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Significance of anti-pre-S antibodies in patients with fulminant hepatic failure

M. IRSHAD, B.M. GANDHI, S.K. ACHARYA, Y.K. JOSHI, and B.N. TANDON
Department of Gastroenterology and Human Nutrition, All india Institute of Medical Sciences, New Delhi-110029, India

Summary: Anti-pre-S antibody was tested in 38 sera from patients with fulminant hepatitis (positive for HBsAg and/or IgM anti-HBc) using a specific solid phase enzyme linked immunosorbent assay (ELISA). Anti-pre-S activity was detected in 50 percent sera samples positive for HBsAg but negative for IgM anti-HBc. There were 12.5% sera positive for both HBsAg as well as IgM anti-HBc and 75% sera negative for HBsAg but positive for IgM anti-HBc. The prevalence of HBV-specific DNA-polymerase activity was high in all the three groups whereas anti-HBs positivity was low. Anti-pre-S activity was observed both in the presence as well as in the absence of DNA-polymerase activity. High-anti-pre-S level in fulminant hepatitis B patients was assumed to be implicated in the fast clearance of HBsAg from circulation. *Gastroenterol Jpn 1990;25:499–502*

Key words: *anti-pre-S; ELISA; fulminant hepatitis; HBsAg clearance*

Introduction

Fulminant hepatic failure due to viral infection is an uncommon but severe complication of acute viral hepatitis[1]. The reason why acute infection in some patients pursues a fulminant course is not yet known. Occurrence of massive hepatic necrosis in patients with fulminant hepatitis B has been attributed to an enhanced immune response against the HBV antigens[2]. Both cell-mediated immunity as well as humoral immunity have been reported to be involved in the pathogenesis of liver[3]. There are several reports[2,3] demonstrating high anti-HBs and anti-HBe levels in sera from patients with fulminant hepatitis B. However, little information is available on the presence and significance of antibodies directed against pre-S proteins (anti-pre-S anti-bodies) in these patients. The present study, therefore, was carried out to detect anti-pre-S antibodies in the sera samples from patients with fulminant hepatitis (positive for IgM anti-HBc and/or HBsAg) by using a specific ELISA technique[4]. Attempts were also made to establish a relation between prevalence of anti-pre-S antibody and HBV-replication during the course of the disease.

Patients and Methods

Patients

A total of 38 patients of fulminant hepatitis, including 28 cases with viral hepatitis B (IgM anti-HBc positive) and 10 cases of non-B hepatitis (IgM anti-HBc negative but HBsAg positive) were admitted to the Rajgarhia Liver Unit of our institute in 1986 and 1987. All patients developed hepatic encephalopathy within 10 days of the onset of symptoms and signs of acute hepatitis in the absence of any pre-existing liver diseases. The sera from these patients were collected within one week of the onset of encephalopathy and kept at −70°C until used for anti pre-S analysis.

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Address for Correspondence: M. Irshad, M.D., Department of Gastroenterology & Human Nutrition, All India Institute of Medical Sciences, Ansari Nagar, NEW DELHI-110029, India.

Table 1 Hepatitis B markers in patients with fulminant hepatitis

Group	No.	Anti-HBs$^+$	Anti-pre-S$^+$	DNA-P$^+$
(a) HBsAg$^+$ and IgM anti-HBc$^-$	10	1 (10%)	5 (50%)	3 (30%)
(b) HBsAg$^+$ and IgM anti-HBc$^+$	8	0 (Nil)	1 (12.5%)	4 (50%)
(c) HBsAg$^-$ and IgM anti-HBc$^+$	20	3 (15%)	15 (75%)	3 (35%)

DNA-P: HBV-specific DNA-polymerase

Serological analysis

HBsAg and anti-HBs in sera samples were tested as described in a previous report[5]. IgM anti-HBc was tested by commercial EIA-kit obtained from Abbott Laboratories, Chicago, USA. HBV-specific DNA-polymerase activity was measured by the modified method of fang et al[6].

Table 2 Relation between anti-pre-S antibodies and HBV-DNA-polymerase in fulminant hepatitis patients

Group	DNA-P positivity in	
	Anti-pre-S$^+$	Anti-pre-S-sera
(a) HBsAg$^+$ and IgM anti-HBc$^-$	1/5 (20%)	2/5 (40%)
(b) HBsAg$^+$ and IgM anti-HBc$^+$	0/1 (Nil)	4/7 (57%)
(c) HBsAg$^-$ and IgM anti-HBc$^+$	5/15 (33%)	2/5 (40%)

ELISA-procedure

The ELISA presently used for the detection of anti-pre-S antibody in serum is the same as described in a previous report[4]. The test is a specific assay system and is based on competitive binding between anti-pre-S and horse radish peroxidase-labelled polymerised human serum albumin (HRPO-pHSA) for the pHSA-receptor site on HBsAg molecules fixed to a solid surface. Pretreatment of HBsAg with anti-pre-S positive serum inhibits the subsequent binding of HRPO-pHSA conjugate to HBsAg. Percent inhibition of OD was used as the measurement of anti-pre-S activity in serum. The preparation of pHSA and its labelling with HRPO was carried out as described previously[5]. In the ELISA procedure the antigen (HBsAg enriched in pHSA-receptor) was coated by addition of 50ul of HBsAg solution (1 ng/ml) in carbonate buffer, pH9.6 to each well of a microtitre plate (Nunc, Denmark). The plate was incubated overnight (18-20 hrs) at room temperature followed by washings with 0.1 M phosphate buffered saline, pH7.2 containing 0.05% Tween-20 (PBS-T), Postcoating was done by the addition of 200ul of 0.5% gelatin in PBS to each well. After washing, 50ul serum sampels were placed in each well and the plate was incubated at 37°C for 2 hrs. Anti-HBs, if present in the test serum, was neutralized as described by Okamoto et al[7] before the addition of serum to the plate. The plate was washed 3 times and 50ul of optimally diluted HRPO-pHSA conjugate, (after proper dilution with PBS), was added to each well and incubated at 37°C for 2 hrs. After washing, color was developed by adding 50 ul of O-phenylene-diamine solution (0.4 mg/ml) in 0.1M phosphate citrate buffer, pH5.0 containing 0.06% H_2O_2 to each well. The reaction was stopped after 15 min by adding 50 ul of 4N H_2SO_4 and the optical density was measured at 492 nm. The results were expressed in terms of percent inhibition of O.D. i.e.:

$$\frac{\text{(O.D. of control)} - \text{(O.D. of sample)}}{\text{(O.D. of control)}} \times 100$$

Sera from healthy persons without HBV-markers served as control. Any test serum causing more than 50% inhibition was considered positive for anti-pre-S activity.

Results

A total of 38 sera samples from the patients with fulminant hepatitis, belonging to three different groups (**Table 1**) was analyzed for anti-pre-S antibody, anti-HBs and HBV-specific DNA-polymerase activity. Anti-pre-S antibody was detected in 5 of 10 sera samples positive for HBsAg but negative for IgM anti-HBc, 1 of 8 sera smaples positive for both HBsAg as well as IgM anti-HBc

and 15 of 20 sera samples negative for HBsAg but positive for IgM anti-HBc. The prevalence of anti-HBs in these patients was very low. HBV-specific DNA-polymerase was present in a significant proportion in each group of patients. A study of the relationship between anti-pre-S positivity and DNA-polymerase activity revealed that DNA-polymerase was present both in the presence as well as inthe absence of anti-pre-S activity without any significant difference (**Table 2**).

Discussion

The pathogenesis of massive hepatic necrosis in fulminant hepatitis B remains enigmatic and seems more related to brisk host immune response than to direct cytopathogenicity or increased replication of hepatitis B virus. There is evidence of high anti-HBs and anti-HBe levels[3] in serum samples of fulminant hepatitis B patients. It is assumed that these antibodies complexed with antigens cause an Arthus reaction in hepatic sinusoids leading to ischemic necrosis of the hepatocytes. The enhanced immune response is also reported to cease HBV-replication after hepatic encephalopathy[1]. However, there is no report available which show the status and the possible role of anti-pre-S antibodies in fulminant hepatitis patients with evidence of present or past HBV infection.

In the present study, anti-pre-S activity was evaluated by the ELISA method, the specificity of which has already been established in a previous paper[4]. The present series of 38 patients with fulminant hepatitis (positive for either IgM anti-HBc and/or HBsAg) demonstrated a low prevalence of anti-HBs but moderately high prevalence of HBV-specific DNA-polymerase. These findings are different from the previous reports[2,3] showing high anti-HBs positivity and low HBV-replication in fulminant hepatitis. A significantly high proportion of sera showed anti-pre-S antibody activity. In non-fulminant hepatitis B, i.e. acute viral hepatitis B cases, anti-pre-S activity was recorded in only one of 24 (4.2%) cases as published in our previous paper[4]. Thus anti-pre-S in fulminant hepatitis is significantly high as compared to non fulminant hepatitis. High prevalence of anti-pre-S activity in fulminant patients has also been reported by Thielmann et al[8], though the number of sera analyzed was very small i.e. only 3. These findings indicate that fulminant patients show a brisk immune response to pre-S protein resulting in an early appearance of anti-pre-S antibodies, i.e. even before the disappearance of pre-S proteins or HBV-replications. The appearance of anti-pre-S in the presence of pre-S proteins and HBV-DNA has been demonstrated in experimentally infected chimpanzees as well as in patients with acute HBV-infection[8]. The low anti-HBs positivity in fulminant patients indicate that a brisk immune response is present exclusively to pre-S proteins and not to HBsAg. This is supported by the findings of Milich et al[9] suggesting that immune responses against pre-S proteins and HBsAg are regulated independently.

Based on HBsAg and IgM anti-HBc status in sera, the patients with fulminant hepatitis were divided into 3 different groups. The presence of anti-pre-S antibody in group (a) patients, which represent either a non-B infection in HBsAg carriers or an early stage of HBV-infection, indicates an early appearanceof anti-pre-S, i.e. before the development of IgM anti-HBc. Presumably, this is the IgM type of anti-pre-S antibody. Since anti-pre-S appears in a biphasic pattern[7] the low level of anti-pre-S in group (b) patients which represent the middle and peak stage of disease, may correspond to the declining phase before it reappears again. In group (c) patients, representing a late stage of the disease, anti-pre-S appeared in the second phase and thus represents the IgG type of anti-pre-S as reported earlier[8]. Absence of HBsAg in group (c) patients may be ascribed to the neutralizing effectof anti-pre-S present in high level. Fast clearance of HBsAg in fulminant hepatitis B patients has been reported earlier[1] and it was attributed to an enhanced immune response resulting in either cessation of HBV-replication or the masking of HBsAg synthesis by massive anti-HBs production. Since in the present cases, anti-HBs positivity is very low and at the same time anti-pre-S is reported to be involved in the clearance of HBsAg[10] high anti-pre-S level in

group (c) patients possibly clears off HBsAg by neutralization. However, anti-pre-S does not arrest HBV-replication, as is evident by high DNA-polymerase activity, suggesting that anti-pre-S clears off only the released HBsAg from hepatocyters without interfering in HBV-replication. The clearance of HBsAg by anti-pre-S predominates over its production.

Anti-pre-S antibody in fulminant patients seems to play a major role of clearing HBsAg from circulation. Its function is different form that of anti-HBs which possibly is involved more in the pathogenesis than the clearance of HBsAg. Anti-pre-S helps in clearing HBsAg by neutralizing it. The present study does not explain the massive liver necrosis in fulminant patients, particularly when anti-HBs positivity is so low. Possibly, factors other than high anti-HBs level are also responsible for the liver damage.

References

1. B rechot C, Bernaun D, Thiers V, et al: Multiplication of hepatitis B virus in fulminant hepatitis B. Br Med J 1984;288:270-271
2. Trepo CG, Robert D, Motin J, et al: Hepatitis B antigen (HBsAg) and/or antibodies (anti-HBs and anti-HBc) in fulminant hepatitis; pathogenic and prognostic significance. Gut 1976;17:10-13
3. Zuckerman AJ: The Enigma of fulminant viral hepatitis. Hepatology 1984;4:568
4. Irshad M, Gandhi BM, Acharya SK, et al: Anti-pre-S antibodies in different groups of patients with hepatitis B virus (HBV) infection. J Gastroenterol Hepatol 1989;4:25-32
5. Irshad M, Gandhi BM, Chawla TC, et al: Studies on HBsAg binding with polymerised human serum albumin by ELISA. J Virol Meth 1987;16:75-85
6. Fang CT, Nath N, Pielech M: A modified technique for the detection of hepatitis B virus specific DNA-polymerase. J Virol Meth 1981;2:349-356
7. Okamoto H, Usuda S, Imai M, et al: Antibody to the receptor for polymerised human serum albumin in acute and persistent infection with hepatitis B virus. Hepatology 1984;5:354-359
8. Theilmann L, Klinkert M.Q, Gmelin K, et al: Detection of antibodies against anti-pre-S proteins in sera of patients with hepatitis B virus (HBV) infection. J Hepatol 1987;4:22-28
9. Milich R, Thronton GB, Neurath H, et al: Enhanced immunogenicity of the pre-S region of hepatitis B surface antigen. Science 1984;228:1195-1199
10. Alberti A, Pontisso P, Schiavon K, et al: An antibody which precipitates Dane particle in acute hepatitis type B: relation to receptor sites which bind polymerised human serum albumin on virus particle. Hepatology 1984;4:220-226