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【 CASE REPORT 】

Hepatitis B Virus Reactivation in a Patient with Nonalcoholic Steatohepatitis 41 Months after Rituximab-containing Chemotherapy

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Abstract:
Hepatitis B virus (HBV) reactivation occasionally occurs long after immunosuppressive therapy. The characteristics of late HBV reactivation remain unclear. We herein present a case of HBV reactivation in a patient with nonalcoholic steatohepatitis (NASH) more than 3 years after rituximab-containing chemotherapy for diffuse large B-cell lymphoma. Increased transaminase levels, which were induced by NASH, were observed after chemotherapy and were alleviated with statin treatment. HBV reactivation was identified incidentally. The patient developed hepatitis that improved with entecavir therapy. Our case might indicate that the presence of NASH is associated with HBV reactivation long after treatment and that statins, as immune-modulatory agents, affect HBV reactivation.

Key words: hepatitis B virus, reactivation, nonalcoholic steatohepatitis, rituximab

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Introduction

Hepatitis B virus (HBV) is a major health problem worldwide; there are 350 million chronic HBV carriers globally, and 30% of the world's population shows evidence of current or past HBV infection (1). In addition to acute and chronic hepatitis, the reactivation of HBV has been recognized as an important disease state (2). The reactivation of HBV is a fatal complication that is induced by long-term chemotherapy or immunosuppressive therapy (3). HBV reactivation usually develops a few months after chemotherapy; however, late-onset HBV reactivation, including reactivation more than 1 year later, is occasionally encountered (4, 5). Early detection and therapy for HBV reactivation can prevent the development of fatal hepatitis (2). The risk factors for HBV reactivation have been reported (5-7); however, the factors associated with late-onset HBV reactivation are unknown. Nonalcoholic fatty liver disease (NAFLD) is also a major health problem (8). The prevalence of NAFLD was estimated to be 27.4% in Asia and 24.6-29.7% in Japan (9). Although several studies have reported an association between NAFLD and HBV infection (10, 11), the influence of NAFLD on HBV reactivation is unknown. We present the case of a patient who experienced HBV reactivation 41 months after rituximab-based chemotherapy. He developed increased transaminase levels, which were induced by NASH after chemotherapy, and his transaminase level was reduced after statin treatment. We also examined the association between HBV reactivation and risk factors.

Case Report

An 82-year-old man was referred to our department after testing positive for hepatitis B surface antigen (HBs-Ag) when he was admitted to our hospital to undergo a prostate biopsy. He had a history of diffuse large B-cell lymphoma (DLBCL). He was diagnosed with DLBCL stage IIA with a bulky mass on the left neck. He underwent 6 courses of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) therapy. His HBs-Ag status was negative, and he did not have a history of liver enzyme ele-

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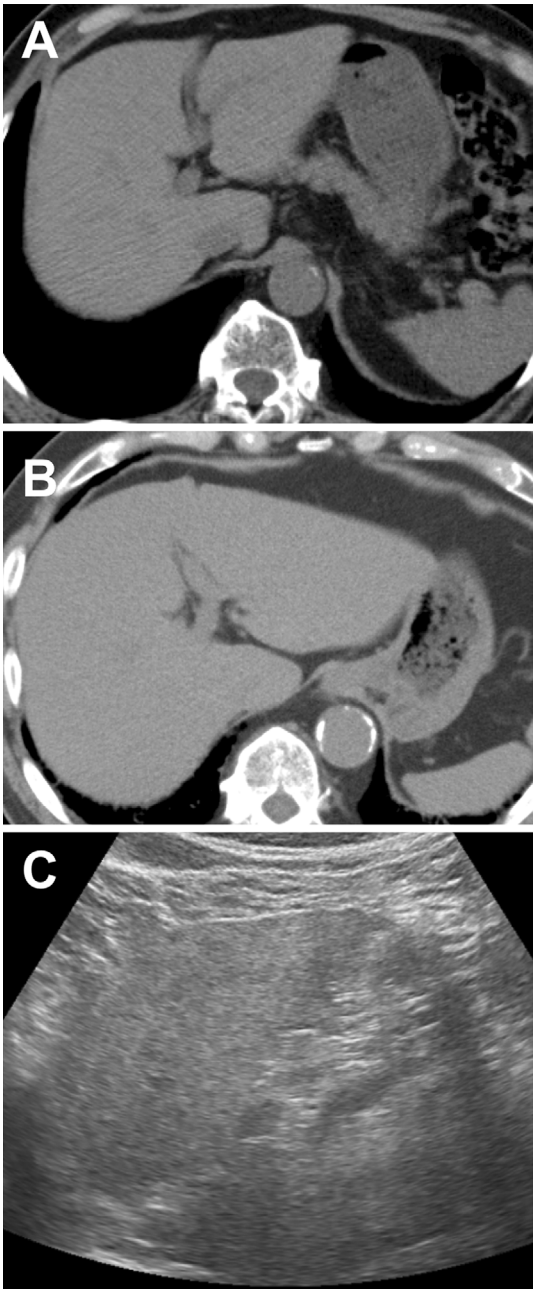

Figure 1. The computed tomography (CT) and ultrasonography findings. The liver/spleen (L/S) ratio, as determined by CT, was lower than that before R-DeVIc therapy. Ultrasonography revealed an increase in echogenicity in the liver. A: CT scans performed during the precontrast phase when the patient underwent R-DeVIc therapy (L/S ratio: 2.8). B: A CT scan during the precontrast phase when he was referred to our department (L/S ratio: 1.1). C: Ultrasonography of liver.

vation. R-CHOP therapy induced a complete remission, but a relapse was observed. He was treated with 6 courses of rituximab, dexamethasone, etoposide, ifosfamide and carboplatin (R-DeVIc). R-DeVIc therapy finished 41 months before he visited our department and induced a second complete remission. Relapse was observed in the right subclavian lymph nodes, and the patient underwent radiotherapy (total 40 Gy/20 fractions). Radiotherapy, which was finished 24 months before the visit to our department, induced a third complete remission. The patient also had history of treatment for diabetes and dyslipidemia. He was not exposed to HBV after chemotherapy through routes such as iatrogenic transmission via blood transfusion or needle-stick injury. He did not display symptoms. His laboratory data were as follows: white blood cell count, 6,900/μL; hemoglobin, 12.9 g/dL; platelet count, 13.5×10^4/μL; prothrombin time, 98.5%; albumin, 4.0 g/dL; total bilirubin, 0.5 mg/dL; aspartate aminotransferase, 29 U/L; alanine aminotransferase, 29 U/L; alkaline phosphatase, 262 U/L; γ-glutamyl transpeptidase, 40 U/L; HbA1c, 6.8%; alpha-fetoprotein, 3.7 ng/mL; soluble interleukin-2 receptor, 496 U/mL; HBs-Ag, 2,500 IU/mL; antibody to HBs-Ag (HBs-Ab) 0.0 mIU/mL; hepatitis B e antigen, unmeasurable; antibody to hepatitis B e antigen, 1,710 S/CO; antibody to hepatitis B core antigen (HBc-Ab), 3.3 S/CO; IgM-HBc-Ab, 0.2 S/CO; IgM-HA Ab, 0.07 S/CO; ferritin, 381 ng/mL; iron, 122 μg/dL; IgG, 1,928 mg/dL; IgA, 393 mg/dL; IgM, 49 mg/dL; cytomegalovirus antigenemia, negative; hyaluronic acid, 62.0 ng/mL; type 4 collagen 7S, 6.9 ng/mL; Epstein-Barr virus viral capsid antigen (VCA)-IgG, 640; VCA-IgM, <10; Epstein-Barr virus nuclear antigen Ab, 80; anti-mitochondrial M2 Ab, <1.5; antinuclear Ab, <80. There were no previous data for HBc-Ab or HBs-Ab. The liver/spleen ratio, as determined by a computed tomography scan, was lower (ratio; 1.1) than the ratio before R-DeVIc therapy (ratio; 2.8). Ultrasonography revealed an increase in echogenicity of the liver, suggesting fatty liver (Fig. 1). The patient's past medical records revealed BMI elevation and slight transaminase elevation after treatment for DLBCL. Pravastatin had been started for dyslipidemia 12 months previously, and the patient's transaminase level had improved to within the normal range (Fig. 2A).

One week later, elevated HBV-DNA (7.9 log copies/mL, real-time polymerase chain reaction) and transaminase levels (AST, 43 U/L; ALT, 44 U/L) were observed (Fig. 2B). HBV genotyping was group C. He was treated with entecavir. The peak transaminase level was observed at 1 month after treatment (AST, 339 U/L; ALT, 477 U/L); however, the transaminase and HBV-DNA levels gradually decreased. After 10 weeks of treatment, his HBc-Ab and IgM-HBc-Ab levels were 9.4 S/CO and 1.0 S/CO, respectively. The patient was diagnosed with hepatitis due to HBV reactivation because of his low IgM-HBc-Ab level and because he had a history of 12 courses of rituximab-based chemotherapy. Additionally, he did not have any opportunities for HBV infection. At four months after treatment, HBV-DNA was not detectable (<2.1 log copy/mL); however, his transaminase level was still above the normal range (ALT 53 U/L). Transient elastography was performed using Fibroscan® (Echosens, Paris, France). The controlled attenuation parameter value was 295 dB/m, and the liver stiffness measurement was 9.1 kPa. Liver biopsy revealed pericellular fibrosis and hepatocyte ballooning. Macrovesicular steatosis was observed in the hepatocytes. The inflammatory cells consisted primarily of lymphocytes infiltrating the hepatic parenchyma. The Matteoni classification was type 3, and the NAS score was 4

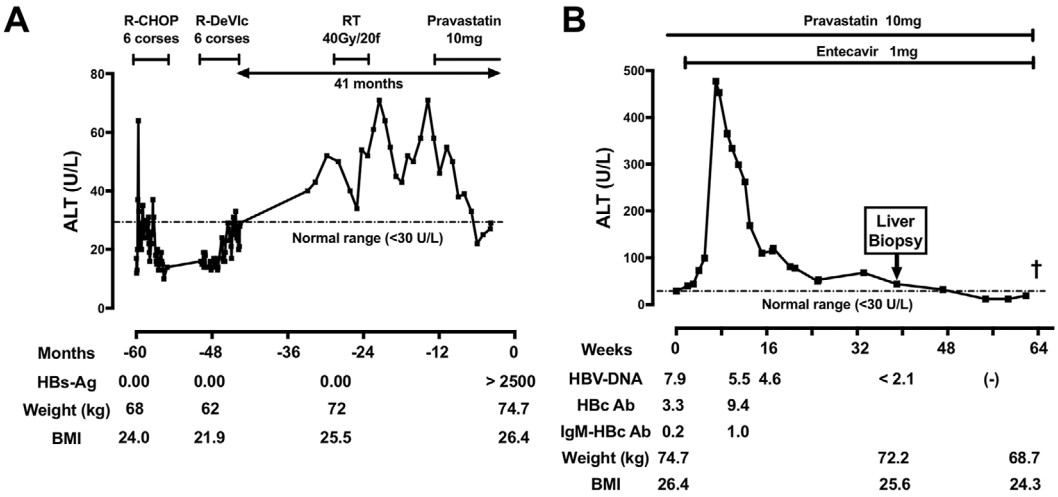

Figure 2. **The clinical course of the patient. A: The clinical course between the diagnosis of lymphoma and visiting our department. B: The clinical course after visiting our department. R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone, R-DeVIc: rituximab, dexamethasone, etoposide, ifosfamide and carboplatin, RT: radiotherapy, HBs-Ag: hepatitis B surface antigen, BMI: body mass index, HBc-Ab: antibody to hepatitis B core antigen**

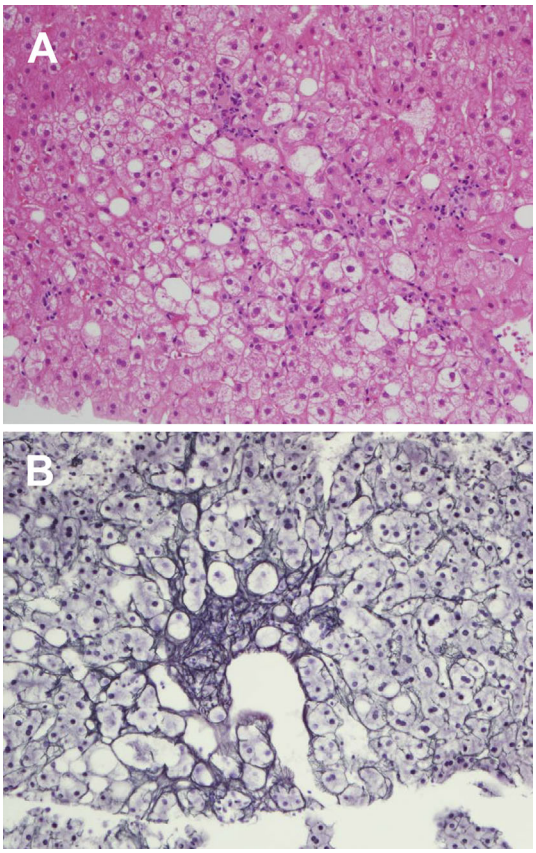

Figure 3. **The histological findings of the liver biopsy specimen. Ballooning, macrovesicular steatosis in hepatocytes and pericellular fibrosis were observed. A: Hematoxylin and Eosin staining, ×100. B: Silver impregnation staining, ×100.**

(Fig. 3). Accordingly, we diagnosed the patient with NASH. NASH-induced increases in transaminase levels were observed after treatment for DLBCL. Furthermore, he developed hepatitis due to HBV reactivation 41 months after rituximab-containing chemotherapy. Treatment with entecavir was continued, and he did not show HBV reactivation. Sixty-four weeks after the diagnosis of HBV reactivation, he died from a relapse of DLBCL.

Discussion

In this case, the patient developed hepatitis due to HBV reactivation 41 months after rituximab-containing chemotherapy. NASH induced an increase in his transaminase level after chemotherapy. HBV reactivation is a common and potentially fatal complication in patients undergoing immunosuppressive therapy. The average period for the development of hepatitis after chemotherapy in HBs-Ag-negative patients is 7.4 months (range: 5-10 months) (12). The monitoring of serum HBV-DNA for 12 months after the completion of chemotherapy is recommended for the early detection of reactivation (2). HBV reactivation is occasionally observed at more than 12 months after chemotherapy. The recognition of risk factors for reactivation after a long duration is important for the clinical setting because of the high fatality rate of *de novo* hepatitis due to HBV reactivation (2). In this case, the patient developed HBV reactivation and hepatitis 41 months after rituximab-containing chemotherapy. His hepatitis was improved by nucleoside analog treatment, but HBV reactivation was found incidentally. Risk factors for late-onset HBV reactivation are important for its early diagnosis and treatment.

In our case, it was difficult to distinguish acute infection from acute-on-chronic infection because we did not investigate the HBc-Ab or HBs-Ab levels before chemotherapy. The usefulness of IgM-HBc-Ab for differentiating between acute infection and acute-on-chronic infection is well

known (13). The IgM-HBc-Ab levels of patients with acute infection have been shown to be significantly higher than those in patients with acute-on-chronic infection (14). Patients with *de novo* hepatitis show low IgM-HBc-Ab levels (4, 15). The low IgM-HBc-Ab level in this patient suggests that he had acute-on-chronic infection. The differentiation of *de novo* hepatitis from resolved HBV infection and hepatitis from occult HBV infection was also a difficult problem in our case because we did not investigate the HBV-DNA level before chemotherapy. The incidence of HBc-Ab and HBs-Ab seropositivity in Japan were reported to be 20% and 22%, respectively, while prevalence of occult HBV is reported to be 0.11% (16, 17). The possibility of *de novo* hepatitis is higher than that of hepatitis from occult HBV infection. If this patient had hepatitis from occult HBV infection, the association between late-onset HBV reactivation and NASH might be important. In general HBV-DNA-positive patients show early-onset reactivation (18, 19). Additionally, the possibility of the exacerbation of a persistent infection after radiotherapy remained. The patient was not exposed to HBV after radiotherapy through routes such as iatrogenic transmission. Serum positivity for IgM-HBc-Ab was found in 82% of the patients with acute hepatitis at 6 months after the onset of illness, and 6 out of 28 patients showed persistent seropositivity for IgM-HBc-Ab after 13-24 months (20). Our patient seronegative for IgM-HBc-Ab, and this serological finding might suggest that his HBV infection was not due to recent HBV transmission.

Recent studies have reported an inverse association between HBV infection and NAFLD. In mice with chronic HBV infection, fatty liver reduced HBV replication (21). HBV infection was associated with a decreased risk of NAFLD (10, 11). Steatosis in patients with HBV infection was associated with low HBs-Ag and HBV-DNA levels (22, 23). Moderate or severe steatosis was associated with a high rate of HBs-Ag seroclearance (24). Although the mechanism underlying these findings is still unclear, the apoptosis of hepatocytes might increase viral clearance in NAFLD patients (22, 25). In this report, patients showed increased transaminase levels induced by NASH after chemotherapy, and the reactivation of HBV did not occur for more than 3 years. The apoptosis of hepatocytes in NASH may also affect HBV reactivation.

Statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, reduce the circulating level of low-density lipoprotein cholesterol and cardiovascular events (26). The efficacy of statins in the treatment of NASH has been recognized. Statins decreased apoptosis in mice with NASH and improved liver injury in patients with NASH (27, 28). Statins have been observed to reduce the serum liver enzyme levels in patients with NASH, even in patients whose weight did not decrease (29). In our case, transaminase was reduced after statin treatment. HBV reactivation developed after the normalization of the patient's transaminase levels. The alleviation of the elevated transaminase levels by NASH might influence HBV reactivation via the reduction of viral clearance induced by NASH.

The role of statins as immune-modulating agents has been reported (29, 30). Research on statins and autoimmune disease has suggested that statins reduce inflammation by modulating immune responses (31). CD4+CD25+ regulatory T cells (Tregs), which play an important role in immune suppression, are increased in the peripheral blood of patients treated with statins (32). Several reports have examined the association between Tregs and HBV replication. The abundance of Tregs in the peripheral blood of chronic hepatitis B-infected patients was greater than that in the peripheral blood of healthy controls (33). The level of Tregs is reported to be associated with the number of HBV copies in the patient's blood (34). An immunological analysis of patients with HBV reactivation revealed that as the number of HBV-specific cytotoxic T lymphocytes increased, the number of Tregs decreased among peripheral blood mononuclear cells during HBV reactivation (35). Statin-induced HBV reactivation was reported, and statins may affect a patient's immune state (36). In our case, the patient was treated with statins before HBV reactivation. Whether statins affected the patient's immune state was unclear. The elucidation of the association between HBV reactivation and Tregs would be useful for determining the mechanism of HBV reactivation.

Radiotherapy is also believed to be a risk factor of HBV reactivation. Radiotherapy was reported to induce HBV reactivation in patients with hepatocellular carcinoma of the liver (37, 38). A previous study suggested that radiation-induced liver toxicity with HBV reactivation arises from a bystander effect on irradiated endothelial cells releasing cytokines (39). In our case, the only the right subclavian lymph nodes received radiotherapy. The effect of radiotherapy on HBV reactivation would be small if present.

The factors associated with late-onset HBV reactivation are still unclear. Yamada et al. reported a case of HBV reactivation 3 years after chemotherapy and reviewed the literature on late HBV reactivation (reactivation taking place one year after the final therapy session) (4). They reviewed 14 cases of late HBV reactivation and found that it was characterized by advanced age, lymphoid malignancy, and multiple and rituximab therapies. However, the researchers suggested that these features were unsatisfactory for the detection of patients at high risk for late HBV reactivation. The late onset of HBV reactivation was thought to be related to a delayed immune recovery due to the prolonged suppressive effects of intensive chemotherapy (5). Recently, the levels of HBs-Ab and HBc-Ab were proposed as potential predictors of HBV reactivation (4, 40). If NASH is negatively associated with the duration of HBV reactivation, the presence of NASH - in addition to HBs-Ab or HBc-Ab - may support continuing the period for which patients are monitored for the development of HBV infection after chemotherapy. Yamada et al. also examined the risk of mortality of typical *de novo* HBV hepatitis and late reactivation (4). They suggested that mortality of late-onset *de novo* hepatitis was low

compared to that of typical de novo hepatitis, but 3 patients died among 13 patients who developed late reactivation. Early detection and treatment for HBV reactivation after a long period would be important, as is the case for typical *de novo* hepatitis.

In summary, we reported a case of HBV reactivation in a patient with NASH 41 months after rituximab-containing chemotherapy. He developed HBV reactivation long after chemotherapy. Although he developed hepatitis, he successfully improved after treatment with a nucleoside analog. We observed a transition in transaminase levels and detected the elevation and alleviation of transaminase levels in relation to NASH. This case suggests the influence of NASH on HBV reactivation. Further investigations are therefore needed to elucidate these associations.

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

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