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Hepatitis B virus replication in patients with chronic liver diseases

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Summary: One hundred and seventy five subjects with chronic liver diseases which included patients with chronic active hepatitis (90), liver cirrhosis (31) and asymptomatic hepatitis B carriers (54), were included in the study. Hepatitis B virus (HBV) specific DNA-polymerase activity and HBe-markers were tested as markers of HBV-multiplicaiton. In HBsAg positive samples, DNA-P activity was positive in 44.4% of the HBV carriers, 52.9% of the patients with chronic active hepatitis and 81.8% of the patients with liver cirrhosis. The corresponding figures for the presence of HBeAg in these groups were 18.5, 26.5 and 45.5% respectively. Virus multiplication was also observed in 41.1 and 44.4% patients with chronic active hepatitis and liver cirrhosis respectively, in the absence of HBsAg. The results of the present study show that hepatitis B virus is the most important etiological factor of chronic liver diseases in India. Most of our patients of chronic liver diseases seems to have contacted HBV infection as young adults and the mode of transmission is likely to be horizontal rather than vertical. The virus replicating markers correlate well with the severity of the liver injury and decreased with the age. DNA-P activity is a more sensitive marker of viral multiplication than HBeAg. Viral multiplication was also found to occur in the absence of the usual HBV markers. Continued viral multiplication in patients with chronic active hepatitis and liver cirrhosis is implicated in continued liver injury and progressive liver disease. *Gastroenterol Jpn 1990;25:258–264*

Key words: *chronic liver disease; DNA-polymerase; HBeAg; HBV*

Introduction

Studies of hepatitis B virus infections have shown a close relationship between the phase of active viral replication and the development of hepatic lesions[1-3]. During active viral replications, liver cell necrosis was assumed to occur as a result of the direct cytopathic effect of HBV on hepatocytes[4,5]. However, recent studies indicated the role of the immune system in the pathogenesis of liver diseases associated with HBV infection[6,7]. Active viral replication has been associated with the presence of hepatitis B antigen (HBeAg) and the underlying active liver disease and its relationship with the presence of hepatitis B virus (HBV) DNA in serum is well documented[8,9]. Detection of HBV-DNA and/or DNA-polymerase in serum is a specific and sensitive method for the determination of intact HBV in serum and the degree of HBV replication[8,10-12] which is independent of HBeAg/anti-HBe system[13-15]. The presence of HBV-associated DNA-polymerase (DNA-P) is direct evidence of the presence of DNA containing HBV[16,17] and an indication of HBV infection.

The present study was carried out to find out the status of HBV replication in HBsAg-positive and HBsAg-negative (also IgM anti-HAV-negative) patients with chronic active hepatitis (CAH), cirrhosis of the liver (CL) and asymptomatic HBV carriers. Comparative values of DNA-P activity and HBe-markers were also as-

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Table 1 HBV-markers in different age groups of patients with chronic liver disease

Age group years	HBsAg			DNA-P			HBeAg		
	HC	CAH	CL	HC	CAH	CL	HC	CAH	CL
10–20	1	3	3	2	0	2	1	0	2
21–30	29	9	2	10	5	3	4	3	1
31–40	10	4	4	5	6	6	2	2	3
41–50	7	7	7	3	3	4	1	2	3
51–60	6	6	5	3	2	3	2	1	1
61–70	1	5	1	1	2	0	0	1	0
Total	54	34	22	24	18	18	10	9	10
Percentage*	100	38	71	44	20	38	19	10	31

HC: HBV carriers; CAH: chronic active hepatitis; CL: cirrhosis of liver
* Values are percentages of the total of patients in each group.

sessed as markers for viral replication.

Materials and Methods

Patients

Sera from 175 consecutive cases of chronic liver disease which included chronic active hepatitis (90), liver cirrhosis (31) and asymptomatic hepatitis B carriers (HBV-carriers) (54), and admitted to the wards of the Department of Gastroenterology at the All India Institute of Medical Sciences, were included in the study. All the samples were tested for HBV infection including the markers of virus multiplication in order to find out the magnitude and role of HBV infection in chronic liver diseases.

The diagnosis was confirmed by standard clinical, biochemical, serological and histological criteria[18,19]. Hepatitis B virus carriers were diagnosed from the voluntary blood donors during screening of their sera for HBsAg by enzyme linked immunosorbent assay (ELISA). In addition, 45 sera samples from healthy students from the University of Bergen, Norway, were tested as a control group.

Serological investigations

HBsAg in serum samples was tested by micro-ELISA technique[20]. HBeAg and anti-HBe in serum samples were assayed by the HBe-EIA kits available commercially from the Abbott Laboratories, North Chicago, Illinois. DNA-polymerase activity was measured in serum samples by a modification of the technique of Fang et al[21]. Details of the modification are described elsewhere[22].

Statistics

The test of proportion was applied to evaluate the level of differences in the groups.

Results

The mean age of the asymptomatic HBV carriers, patients with CAH and the patients with liver cirrhosis (CL) was found to be 33.4+12.7 (range 15-54), 39.1+15.9 (range 9-66) and 41.2+13.7 (range 14-67) years respectively. The corresponding male/female ratios in these groups were 2.1:1, 3.1:1 and 5.7:1 respectively. The patients with CAH and CL belonged to a higher age group in comparison to asymptomatic HBV-carriers ($P<0.05$). The HBV viral markers in different age groups are presented in **Table 1**.

All the 45 sera samples from healthy students from the University of Bergen, Norway, were negative for HBsAg and markers of viral replication. Thirty four of 90 patients with CAH and 22 of 31 patients with CL were positive for HBsAg. While estimating the proportion of patients with HBV multiplication, the patients with chronic liver diseases and those without any markers of HBV-infection were excluded form the analysis for all practical purposes. All the patients with

Table 2 Infection due to hepatitis B virus in patients with chronic liver diseases

Disease	Number	HBsAg +ve	HBsAg −ve DNA-P +ve	Number with HBV markers
CAH	90	34	23	57 (63.3%)
CL	31	22	4	26 (83.8%)
Total	121	56 (46.3%)	27 (22.3%)	83 (68.6%)

CAH: Chronic active hepatitis, CL: Cirrhosis of the liver

Table 4 Status of HBe-markers in samples positive for DNA-P activity

Groups	DNA-P +ve	HBeAg +ve	anti-HBe +ve	HBe-markers −ve
HBV-carriers	24	6 (25.0)	7 (29.2)	11 (45.8)
CAH	18	8 (44.4)	7 (38.9)	3 (16.7)
Cirr. liver	18	9 (50.0)	3 (16.7)	6 (33.3)
Total	60	23 (38.4)	17 (28.3)	20 (33.3)

Values of parenthesis show percent values

chronic liver diseases are enumerated at **Table 2** in order to provide a holistic picture of HBV status in these patients. In all about 63% of the CAH patients and 84% of the cirrhotics had markers of HBV infection in their sera either in the form of HBeAg and/or DNA-polymerase. The overall evidence of HBV infection in patients with chronic liver disease was 68.6%.

The status of viral replication in various groups of patients with HBV infection is shown in **Table 3**. Among the asymptomatic HBV carriers, DNA-P activity was found to be present in 44.4% of the subjects in comparison to HBeAg positivity in 18.5% of the same subjects. Among the chronic active hepatitis patients, positive for HBsAg, 52.9% showed DNA-P activity while only 26.5% were positive for HBeAg. In patients with liver cirrhosis, 81.8% of HBsAg positive subjects showed DNA-P activity in comparison to the presence of HBeAg in 45.5% of the patients. Out of a total of 110 samples positive for HBsAg, 54.5% showed positive DNA-P activity while 26.4% of these subjects were positive for HBeAg.

DNA-P activity was found to be present in a significantly large number of patients with the liver cirrhosis in comparison to asymptomatic carriers ($P<0.01$) and the CAH group ($P<0.05$). However, the difference between the cirrhotics and the asymptomatic HBV carriers in relation to the CAH group was not significant.

Among the serum samples positive for DNA-P activity (**Table 4**), 38.3% were found to be associated with the presence of HBeAg, 21.7% were associated with anti-HBe, while in the remaining 33.3%, DNA-P activity was present in the absence of any of the HBe-markers.

The present study shows the prevalence of HBsAg in over 55% of the patients with chronic liver diseases over the age of 40 whereas DNA-P activity and HBeAg were found to be present in 38.9 and 42.1% of these patients respectively (**Fig. 1**). However, there was no significant difference in the presence of HBsAg, DNA-P activity and HBeAg in asymptomatic HBV carriers between patients above and below the age of 40 (**Fig. 2**).

Among the 56 subjects with CAH negative for HBsAg, 23 (41.1%) showed the presence of

Table 3 Status of DNA-P and HBeAg among different groups of HBV infection

Groups	Number	HBsAg +ve	DNA-P +ve	HBeAg +ve
HBV carriers	54	54/54 (100.0)	24/54 (44.4)	10/54 (18.5)
CAH	90	34/90 (37.8)	18/34 (52.9)	9/34 (26.5)
Cirrhosis of liver	31	22/31 (71.0)	18/22 (81.8)[bc]	10/22 (45.5)[a]
Total	175	110/175 (62.9)	60/110 (54.5)	29/110 (26.4)

Values in parenthesis show per cent values

a: Difference significant ($P<0.05$) in comparison to HBV-carriers

b: Difference significant ($P<0.01$) in comparison to HBV-carriers

c: Difference significant ($P<0.05$) in comparison to CAH group

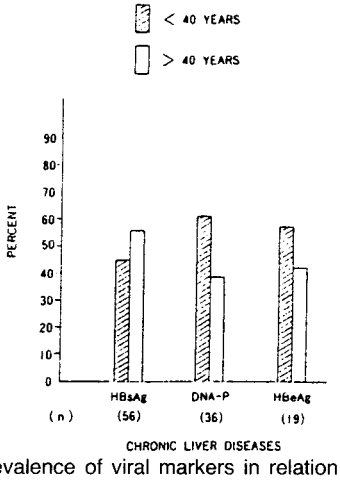

Fig. 1 The prevalence of viral markers in relation to age in patients with chronic liver diseases. Values in parenthesis show the number of patients tested for each parameter. Shaded columns shows the patients aged less than 40 while the open columns show patients aged more then 40.

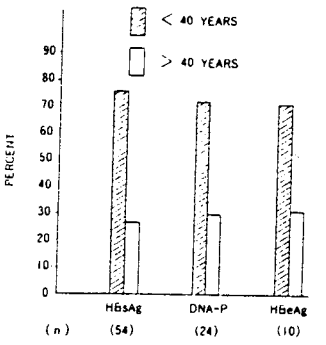

Fig. 2 The prevalence of viral markers in relation to the age in hepatitis B virus carriers. Values in parenthesis show the number of patients tested for each parameter. Shaded columns show the patients aged less than 40 while the open columns show patients aged more than 40.

DNA-P activity while among HBsAg negative patients with cirrhosis of liver DNA-P activity positivity was 44.4% (4/9 subjects).

Discussion

The mean age of HBV carriers, patients with CAH and patients with cirrhosis in the present study was 33.4, 39.1 and 41.2% years respectively. The corresponding evidence of HBV replication as tested by DNA-P activity in these patients was observed in 44, 53 and 82% of the subjects respectively. The presence of replicating HBV in the liver of the patients age over 40 indicate that they have contacted the infection in their adolescence or as young adults. Vertical transmission has been shown not to be a major mode of HBV transmission in India[23]. Most of the Indian patients seems to contact HBV infection by other modes such as the parenteral route or prolonged contact, particularly because most of the transfusion services in this country do not have screening facilities for HBsAg and sanitation is a persistent and continuing problem.

Markers of HBV infection were present in about 69% of the patients with chronic liver diseases, implicating HBV to be the single major cause. This observation was supported by the fact that about 84% of the histologically proved cirrhotics has either HBsAg or DNA-P in their sera (**Table 2**). Even though it was believed that HBV was the most important agent inducing chronic liver disease in India, it has not been well documented, except for one study with very few cases[24] where HBV was demonstrated to be the sole cause of cirrhosis in India.

In all the three goups i.e. asymptomatic HBV-carriers, patients with chronic active hepatitis and patients with liver cirrhosis, DNA-P activity was present in significantly more of cases compared to HBeAg. The presence of HBV-DNA has already been shown to be present in the serum at various stages of HBV-infection regardless of HBeAg detection[25,26], and the absence of HBeAg in these patients does not necessarily reflect low levels of viral particles in the serum or the occurence of HBV genome varients. The relative insenstivity of radioimmunoasay for HBeAg rather than the absence of HBeAg/anti-HBe has been shown to reflected by the DNA-P activity[27]. The testing of the sera for HBeAg/Ab to show infectiousness and to decide the prognosis has already been questioned[26,28] specifically in CAH and cirrhosis of liver[29]. The assay of DNA-P has been shown to be useful in predicting the chronic sequelae in patients with acute viral hepatitis[30]. In asymptomatic HBV-carriers, the DNA-P activity was found to be present in 44.4% cases, in comparison

to 18.5% positivity of HBeAg. Kam et al[31] have demonstrated significant quantitative and qualitative differences among the asymptomatic HBV-carriers in relation to the presence of hepatitis B virus DNA (HBV-DNA) and HBeAg. DNA-P activity has also been shown to be present in as low as 5.5% of the asymptomatic HBV-carriers[21]. The frequency of HBeAg in asymptomatic HBV-carriers in various countries has been shown to range from 0% to as high as 50%[32,33] and anti-HBe between 18.6 to 74%[32].

The finding of evidence of viral replication in 44.4% of HBV-carriers is of concern, as this may lead to chronic liver disease in due course of time. HBV-DNA has been found to integrate into hepatocyte DNA in cases of CAH[34,35] and liver cirrhosis[34]. The integration of viral sequences and persistant surface antigen production contributes to the development of progressive liver disease and the cirrhosis is observed in clinically asymptomatic carriers[36].

Another important observation of the present study was the presence of HBV replication in a significantly higher proportion of the patients with cirrhosis in comparison to patients with CAH and HBV-carriers. Our data confirms the earlier findings of increased activity of DNA-P[30] and HBV-DNA[9] even up to 76% in patients with CAH and HBV-DNA in 70% of the patients which progressed from chronic active hepatitis to cirrhosis of liver[37,38]. However it is difficult to assert from the available data based on detection of DNA-P or HBeAg, whether ther cytopathic effect of virions or the immune system is involved in the mechanism of hepatic necrosis in chronic liver diseases.

Viral replication was also found to be present in 44.4% of the patients with cirrhosis of liver and 41.1% of the patients with chronic active hepatitis which were negative for HBeAg. This data strengthens the conclusion that HBV multiplication is recorded in extremely high proportions in patients with chronic active hepatitis and cirrhosis of liver. Higher frequency of DNA-P positivity in HBsAg negative patients with liver cirrhosis and chronic active hepatitis suggest that multiplication of HBV may occur in the absence of conventional HBV markers. Similar observations were made in these groups by Brechot et al[34] and Nalpas et al[39]. The explanation given for such an event was that HBsAg could be masked by immune complexes or be present in the serum at very low levels.

The dominance of DNA-P activity in the serum over the HBe-markers as a positive marker of viral replication has been further emphasized by the fact that in DNA-P positive samples, on an average, HBeAg positivity was 38.3% and anti-HBe in 22.7%. In 33.3% of the DNA-P positive samples, both HBeAg and anti-HBe wre absent. The percentage being as high as 45.8% in HBV-carriers (**Table 4**). In the group of HBV-DNA positive patients, the presence of liver disease has been shown to be significantly connected with the absence of HBeAg in serum and suggested that the HBeAg/anti-HBe status in HBV-DNA positive sera distinguish two different types or phases of chronic type B hepatitis[14]. There is an inconsistent relationship between the time of seroconversion of HBeAg to anti-HBe and the disappearance of HBV-DNA[15]. In CAH, DNA-P activity seems to be a good marker of positive viral replication in comparison to HBeAg. The presence of DNA-P in the serum of some HBeAg/anti-HBe positive carriers (29.2) suggest that they may be infective[12,41]. The absence of HBe-markers in large number of samples may be due to the relative insensitivity of the enzyme immunoassay for detection of HBeAg/anti-HBe or "e" window period[27]. In contrast to earlier reports[8,26,42], only 50% of our DNA-P positive patients with cirrhoris of the liver and none with chronic active hepatitis, showed the presence of HBeAg. Continued viral replication has been shown to be present in a significant large number of patients after HBeAg seroconversion in Taiwan[37]. The presence of anti-HBe has been shown to be a marker of low viral replication, rather than the complete absence of viral replication[9].

The present study reports evidence of virus multiplication in a very high proportion in patients with liver cirrhosis and chronic active hepatitis. DNA-P activity was found to decrease with age. In addition to individual host immune fac-

tors[43], inflammatory changes in the liver of chronic HBV infection patients have been found to be associated with high levels of viral production maintained for long periods[37,44,45], leading to progressive disease. High viral multiplication of HBV virus in these patients also implies the lack of viral integration in these groups whcih may be due to the shorter duration of the disease process or variable infectivity. An unpublished report from this center revealed hepatocellular carcinoma with cirrhosis in 5 patients in comparison to hepatocellular carcinoma with normal liver in 11 patients as observed by laparoscopy and multiple biopsies under direct vision. A low incidence of hepatocellular carcinoma associated with cirrhosis in our population might be consistent with the present observations.

Our findings are significant in relation to understanding of pathogenesis with particular need for treatment with anti-viral agent to stop or arrest the process of replication, in order to prevent the liver injury. The carrier state may also be not as benign for individuals as considered in the past and raises the question of anti-viral therapy in them.

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