Journal of Gastroenterology and Hepatology (1988) **3**, 139–142

LIVER AND BILIARY

Evidence of ongoing virus multiplication in type B fulminant hepatitis

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Accepted for publication 7 September 1987

Abstract The presence of HBeAg- and HBV-specific DNA-polymerase (DNA-P) activity — regarded as markers of HBV multiplication — were studied in 40 patients with fulminant hepatitis B within 1–5 days of the onset of hepatic encephalopathy. HBeAg and DNA-P were detected in three (7 %) and 21 (51%) of the patients respectively. Only three (7%) were positive for anti-HBs. DNA-P activity was present in 38.1 % even 10 days after the appearance of icterus. Present findings confirm an ongoing HBV multiplication in about half of the patients of fulminant hepatitis B.

Key words: DNA-polymerase activity, fulminant hepatitis B, hepatitis B, hepatitis B e antigen, hepatitis B virus.

INTRODUCTION

Fulminant hepatitis is a serious and very often a fatal presentation of HBV infection.[1,2] The mechanism of extensive hepatocellular necrosis and failure of hepatocyte regeneration leading to the acute liver failure in this condition is not known. Brechot *et al.* suggested that prolonged virus replication is not an important factor in the pathogenesis.[3] However, 2 years later investigators from the same laboratory concluded that spread of HBV infection within the liver increases the area of destruction in the liver, consequently decreasing the residual functional hepatocytes, which may be the underlying cause of acute liver failure.[4] The present study was therefore carried out to determine the evidence, if any, for ongoing HBV replication in fulminant hepatitis B.

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METHODS

Forty patients with fulminant hepatitis B, comprised of 17 males and 23 females with a mean age of 26.4 years (s.d.=7.4; range 17–44 years), admitted to the Rajgarhia Liver Unit of the Department of Gastroenterology at All India Institute of Medical Sciences, were studied. The diagnosis of fulminant hepatitis was established according to accepted criteria.[5] These patients belonged to the average socio-economic class and had no evidence of moderate or severe undernutrition. The mean values for total serum proteins and albumin were 7.8 g (s.d.= 0.16) and 3.6 g (s.d.=0.21) respectively. Acute HBV infection was established as the aetiological agent on the basis of the following three findings: positive IgM anti-HBc, negative IgM anti-HAV, and a negative history for intake of any hepatotoxic substance. Blood was collected within 10 days of the onset of illness in 22 patients, and from 10 to 15 days in the remaining 18 cases. Serum was separated and stored at −70°C. IgM anti-HBc, IgM anti-HAV, HBeAg and anti-HBs were tested by EIA diagnostic kits from Abbott Laboratories. HBsAg was detected by micro-ELISA technique.[6]

DNA-polymerase (DNA-P) was tested by the following modified technique of Fang *et al.*[7] A portion of each serum sample (25 µl) was mixed with 10 µl of detergent nonidet P-40 (10%) and β-mercaptoethanol (3%) in microfuge tubes (1.5 ml). After 30 s, 100 µl of freshly prepared reaction mixture — 1.6 mol/l Tris (hydroxymethyl) aminomethane, 0.4 mol/l $MgCl_2$, 1.2 mol/l NH_4Cl, 5 mmol/l each of D-CTP, D-GTP, and D-TTP (Sigma Chemicals), and 5 mmol/l of tritiated ATP ammonium salt (Bhabha Atomic Research Centre, Trombay, Bombay) — was added to each tube. The tubes were vortexed for 30 s and incubated at 37°C for 4 h in a shaking water bath. After incubation, 50 µl of reaction mixture was spotted on glass microfibre filters (2.4 cm diameter), pretreated with 200 µl of trichloroacetic acid (5%). The filters were washed three times with trichloroacetic acid for 15 min each. The final washings were given with methanol and acetone for 5 min each. The filter discs were dried at 37°C and counted in a scintillation counter with 10 ml of scintillation counting fluid (4.0g PPO and 0.1 g POPOP per litre of toluene). A sample was considered positive for DNA-P if its count/min was higher than the mean count/min of negative controls plus three standard deviations (mean + 3 s.d.).

RESULTS

The serological profile of HBV markers in patients of fulminant hepatic failure is presented in Table 1. Fourteen patients were positive of HBsAg. All were positive for IgM anti-HBc and negative for IgM anti-HAV. HBeAg and anti-HBs were each present in three patients.

DNA-P was detected in 21 patients (51%), 13 of whom were ill for less than 10 days and eight had the disease for 10–15 days. DNA-P and anti-HBs were both positive in only one patient.

Thirty-three patients had a fatal outcome which included 17 with a positive DNA-P activity. Seven patients survived, four of whom were positive for DNA-P.

Table 1 Status of HBsAg, DNA-Polymerase, HBeAg and anti-HBs in 40 patients with fulminant hepatitis B positive for IgM anti-HBc

Number of patients	HBsAg	DNA-P	HBeAg	Anti-HBs
3	+	−	−	−
8	+	+	−	−
3	+	+	+	−
14	−	−	−	−
9	−	+	−	−
1	−	+	−	+
1	−	−	−	+

+: positive; − : negative.

DISCUSSION

The present study records the evidence of ongoing HBV multiplication in 51% of the patients with fulminant hepatitis B, as indicated by positive DNA-P activity. The tests for detection of HBV multiplication in ascending order of sensitivity are: HBeAg, DNA-P, and molecular hybridization technique for HBV-DNA.[8-10] It is possible that the more sensitive molecular hybridization technique for HBV-DNA might have detected a higher proportion of patients with evidence of viral replication in this group. Brechot *et al.* reported HBeAg positivity of 37% in a series of 64 patients of fulminant hepatitis.[3] However, the more sensitive test for virus multiplication, i.e., HBV-DNA by molecular hybridization, was positive only in 9% of these patients. The criteria for diagnosis of fulminant hepatitis in the present series was different from those used by Brechot *et al.*[3] All of the patients in the present series had disease for less than 15 days with manifestations of hepatic encephalopathy 2-3 days after the onset of jaundice. Compared with this, the duration of disease in the series reported by Brechot *et al.* was 1-58 days and the development of hepatic encephalopathy took up to 2 months after the onset of jaundice.[3] Therefore, the results of the study of Brechot *et al* which according to the definition in the present study may have included patients of subacute hepatic failure cannot be strictly compared with the present study.[11]

HBsAg was positive only in 14 patients. However, positive IgM anti-HBc confirmed virus B aetiology of all patients. Presence of DNA-P in the absence of HBsAg may be due to low levels of circulating HBsAg particles undetectable by the presently available ELISA technique using polyclonal antibody, and/or brisk humoral immune response to the HBV, leading to the clearance of circulating HBsAg, despite ongoing virus multiplication.[3,6] Evidence for HBV replication has been shown in the absence of conventional HBV markers by other investigators.[12,13]

Only three out of the 40 patients were positive for HBeAg and all of them were also positive for DNA-P. Only three patients (7 %) had anti-HBs in their sera. Anti-HBs positivity has been considered as evidence of enhanced immune response which stops the multiplication of HBV.[14,15] The absence of enhanced immune response in the present series may have provided an opportunity for continued multiplication of HBV and consequent spread of the infection within the liver, resulting in submassive necrosis. The remaining functioning hepatocytes may be too few in number for efficient regeneration, as earlier suggested by Bernuau *et al.*[4] The mechanism of hepatic necrosis in fulminant hepatitis B is not elucidated by the present study.

Foscarnate, an anti-viral drug, has been recently reported to give some promising results in patients with fulminant hepatitis B.[16,17] Furthermore, at an international symposium on hepatitis δ virus held in Turin, 19 June 1986, Dr Hedin and colleagues reported successful results for Foscarnate used in five consecutive patients of fulminant hepatitis. A control trial of this anti-viral drug is needed to establish its utility in fulminant hepatitis B, a serious disease carrying a mortality of about 80%.[18]

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