Reactivation of Occult Hepatitis B Virus Infection 27 Months after the End of Chemotherapy Including Rituximab for Malignant Lymphoma

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Abstract

A 68-year-old man with occult hepatitis B virus (HBV) infection was diagnosed with malignant lymphoma and achieved complete remission after treatment with a chemotherapy regimen including rituximab for 5 months. Entecavir (ETV) was also used during and after chemotherapy and was ended at 14 months after chemotherapy. However, reactivation of HBV was observed in blood tests, which showed not only elevation of HBV-DNA but also HBsAg and HBeAg, at 27 months after the end of chemotherapy. After restarting ETV, the HBV-DNA levels immediately subsided. In addition, anti-HBs became and remained positive at 31 months after chemotherapy. ETV was re-discontinued at 36 months after chemotherapy.

Key words: entecavir, HBV, reactivation, rituximab

Introduction

Hepatitis B virus (HBV) infection is a major health problem in many countries (1). Approximately 2 billion people worldwide have serologic markers for HBV, and nearly 300 million of these have chronic HBV infection. Most cases of HBV infection are identified by the presence of serum hepatitis B surface antigen (HBsAg), but some patients are positive for serum hepatitis B core antibody (anti-HBc) and have low levels of HBV-DNA but are negative for HBsAg. They are classified as having “occult HBV infection (OBI)” (2).

Reactivation of HBV under immunosuppressive treatment for malignant or autoimmune disease often becomes life threatening in HBsAg-positive patients (3, 4). It is recommended that such patients receive nucleotide analogue prophylaxis for 12 months after the end of immunosuppressive treatments (5). Although less frequently than in HBsAg-positive patients, OBI patients can also experience the reactivation of HBV under immunosuppressive conditions (3). Therefore, prophylaxis with nucleotide analogues is also recommended in OBI patients. However, it is unclear how long such patients should receive preventive treatment for HBV reactivation, and there are some reports of reactivation occurring in OBI patients more than 12 months after the end of immunosuppressive treatments (3, 6, 7).

We herein report a case of HBV reactivation in an OBI patient with non-Hodgkin’s lymphoma that occurred 24 months after rituximab discontinuation despite nucleotide analogue prophylaxis covering the 5 months of rituximab administration and the subsequent 14 months.

Case Report

A 68-year-old man visited our hospital because of rapid enlargement of the cervical lymph nodes in 2011. Although he had gone to a hospital regularly for treatment of hypertension and ischemic heart disease since the age of 60, he had never had an abnormal liver function test. His tonsils and cervical, axillary and abdominal lymph nodes were enlarged. A tonsil biopsy revealed malignant lymphoma (diffuse large B-cell type according to the WHO classification). Because the cytospin examination of the cerebrospinal fluid identified large atypical cells, he was diagnosed as clinical...
stage IVA and at high risk, according to the revised interna-
tional prognostic index (R-IPI). His laboratory findings were
negative for HBsAg [chemiluminescence enzyme immunoas-
say (CLEIA)] and anti-HBs (CLEIA) and positive for anti-
HBc (CLEIA). His serum levels of HBV-DNA [real-time
polymerase chain reaction (RT-PCR)] were 2.6 log copies/
ml. Computed tomography showed a normal liver (Fig. 1).
Therefore, he was also diagnosed with OBI. Entecavir
(ETV) was instituted for the prevention of HBV reactivation
due to chemotherapy. We performed R-THP-COP therapy
[rituximab 375 mg/m$^2$ (610 mg/body), cyclophosphamide
460 mg/m$^2$ (750 mg/body), doxorubicin 30 mg/m$^2$ (50 mg/
body), vincristine 0.9 mg/m$^2$ (1.4 mg/body) and predniso-
lone 1.0 mg/kg (60 mg/body)] with intrathecal administra-
tion (methotrexate 10 mg, prednisolone 20 mg and cyta-
rabine 20 mg). R-THP-COP therapy was performed every 4
weeks, 6 times in total, 2 sessions of which were intrathecal
administrations (Fig. 2). He achieved complete remission
with chemotherapy at five months. During the period of che-
motherapy, his serum levels of HBV-DNA remained unde-
tectable.

After chemotherapy, he continued ETV and was followed
up regularly every 1-2 months (Fig. 2). ETV was discontin-
ued at 14 months after the end of chemotherapy because the
serum HBsAg, HBV-DNA and hepatitis B core-related anti-
gen (HBcrAg, CLEIA) were all negative. After discontinua-
tion of ETV, his serum HBV-DNA remained undetectable,
and his serum gammaglobulin values were within the nor-
mal range for 10 months. However, his serum HBV-DNA
levels became positive at 24 months after the end of chemo-
therapy (Fig. 2, 3), increasing to 3.3 log copies/mL. HBsAg
and HBcrAg also became positive at 27 months after the
end of chemotherapy. Two weeks later, laboratory data
showed a further increase in the serum levels of HBV-DNA
and reactivation of hepatitis B envelope antigen (HBeAg,
CLEIA) without elevation of serum alanine aminotransferase
(ALT) (Fig. 3). Although we recommended he restart ETV,
the patient refused and requested a reexamination two weeks
later. Laboratory data at 28 months after the end of chemo-
therapy showed normal liver function test findings and fur-
ther elevations of the HBV-DNA and HBsAg levels (Table
and Fig. 3). The genotype of HBV could not be identified
because serum level of HBsAg was very low. He restarted
ETV at 28 months after the end of chemotherapy; the serum
HBV-DNA, HBsAg, HBeAg and HBcrAg findings immedi-
ately turned negative (Fig. 3). In addition, anti-HBs became
positive for the first time at 31 months after the end of che-
motherapy (Fig. 2). ETV was re-discontinued at 36 months
after the end of chemotherapy, and anti-HBs has remained
positive for 12 months.

**Discussion**

Although several definitions of OBI have been prop-
osed (8), the strict definition of OBI as the presence of
HBV-DNA in the liver, regardless of the presence of serum
HBV-DNA, without HBsAg was adopted in Italy in 2008 (9).
The most accurate method for diagnosing OBI is the
detection of HBV-DNA in DNA extracted from the liver.
However, it is invasive and difficult to obtain hepatic HBV-
DNA from the liver (8). As an alternative, recent RT-PCR-
based assays for serum HBV-DNA have been shown to have
adequate sensitivity (9). In clinical practice, OBI is defined
as the presence of serum HBV-DNA without detectable
HBsAg. Therefore, strictly defined OBI is divided into clin-
ical OBI and resolved hepatitis B, with or without serum
HBV-DNA, respectively (1). The present case was diagnosed
with clinical OBI because the laboratory findings before the
chemotherapy were negative for HBsAg and positive for
HBV-DNA.

The natural course of chronic HBV infection depends on
the interaction between virus replication and the host’s im-
mune response (10, 11). It consists of an immune tolerance
phase, an immune clearance phase, an inactive HBV carrier
phase and a reactivation phase. In the inactive HBV carrier
phase, HBV replication and production of HBsAg are
gradually suppressed. Generally, serum HBV-DNA disap-
ppears first, followed by HBsAg in most cases. However,
even when HBsAg decreases to undetectable levels, HBV-
DNA often remains detectable, more so in the liver than in
the serum (3). Therefore, most OBI patients diagnosed un-
der the clinical definition are considered to be in the inac-
tive carrier phase. Our case was also considered to be in the
inactive carrier phase but not to have resolved hepatitis B,
because serum HBV-DNA was positive before the chem-
otherapy.

OBI has some major clinical significance (8). First, there
is a risk of OBI transmission through sexual activity or
medical procedures (transfusion, needle sticking accidents or
organ transplantation) (9). Second, OBI can be associated
with the progression of hepatic fibrosis, especially in pa-
patients with chronic hepatitis due to hepatitis C virus
(HCV) (12). Third, OBI may carry a risk of inducing hepa-
toellular carcinoma (13). Finally, OBI may reactivate in im-
munocompromised patients or those receiving chemother-
apy (14-16). In OBI patients, HBV activity is strongly sup-
pressed by the host’s immune surveillance and is preserved

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**Figure 1.** On contrast abdominal computed tomography, the
liver and spleen were normal in size and shape.
patients under immunosuppressive treatment is well-known for any kind of immunosuppression. Reactivation of HBV in this balance between the host and virus can be disrupted by the balance of hepatitis for a long period of time (17). However, as covalently-closed-circular DNA (cccDNA) in the nuclei of the hepatocytes for a long period (17). However, this balance between the host and virus can be disrupted by any kind of immunosuppression. Reactivation of HBV in patients under immunosuppressive treatment is well-known and can be life-threatening in both HBsAg-positive (overt HBV infection) and clinical OBI patients (3, 4). Although viral reactivation in OBI patients is rarer than in HBsAg-positive patients, not only symptomatic hepatitis but also HBsAg re-seroconversion may occur (14-16). Therefore, recent major guidelines, such as AASLD (1), EASL (18), APASL (19) and JSH (5), recommend the administration of

Figure 2. The patient's clinical course. Serum HBsAg was negative, and HBV-DNA was present at 2.6 log copies/mL before chemotherapy. Entecavir (ETV) was used during R-THP-COP chemotherapy and the subsequent 14 months. Serum HBV-DNA levels remained undetectable, and serum gammaglobulin levels were within the normal range. ETV was discontinued at 14 months after the end of chemotherapy. However, serum HBV-DNA became positive at 24 months and increased to 3.3 log copies/mL at 27 months. In addition, serum HBsAg also reverted. After restarting ETV at 28 months, serum HBV-DNA and HBsAg immediately turned negative. Anti-HBs became positive for the first time at 31 months and remained positive at 46 months, whereas ETV was re-discontinued at 36 months.

Figure 3. Details of the clinical course from 24 to 29 months after the end of chemotherapy. Serum HBV-DNA became positive at 24 months and increased to 3.3 log copies/mL at 27 months. HBsAg, HBeAg and HBcAg reverted at the same time. Two weeks later, laboratory data showed a further increase in the serum HBV-DNA levels and reversion of HBeAg without elevation of serum ALT levels. The patient started taking entecavir (ETV) again at 28 months. After restarting ETV, serum HBV-DNA, HBsAg, HBeAg and HBcAg immediately turned negative.
nucleotide analogues for prophylaxis of HBV reactivation not only in patients with overt infection but also in those with clinical OBI.

These guidelines also suggest extending the prophylaxis 12 months after the end of immunosuppressive treatment to prevent immunosuppressive therapy- or chemotherapy-induced reactivation of HBV infection (1, 5, 18, 19). Although our case had continued ETV for 14 months after the end of chemotherapy, reactivation of HBV was observed 24 months after the discontinuation of immunosuppressive treatment in patients receiving rituximab-based chemotherapy. A prospective study on the efficacy of extended prophylaxis after immunosuppressive treatment should be undertaken, especially in patients who receive rituximab-based chemotherapy.

There have been some reports that the absence or presence of low titers of baseline anti-HBs in clinical OBI is associated with a risk of HBV reactivation among lymphoma patients receiving rituximab-containing treatments (22-25). The present case was also negative for serum anti-HBs at baseline. Interestingly, the present case became positive for anti-HBs for the first time after restarting nucleotide analogue treatment (Fig. 3). Although the mechanisms of sero-

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Table. Laboratory Findings at 28 Months after the End of Chemotherapy.

| Hematology     | Serology     |
|----------------|--------------|
| WBC 6,700 μL   | CRP 0.49 mg/dL |
| Neutro. 50.0 % | IgG 1,972 mg/dL |
| Lympho. 33.0 % | IgA 256 mg/dL |
| RBC 505x10^4 /μL | IgM 30 mg/dL |
| Hb 14.2 g/dL   |              |
| Ht 42.3 %      | Tumor marker |
| Plt 18.3x10^4 /μL | sIL-2R 1,381 U/mL |

Biochemistry

| Biochemistry | Coagulation     |
|--------------|-----------------|
| TP 8.0 g/dL  | PT% 96.0 %      |
| Alb 4.1 g/dL | PT-INR 1.02     |
| T-bil 0.4 mg/dL | APTT 39.6 sec |
| AST 21 IU/L  | Fibrinogen 501 mg/dL |
| ALT 14 IU/L  |                 |
| LDH 105 IU/L | HBV markers     |
| ALP 445 IU/L | HBsAg 19.5 COI  |
| GGT 42 IU/L  | Anti-HBs (-) mIU/mL |
| T-cho 188 mg/dL | HBeAg 9.6 COI |
| BUN 20 mg/dL | Anti-HBe 51.7 %  |
| Cre 1.08 mg/dL | HBV-DNA 4.4 log copies/mL |
| UA 7.0 mg/dL | HBcAg 5.6 log U/mL  |
| FPG 106 mg/dL | Genotype indeterminate |
|              | PC/CP type wild/wild |

WBC: white blood cell, RBC: red blood cell, Hb: hemoglobin, Ht: hematocrit, Plt: platelet, TP: total protein, Alb: albumin, T-bil: total bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, GGT: gamma glutamyl transpeptidase, T-cho: total cholesterol, TG: triglyceride, Amy: amylase, BUN: blood urea nitrogen, Cre: creatinine, FPG: fasting plasma glucose, CRP: C-reactive protein, sIL-2R: soluble interleukin-2 receptor, PT: prothrombin time, APTT activated partial thromboplastin time, HBsAg: hepatitis B surface antigen, Anti-HBs: hepatitis B surface antibody, HBeAg: hepatitis B e antigen, Anti-HBe: hepatitis B e antibody, HBcAg: hepatitis B core related antigen, PC: pre-core, CP: core promoter.
conversion during antiviral therapy are unknown, the reactivation of HBV after chemotherapy may affect the balance between virus replication and the host’s immune response.

In summary, clinical OBI patients can develop HBV reactivation even 27 months after discontinuation of immunosuppressive treatment. It is very important to conduct careful follow-up, being alert for HBV reactivation, after discontinuation of nucleotide analogue prophylaxis, and prompt antiviral treatment after the diagnosis of reactivation can be life-saving.

The authors state that they have no Conflict of Interest (COI).

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