cause of hepatitis in patients given blood transfusions and in drug addicts. Control of the disease is relatively difficult as very little is known about the characteristics of the virus. It is not known how to kill or to attenuate the virus, how to treat water to reduce or eliminate the risk of infection, or whether human immune serum globulin can be used for prophylaxis of infection.

Hepatitis B virus was noted as the next most important cause of hepatitis in adults in this study. A total of 42% of cases of sporadic viral hepatitis in adults were caused by HBV, while only 9% of the children with this disease had HBV infection. Reports in the literature have recorded HBV infection in 28–80% of patients with sporadic hepatitis (*1*). The high prevalence of viral hepatitis B in adults compared with children may be related to increased exposure of adults to infection in their living and working conditions.

HBV was also an important cause of acute (33%) and subacute hepatic failure (45%). Acute liver failure has been reported to be due to fulminant viral hepatitis B in 40–60% of cases in different studies (*15–18*). These figures are higher than the 33% recorded in the present series. Some of the factors that promote the spread of hepatitis B virus infection, such as drug addiction, alcoholism, and homosexuality, are not as common in India as in several other countries with a higher frequency of viral hepatitis B. This may explain the lower rate of acute liver failure due to hepatitis B virus in Indian patients.

Hepatitis A virus was found to be the major cause of hepatitis in children; 67% of acute sporadic hepatitis and a single epidemic of hepatitis in schoolchildren were due to HAV infection. HAV was not a common cause of hepatitis in adults. Only 14% of adult acute sporadic hepatitis, 12% of acute liver failure, and 4% of subacute hepatic failure were due to hepatitis A virus infection. In contrast with the findings of the present series, HAV has been reported to be a more frequent cause of hepatitis in adults than in children in most developed countries (*1*, *20*). It has been suggested that local unhygienic conditions in developing countries favour HAV infection in young age groups, while frequent travel to developing countries is favourable for hepatitis A virus infection in adults without adequate immunity (*12*, *20–22*).

The poor environmental and hygienic conditions in developing countries favour the spread of water- and food-borne infections of hepatitis A virus. Generally, viral hepatitis A is a relatively benign disease, but it was noted as the cause of acute and subacute hepatic failure in a proportion of our patients. Mathiesen et al. have also reported acute liver failure due to viral hepatitis A (*1*, *15*).

Disregarding the high-risk hospital workers, the hepatitis B virus carrier rate in the present series was 5%. India can thus be classified along with some countries of eastern and southern Europe as having a medium carrier rate of 5–10% (*23*). The carrier rate among doctors and other workers in the blood bank, blood-handling laboratories, gastroenterology wards, and renal dialysis unit was quite high, and in line with the previously reported incidence for high-risk professional groups (*24*). This finding is stressed to draw the attention of hospital administrators and technical staff to the problem, so that all possible preventive measures may be instituted in order to reduce the incidence of hepatitis B virus infection and carrier rate. The recently developed hepatitis B vaccine will be ideal for prophylaxis but its high cost may be a limiting factor. It has been suggested that immune serum globulin could be useful for pre-exposure prophylaxis of viral hepatitis B (*27*). We would recommend the administration of immune serum globulin to high-risk groups such as that described in the present report.

Anti-HBs was found in 10–38% of healthy persons in the different categories. The tribal population, who lived away from the cities and were not greatly exposed to the transmission of hepatitis B virus infection, had an anti-HBs positivity rate of 16% compared with 25% in the technical staff working in the blood bank and gastroenterology laboratory, and 38% among the nurses working on the hospital wards. Anti-HBs prevalence in the general population in India was found to be similar to those found in some devleoped countries such as the German Democratic Republic (16.2%) and Japan (16.6%) and is much lower than the prevalences of 31–77% reported from some developing and tropical countries (*25*).

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RÉSUMÉ

ÉVENTAIL ÉTIOLOGIQUE DE L'HÉPATITE VIRALE ET PRÉVALENCE DES MARQUEURS DE L'INFECTION PAR LE VIRUS DE L'HÉPATITE A OU B DANS LE NORD DE L'INDE

L'article présente des données sur l'éventail étiologique de l'hépatite virale et sur la prévalence des marqueurs de l'infection par le virus de l'hépatite A ou B dans une population en bonne santé habitant le Nord de l'Inde.

Le virus de l'hépatite A (HAV) s'est révélé la cause la plus fréquente d'hépatite aiguë chez les enfants (67%). Il était moins souvent en cause chez les adultes (14%). Ce virus n'a que rarement été responsable d'une insuffisance hépatique aiguë (12%) ou subaiguë (4%). Il a pu être identifié comme étant l'agent étiologique lors d'une épidémie parmi des écoliers. L'exposition au virus de l'hépatite A survient précocement au cours de l'enfance et, à l'âge de 10 ans, 90% des sujets bien portants présentent des signes sérologiques d'infection par l'HAV.

Le virus de l'hépatite ni A-ni B s'est révélé la cause de l'hépatite aiguë chez 44% des adultes et 24% des enfants malades. Ce virus était en outre l'agent étiologique le plus important dans les cas d'insuffisance hépatique aiguë (55%) ou subaiguë (51%). C'est à lui qu'on a pu imputer la totalité des épidémies d'hépatite dans la population générale.

Quant au virus de l'hépatite B (HBV), il était à l'origine de 9% seulement des cas d'hépatite chez les enfants, mais de 42% chez les adultes. L'HBV a été trouvé dans 33% des cas d'insuffisance hépatique aiguë et 45% des cas d'insuffisance subaiguë. Le taux de portage chronique de l'HBV était de 5% et les sujets porteurs d'anticorps dirigés contre l'antigène de surface de l'hépatite B (HBsAg) pouvaient représenter jusqu'à 38% de la population générale.

L'étude actuelle a permis d'établir que le virus de l'hépatite ni A-ni B est l'agent étiologique le plus important de l'hépatite épidémique ou sporadique et de ses complications, comme une hépatite fulminante ou une insuffisance hépatique subaiguë. A Delhi, l'HBV s'est révélé, par ordre d'importance, le second agent à l'origine de l'hépatite sporadique et de ses complications. La principale cause d'hépatite chez les enfants est le virus de l'hépatite A. L'exposition à ce virus intervient précocement au cours de l'enfance. L'HBsAg et l'anti-HBs ont, dans la population générale, une fréquence du même ordre que celles qu'on observe dans certains pays développés.

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