Serum Neopterin Monitoring in Hepatitis B e Antigen Positive Chronic Type B Hepatitis with Recurrent Acute Exacerbation

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Summary

Activation of cellular immunity, mainly cytotoxic T cells, plays an important role in the mechanisms of cyclic acute exacerbation of hepatocellular damage (AE) in hepatitis B virus (HBV) carriers. Monitoring of the dynamics of immunoreactivity is clinically important to manage the patients and recognize the mechanism of liver damage. Serum neopterin has been widely recognized as a marker of activation of cell-mediated immunity. We observed the clinical course of five hepatitis Be antigen (HBeAg) positive HBV carriers with recurrent AE for mean eighteen months by monitoring of serum neopterin using a RIA kit. Alanine amino transferase (ALT) and HBeAg were also measured serially as markers for hepatocyte necrosis and viral replication, respectively. During follow-up period, each patient had several episodes of AE alternating with normal or nearly normal liver function. The levels of serum neopterin changed dynamically related to those of HBeAg and ALT. Temporal analysis of these makers before, during and after the episodes of AE showed a close involvement of the viral replication prior to the hepatocellular injury as well as the activation of cellular immunity following the viral replication. Immunological monitoring of serum neopterin could provide useful information about the dynamic interaction between virus replication, cellular immunoreactivity and hepatocellular damage. It was thought that serum neopterin can be used as a complementary marker to follow and manage chronic hepatitis B patients.

Key words : Neopterin, Chronic hepatitis B, Acute exacerbation of hepatic damage, Cell-mediated immunity, Immunological monitoring

Introduction

Hepatitis B virus (HBV) is a DNA virus that replicates in infected hepatocytes by reverse transcrip-

tion similar to the process of retroviruses (1). HBV causes transient and chronic infection, the latter mainly caused by the vertical transmission from a mother to her infant.

This chronic infection is represented by a wide spectrum of disease entities ranging from the asymptomatic virus carrier (ASC) to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (2, 3). Regular follow-up is important, especially in hepa-

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titis Be-antigen (HBeAg) positive patients because the virus is actively replicating in those patients and the patients undergo recurrent and progressive exacerbation of hepatic injury (4-6).

Observing the natural course of the HBV carriers with positive HBeAg reveals the following cyclic patterns: normal liver function stage (ASC stage), gradual deterioration of the liver function, acute hepatitis-like hepatocellular damage (acute exacerbation stage), and recuperation (7). Activation of the cellular immunity, mainly cytotoxic T cells, following viral replication is thought to play an important role in the mechanisms of this cyclic acute exacerbation of hepatocellular damage (8-13). Therefore, immunological monitoring of HBV carriers is clinically important to manage the patients and understand the relationship between HBV replication, activity of cellular immunity and hepatocellular damage.

Alanine amino transferase (ALT) and HBeAg are widely used as clinical indices for hepatocyte necrosis and viral replication, respectively. However, there has not been an appropriate marker for the activated cellular immunity, and there are few reports monitoring HBV carriers from immunological viewpoints.

Neopterin belongs to a group of pteridine compounds synthesized *in vivo* from guanosine triphosphate (GTP) via GTP cyclohydrolase I (14), and is produced mainly by activated macrophages under stimulation by interferon gamma (IFN- γ) delivered from activated T-cells (15). As a positive correlation between increased serum neopterin levels and immune response has been reported *in vivo* and *in vivo* (16), neopterin is widely recognized as a biological marker for the activated cell-mediated immunity (17).

The aim of this study is to investigate the clinical course of HBV carriers with recurrent exacerbation of liver injury and their dynamic interaction between viral replication, cellular immunoreactivity and hepatocellular damage, focusing on serum neopterin monitoring.

Patients and Methods

Patients

Five adult chronic HBV carrier patients with both HBsAg and HBeAg-seropositivity were investigated in this study. All of them were histologically diagnosed as chronic active hepatitis.

They were negative for anti-IgM HA, anti-delta, anti-human immunodeficiency virus, anti-HCV antibodies by the commercial serological tests and HCV-

RNA by the reversetranscription polymerase chain reaction (RT-PCR) assay, and showed no evidence of alcoholic, autoimmune or drug-induced liver damage. None of these patients or Epstein-Barr virus, and received anti-viral or immunosuppressive therapy before is study.

In the present study, the period when the serum ALT levels were markedly elevated more than 300 IU/ml, was defined as "acute exacerbation of liver damage (AE) state". On the other hand, the period when the serum ALT levels were normal or nearly normal preceding or after AE period, was defined as "asymptomatic carrier (ASC) state".

If they needed anti-viral therapy during their investigated periods, they were treated with interferon beta (IFN- β ; Feron, Toray Industries, Inc., Tokyo, Japan) 6 MU per day intravenous administration for 4 consecutive weeks.

Informed consent was obtained from each patient before entry into the study.

Methods

They were followed by serial biochemical and serological tests at various time points of each ASC state and during the periods from two months before AE through the peak still two months after AE. At the same time, serial serum samples were also collected and were stored immediately at -30°C for assay of serum neopterin levels.

Serum ALT level was measured with a multi-channel autochemical analyzer (Hitachi 736-60E Hitachi Co., Ltd., Tokyo, Japan) and expressed as international units per liter (IU/l).

HBeAg and antibody to HBeAg (HBeAb) were analyzed by using available commercial radioimmunoassays (RIAs; Dinabot Co., Ltd.) and expressed as a cut-off index and percentage (%), respectively. HBV DNA polymerase, which is a direct indicator of HBV replication, was assayed by using an available commercial serological assay and expressed as cpm.

Serum neopterin level was measured by using a RIA kit "IMMU test Neopterin" (Henning Berlin GMBH, Berlin, Germany). Radioactivity was counted by a programmed gamma-counter "ANSR" (Dinabot Co., Ltd., Tokyo, Japan) and its value was expressed as nmol/l.

To determine the normal level of serum neopterin, forty five serum samples of healthy adult blood donors (25 men and 20 women, age ranged from 23 to 66 yyears, mean age of 42 years) were assayed. The mean level of serum neopterin (mean $\pm$ SE) was 4.4 ± 0.8 nmol/l $\pm$ 4.3 ± 0.7 nmol/L

for the men and 4.5 ± 0.8 nmol/l for the women, respectively). There were no significant differences in the mean serum neopterin levels attributable to sex or age, thus suggesting that serum neopterin level does not fluctuate between the sexes within this age range. Therefore, the mean levels of serum neopterin was used as normal level.

Comparison between mean values were determined by Student's test for paired data. A *P* value of <0.05 was considered as statistically significant.

Results

All the patients had initially both HBsAg and HBeAg in their serum. They were observed for mean eighteen months (from twelve months to twenty four months) period and showed a cyclic pattern characterized by high ALT elevations (AE state) alternating with normal or nearly normal liver function (ASC state). In the follow up period, there were total fourteen times episodes of AE in five patients. Three (No. 2, 3, 4) of five patients were treated with IFN- β consequently after their AE state. Two patients (No. 3, 5) became to anti-HBe antibody seropositive, and another one (No.1) had transiently a seronegative period, but others had consistently HBeAg throughout their clinical course (Table 1).

Through the clinical course of each patient, their levels of serum neopterin changed dynamically related to those of HBeAg and ALT. The result of a one-year follow up study by serum neopterin monitoring on a treatment-free patient (No. 1) is shown in Figure 1. This patient shows a cyclic pattern characterized by AE alternating with normal liver function during the study. The change of serum neopterin was closely related to that of HBeAg and ALT.

The chronological changes in the levels of serum neopterin and HBV markers before and after AE on another patient (No. 3) is shown in Figure 2. The change in HBV DNA-polymerase correlated with the change in HBeAg. As HBeAg rapidly increased, neopterin increased and apparent hepatocellular

Figure 1. Monitoring of serum neopterin (nmol/l), serum HBeAg (cut off index), and ALT levels (IU/l) in a chronic hepatitis B patient (No.1) showing several acute exacerbations of liver injury for a 12-months period. Serum neopterin levels changed dynamically relative to HBeAg and ALT levels.

damage (namely AE) occurred. ALT, after it had peaked, decreased along with a rapid decrease of HBV. Neopterin decreased as well and anti-HBe antibody (HBeAb) increased after that.

Three patients (No. 2, 3, 4) were administered IFN- β for four weeks consequently following AE. The clinical course of a patient (No. 3) who received IFN- β therapy following AE is demonstrated in Figure 2. During the treatment, the serum HBV disappeared, the ALT level returned to normal, and the serum neopterin level was high. Treatment of the other patients with IFN- β resulted in higher levels of neopterin as well during IFN administration.

Two (No. 3, 5) of five patients developed anti-HBe antibodies. The follow-up of a patient (No. 5) is shown in Figure 3. In the patient, however HBeAb turned positive (100%) and HBeAg disappeared after 250 days of the study, an acute hepatocellular damage reoccurred after seroconversion. Because HBV-DNA polymerase level increased highly again even after HBeAg had diminished, it is suggested that this AE was caused by the mutant type of HBV. Serum neopterin changed in correlation with HBV-DNA polymerase and ALT even after disappearance of HBeAg.

According to the total of fourteen times episodes of AE in five patients, the detailed dynamics of HBeAg, serum neopterin and ALT levels preceding,

Table 1. Profiles of five patients with chronic hepatitis B in the present study

Patients No.	Age M/F (yrs)	Follow-up duration (mo)	Episodes of AE* (times)	Peak of ALT (IU/l)	Range of HBeAg (C.I [†])	IFN treatment	seroconversion
1	33 F	12	3	936	0-10.7	no	no
2	30 M	20	2	2281	1.3-8.1	yes	no
3	27 M	18	3	1917	0-12.5	yes	yes
4	28 M	24	3	2456	3.3-11.0	yes	no
5	17 M	16	3	425	0-20.0	no	yes

* AE, acute exacerbation of liver damage

[†]mo, months [‡]C.I, cut-off index

Figure 2. Time course changes of (A) serum neopterin levels (nmol/l) and ALT value (IU/l), and (B) HBeAg (cut-off index), HBV-DNA polymerase (cpm) and HBeAb (%) in a chronic hepatitis B patient (No.3) demonstrating the episodes of AE. The patient was treated with IFN- β after the second AE.

during and after episodes of AE state were analyzed (Figure 4). There were no significant differences in the mean level of serum neopterin between healthy subjects and those of patients at ASC state (4.4 ± 0.8 nmol/l vs. 4.9 ± 0.7 nmol/l, $P > 0.05$). Two months before AE, at ASC state with normal ALT level, no increase of serum neopterin could be detected. Then serum HBeAg increased gradually with an increase

Figure 4. Temporal analysis of serum HBeAg, serum neopterin and ALT levels during the episodes of acute exacerbation (AE) of liver injury. The mean $\pm$ SE for of serum HBeAg (cut-off index), serum neopterin levels (nmol/l) and ALT value (IU/l) before (8 weeks, 4 weeks), during (peak, two weeks after peak), and after (4 weeks, 8 weeks) AE are shown. The dynamics of HBV replication, immunoactivation and liver injury was demonstrated during the period of AE. * $P < 0.01$; ** $P < 0.05$; ***Not significant.

in serum neopterin level 4 weeks before AE. Concurrently, rapid destruction of hepatocytes occurred (i.e., the peak of AE) with high levels of serum neopterin. Two weeks after the peak of AE, HBeAg was significantly reduced already and the hepatocellular damage diminished as the neopterin level decreased.

Discussion

The present study showed that the levels of serum neopterin changed dynamically related to those of HBeAg and ALT following chronic hepatitis B patients. This data suggest that serum neopterin monitoring enabled to observe the interaction between HBV replication and hepatocellular damage in terms of the activity of cellular immunity.

Many hepatitis B patients with positive HBe antigen (HBeAg), when observed for long periods, undergo recurrent acute hepatitis-like liver dysfunction intermitted by periods without hepatocellular damage (4-6). The hepatitis B virus is not directly cytopathic for the infected hepatocyte, and HBV-specific liver cell injury and subsequent viral clearance is thought to be mediated by immune mechanisms (9, 18). Recently, both increased proliferation and interferon gamma (IFN- γ) production in response to HBeAg or HBeAg was reported during AE of chronic hepatitis B patients (18-20). In addi-

Figure 3. Monitoring of (A) serum neopterin (nmol/l) and ALT value (IU/l), and (B) serum HBeAg (cut-off index) and HBV DNA polymerase (cpm) in a chronic hepatitis B patient (No. 5) showing AE caused by HBV mutant type after seroconversion.

tion, AE and the seroconversion to anti-HBe in both chronic and acute HBV infection are associated with an elevated production of INF- γ by PBMC upon stimulation with HBeAg or HBcAg (21). On the other hand, neopterin is widely recognized an indicator for activation of cellular immunity, for endogenous INF- γ production and for monocyte/macrophage activation (16, 22). Therefore, it is reasonable to monitor serum neopterin level serially along with markers for hepatocyte necrosis and viral replication to follow-up HBV carriers with recurrent AE.

The results of our kinetic studies on HBeAg, neopterin, and ALT relative to AE demonstrated a close involvement of viral replication prior to acute liver injury as well as an activation of the cellular immunity following the viral replication. The detailed mechanisms of AE in HBV carriers are yet to be elucidated (4, 6, 18, 23). Previous reports suggested a cascade of mechanisms as follows; at first, something triggers viral replication resulting in an imbalance of immunity, the this imbalance of immunity induces activation of the cellular immunity, mainly cytotoxic T cells which destroy the infected hepatocytes eliminating the virus. Although the first trigger causing HBV replication has not been known yet, the present study shows that serum neopterin monitoring is helpful to understand the relation between the the cellular immunity, hepatocellular damage and HBV replication according to AE in HBV carriers.

It is known that liver damage in HBV carriers subsides following seroconversion from HBeAg positivity to HBe antibody (HBeAb) positivity (7, 24, 25). However, it has been recently reported that mutant HBV causes liver injury which persists even after the seroconversion to HBeAb positivity. In the present study, the mutant HBV replication with AE after seroconversion was found in one of the patients, and the serum neopterin level in this patient was high along with the liver damage. This suggests that liver damage induced by HBV mutant type as well as HBV wild type closely relates to the activated cellular immunity, and it is possible to monitor hepatitis B virus carriers in terms of cellular immunity independently of HBeAg positive or negative by measuring serum neopterin level.

Interferon is widely used for the treatment of chronic hepatitis B. The efficiency of IFN was influenced by some factors such as age, sex, HBV load, histological findings of liver biopsy. The cellular immune response is also thought to be one of the important factors for successful IFN treatment. In this study, the patients treated with IFN- β showed

high levels of serum neopterin throughout the treatment. This suggests the possibility that IFN- β directly or indirectly through a cytokine network affects the cellular immunity. Therefore, serum neopterin monitoring may be applied to estimate the effects and the reversed effects of IFN therapy. This intriguing possibility requires further investigation.

In conclusion, this study shows the clinical usefulness of serum neopterin as an index for monitoring HBV carriers with recurrent exacerbation of liver damage. Serum neopterin monitoring could provide immunological information about the dynamic interaction between HBV replication, cellular immunity and liver injury. Serum neopterin may represent to be a complementary marker to follow and manage chronic hepatitis B patients.

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