Case Report:

Fulminant hepatitis after allogenic bone marrow transplantation caused by reactivation of hepatitis B virus with gene mutations in the core promotor region

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Running title: Fulminant hepatitis after BMT

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Abstract

Under immunosuppressive conditions after hematopoietic stem cell transplantation (HSCT), even if hepatitis B virus (HBV) antigen is negative but hepatitis B surface antibody (HBsAb) or hepatitis B core antibody (HBcAb) is presented, HBV reactivates and sometimes causes fulminant hepatitis. However, it remains unclear which patients will develop fulminant hepatitis, or whether fulminant hepatitis is caused by host-related factors or by virus-related factors. A 30-year-old man with a history of aplastic anemia since three years of age underwent allogenic BMT, when HBsAb and HBcAb were positive but HBs antigen (HBsAg) was negative. The donor was negative for HBsAg, HBsAb, and HBcAb. After transplantation, the patient was complicated by acute GVHD, cytomegalovirus infection, intestinal thrombotic microangiopathy, and aspergillus colitis. Chronic GVHD was well controlled by FK506 and prednisolone. Twenty months after transplantation, the patient was admitted with general fatigue and liver dysfunction and was found to be positive for HBsAg and HBeAg. His serum HBV-DNA level was more than 8.8 LGE/ml. Therefore, he was diagnosed as having hepatitis B caused by HBV reactivation, and 100 mg/day lamivudine treatment was started. However, jaundice and hepatic failure deteriorated and became fatal. On analysis of the HBV-DNA, two adjacent gene mutations in the core promoter region (T1762/A1764) were detected. Increased replication of the mutated HBV might have caused HBV reactivation which progressed to fulminant hepatitis.

Key words: Fulminant hepatitis; Bone marrow transplantation; Hepatitis B virus

1. **Introduction**

Hematopoietic stem cell transplantation (HSCT) has greatly contributed to prognostic improvement in patients with hematological neoplasm and hematopoietic stem cell disorders. Under immunosuppressive conditions after HPCT, not only patients with pretransplant hepatitis B surface antigen (HBsAg) but also those with hepatitis B surface antibody (HBsAb) and hepatitis B core antibody (HBcAb), must be considered at risk of hepatitis B virus (HBV) reactivation [1]. This is related to the intense chemotherapy with or without total body irradiation, and the coexistence of acute graft-versus-host disease (GVHD) [2]. The frequency of HBV reactivation in HBsAb-positive patients (i.e., seroreversion) ranges between 14% and 50% [3]. After the HBV reactivation, some of these patients develop fulminant hepatitis [4-7]. However, it remains unclear how HBV reactivation progresses to fulminant hepatitis. It has been demonstrated that particular viral mutations may affect the clinical course of the infection and are associated with progressed to fulminant hepatitis B [8, 9]. We encountered an HBcAb- and HBsAb-positve patient who developed fulminant hepatitis due to HBV reactivation after BMT and we analyzed the HBV in this patient.

2. Case Report

A 30-year-old man had a history of aplastic anemia since three years of age and had been dependent on blood transfusion for three years prior to the present admission. Immunosuppressive therapy using anti-thymocyte globulin and cyclosporine A did not improve pancytopenia. He had undergone allogenic BMT in August 2002, when HBsAb and HBcAb were positive but HBs antigen (HBsAg) was negative (Table 1). The donor was negative for HBsAg, HBsAb, and HBcAb. Conditioning regimen was total body irradiation (total 12 Gy) and cyclophosphamide (total 120mg/kg). GVHD prophylaxis was tacrolimus and short-term methotrexate. After transplantation, the patient was complicated by acute GVHD (grade III), cytomegalovirus infection, intestinal thrombotic microangiopathy, and aspergillus colitis and was treated with cyclosporin A, mycophenolate mofetil (MMF), antibiotics, antifungal and antiviral drugs. Chronic GVHD was well controlled by 0.4 mg/day FK506 and 5 mg/day prednisolone. Aspartate aminotransferase and alanine aminotransferase were normal and HBsAg was negative on examination at seventeen months after transplantation (Table 1). Twenty months after transplantation, the patient was admitted to our hospital because of general fatigue and liver dysfunction. On admission, he was found to be positive for HBsAg and HBeAg, and his serum HBV-DNA level was more than 8.8 LGE/ml (Table 1). The HBV genotype was type C at that time. The patient was diagnosed as having hepatitis B caused by HBV reactivation, and 100 mg/day lamivudine treatment was started. However, jaundice and hepatic failure gradually deteriorated, and the patient was complicated by infectious colitis followed by paralytic ileus and sepsis on the 35th hospital day. His condition was improved by adsorption of

endotoxin two times using a polymyxin B-immobilized fiber column and antibiotic therapy. However, jaundice and hepatic failure progressed to hepatic coma (Table 1). He finally died of respiratory and renal failure.

3. Molecular Analysis of HBV DNA

The complete genome sequence was determined using a direct sequencing method as reported previously [10]. A serum sample collected on admission was adopted for the determination. The sequences obtained were aligned and compared with the reference HBV sequence (GenBank accession number AF479684). Of the two mutations that have been reported to be associated with an occurrence of severe hepatitis [11], a double mutation in the core promoter region (T1762/A1764) was detected but there was no stop codon mutation in the precore region (A1896) in the present case. There were no other significant mutations including lamivudine-resistant mutation in the polymerase region such as YMDD[12].

4. Discussions

We here reported a case of fulminant hepatitis caused by HBV reactivation after allogenic BMT in an HBsAg-negative but HBcAb- and HBsAb-positive aplastic anemia patient. A retrospective study of 37 patients with HBsAb and HBcAb before transplantation demonstrated four cases of HBV reverse seroconversion; anti-HBs and anti-HBc were lost, and HBsAg, HBeAg, anti-HBc IgM, and HBV DNA emerged together with acute hepatitis, after cessation of immunosuppression [1]. A recent study in an HBV-endemic area showed that anti-HBc and anti-HBs status had no significant correlation with the presence of occult HBV infection [13]. Therefore, even though the present donor was negative for HBsAb and HBcAb in addition to HBsAg-negative status, there is still a possibility that occult HBV infection in the donor, which would only be detectable by PCR assays, might have been transmitted to the recipient via HSCT, However, we speculate that HBV in the recipient might have been reactivated to cause hepatitis following HSCT, since the present recipient was already positive for HBcAb and HBsAb. HBV reactivation in this setting has been considered to results from immunosuppression following decline in recipient-derived immunoglobulin G containing anti-HBS over 20 months after allo-HSCT [3].

Recently, the serological and liver-related outcome of 803 patients who received allogenic HSCT was examined [7]. Two of the 721 HBsAg-negative recipients and 16 of the 82 HBsAg-positive recipients were complicated by HBV-related hepatitis. In three of the HBsAg-positive patients, complication by fulminant hepatic failure was observed and two of these three patients died. To clarify the risk factors for hepatitis due to HBV reactivation after autologous HSCT, 137 patients who underwent HSCT (23 positive for HBsAg, 37 positive for HBsAb, and 77

negative for HBV) were examined, and it was demonstrated that a high HBV DNA level was the most important risk factor for HBV reactivation [14]. However, it remains unclear which patients will develop fulminant hepatitis, or whether fulminant hepatitis is caused by host-related factors or by virus-related factors.

The association of an HBV variant with outbreak of fulminant hepatitis B has previously been reported. Mutations in the core promotor/enhancer region of HBV were found in immunosuppressed patients with severe liver damage [15]. The two adjacent core promotor mutations were also identified in an HBV strain associated with fulminant hepatitis, in whom enhanced viral replication was observed [16]. In the present case, two adjacent mutations (at nucleotides 1762 and 1764) in the core promotor lesion of HBV were demonstrated. Mutations in the HBV core promotor region might be responsible for enhanced replication of the fulminant hepatitis strain. A specific insertion in the basal core promotor region of HBV DNA may also enhance viral replication by creating a novel hepatocyte nuclear factor-1 binding site, leading to fulminant hepatitis [17].

The present case showed a remarkable increase in HBV DNA on admission and was treated with lamivudine. Although the amount of HBV DNA was decreased by the therapy, high levels of DNA persisted for more than two months. The usefulness of lamivudine treatment for reverse seroconversion of HBV ia well recognized [18], however, the efficacy of lamivudine seemed to be limited to prevention and management of HBV reactivation in allogenic HST [19]. Lamivudine should be considered preemptively before or at the initiation of immunosuppressive treatment [20]. Further studies are clearly needed to clarify the pathogenesis and clinical course of fulminant hepatitis arising from HBV mutations.

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Table 1. Clinical signs, serological and laboratory markers before and after HSCT in a patient with HBV infection.

	July, 2002 One month before HSCT	Jan, 2004 17 months after HSCT	Apr, 2004 20 months after HSCT (On admission)	Jun, 2004 22 months after HSCT
Clinical symptoms	Anaemia Purpura	Dry skin	General fatigue Anorexia	Icterus Ascites Somnolence
Serological markers				
HBsAg	-	-	+	NT
Anti-HBc Ab	+	NT	+	NT
Anti-HBs Ab	+	NT	-	NT
Anti-HBc IgM Ab	NT	NT	+	NT
HBeAg	-	NT	+	NT
Anti-HBe Ab	-	NT	-	NT
HBV DNA, copies x 10^6/mL	<2.6	NT	>8.8	5.6
Laboratory data				
White blood cells, /μL	1,700	8,280	13,090	22,390

Haemoglobin, g/dl	7.4	14.3	16.0	12.0
Platetets, $x10^4/\mu L$	3.2	27.6	13.9	6.6
Reticulocytes, $x10^4/\mu L$	NT	8.6	6.2	17.3
Aspartate aminotransferase, U/L	50	33	221	81
Alanine aminotransferase, U/L	55	18	181	21
Alkali phosphatase, U/L	507	245	414	777
Total bilirubin, mg/dl	0.7	0.6	2.8	37.2
Prothrombin time, %	127	116	60	27
Immunoglobulin M, mg/dl	102	62	800	NT

HSCT, hematopoietic stem cell transplantation; NT, not tested