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Changes in Serologic Markers of Hepatitis B Following Autologous Hematopoietic Stem Cell Transplantation

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ABSTRACT

Korea is an endemic area for hepatitis B virus (HBV) infection. Reactivation of HBV is a well-recognized complication in patients with chronic HBV infection undergoing cytotoxic or immunosuppressive therapy, and there are some reports of hepatitis B reverse seroconversion after HSCT. This study evaluated changes in HBV serology after HSCT. We reviewed the medical records of 141 patients who had available HBV serologic data after autologous HSCT. Patient information was retrospectively collected from the BMT database. Before transplantation, 12 patients were positive for hepatitis B surface antigen (HBsAg) and received lamivudine prophylaxis. There was 1 case of reactivation of HBV among these patients. One hundred twenty-nine patients were negative for HBsAg before HSCT, of whom 110 were positive and 19 were negative for hepatitis B surface antibody (anti-HBs). Sixty-two of the 110 patients who were positive for anti-HBs were also positive for hepatitis B core antibody (anti-HBc). Eight patients were negative for anti-HBs and anti-HBc. Seven patients who were initially negative for HBsAg were identified as positive after HSCT, and 5 of those 7 patients developed acute hepatitis, thus indicating reverse seroconversion. Univariate analysis showed that reverse seroconversions were observed more frequently with multiple myeloma than another disease ($P = .005$; relative risk, 11.854; 95% confidence interval, 1.381-101.770). Other factors, such as age, sex, and presence of HBcAb before HSCT, had no statistically significant affect on reverse seroconversion. In conclusion, reverse seroconversion of HBV is not a rare complication of autologous HSCT, and the risk of reverse seroconversion after treatment is a serious concern due to possible complications arising from patients' suppressed immune systems.

KEY WORDS

Autologous hematopoietic stem cell transplantation • Hepatitis B virus • Hepatitis

INTRODUCTION

HSCT is performed worldwide to treat various diseases, mainly hematologic malignancies. Intensive chemotherapy and immunosuppression in patients who undergo HSCT can result in immune dysfunction and these patients are at risk for various infections, including viruses. The risk of fatal hepatitis B virus (HBV) disease has been reported to be as high as 12% after allogeneic transplantation [1,2]. Allogeneic HSC transplants from donors with HBV carries a high risk of HBV infection [1], and because of the immunocompromised state of recipients of allogeneic or autologous HSC transplants, HBV reactivation has become a major hepatic complication of HSCT in patients with hepatitis B surface antigen (HBsAg), especially in areas of prevalent HBV infection [3-6].

Korea has been an area of the world to which HBV infection is endemic. Although the prevalence of HBsAg has been decreasing since the 1980s because of vaccination, a national health and nutrition survey in 1998 reported that the seropositivity of HBsAg in Korea was 5.1% (95% confidence interval, 4.5%-5.7%) in males and 4.1% (95% confidence interval, 3.6%-4.6%) in females [7].

There have been several reports of reverse seroconversion/HBV reactivation in patients previously positive for hepatitis B surface antibody (anti-HBs) after allogeneic or autologous HSCT [3-6,8-11]. However, the risk of reverse seroconversion after HSCT, especially autologous HSCT, has not been well established.

In the present study, we conducted a review of serologic HBV status of patients before and after autologous HSCT and the analyzed risk factors for HBV reactivation and reverse seroconversion.

METHODS

Patients

Two-hundred fifty-one autologous HSCTs were performed in the Department of Medicine, Samsung Medical Center (Seoul, Korea) between March 1996 and December 2005. Serologic markers of HBV were tested before and after autologous HSCT, and the presence of HBsAg and anti-HBs was validated before transplantation in all patients. HBV markers of 141 of 251 patients could be verified after transplantation, and we analyzed serologic changes in hepatitis B and demographics of these 141 patients with post-transplantation HBV markers. We retrospectively reviewed their medical records and the BMT database.

Hepatitis B Markers

We confirmed the hepatitis B infection of all patients before proceeding with autologous HSCT, in accordance with pretransplantation protocol. HBsAg, anti-HBs, and hepatitis B core antibody (anti-HBc) were tested routinely as indicators of HBV infection. All patients enrolled in the present study, except for 4 patients who underwent autologous HSCT before the current transplantation protocol, were completely characterized for pretransplantation status of HBsAg, anti-HBs, and anti-HBc. These 4 patients were not excluded from the analysis because their pretransplantation status for HBsAg and anti-HBs were available. Patients with HBsAg also were tested for HBeAg and anti-HBe, and the titer of quantitative HBV DNA used the Digene Hybrid Capture 2 (Digene Corporation, Gaithersburg, Md, USA) assay before and after transplantation [12] and were followed up every 3 mo. Assessment of changes in HBsAg and anti-HBs of patients without pretransplantation HBsAg was usually accomplished within 1 yr, at which time the vaccination was started.

Immunosuppressive Agents

In present study, patients did not have to receive immunosuppressive agents such as cyclosporine A or tacrolimus for GVHD prophylaxis during and after autologous HSCT. However, some patients with multiple myeloma underwent maintenance treatment of thalidomide and dexamethasone therapy. Oral medication of thalidomide 200 mg was taken daily and dexamethasone 40 mg/d was administered orally or i.v. from day 1 to day 4. The next cycle started 29 d from the starting date of dexamethasone of the previous cycle.

Definitions

Hepatitis was defined as a serum alanine aminotransferase level >100 IU/mL on 2 consecutive determinations ≥ 5 d apart [13]. HBV reactivation of patients positive for HBsAg before transplantation was defined as an increase in titer of serum HBV DNA [6], and reverse seroconversion was defined as appearance of HBsAg and disappearance of anti-HBs after HSCT in patients who had no HBsAg but did have anti-HBs or anti-HBc before transplantation [5,11].

Statistical Analysis

Relative risks for changes in HBV markers were calculated by the Pearson chi-square test or Fisher exact test. Multivariate analyses were performed by logistic regression. $P < .05$ was considered statistically significant.

RESULTS

Patient Characteristics

Patients' baseline characteristics are presented in Table 1. Median follow-up duration after HSCT was 38.6 mo (range, 1.7-113.9 mo), and median time of first follow-up testing for HBV markers was 12 mo (range, 1-63 mo). The median patient age at time of HSCT was 45 yr (range, 16-74 yr) and the numbers of females and males were similar (72 versus 69 patients). Patients with multiple myeloma comprised the most common subgroup (53 patients, 38%).

Nineteen of 54 patients with multiple myeloma received thalidomide and dexamethasone therapy after autologous transplantation for maintenance treatment, and only 3 of 19 patients actually developed reverse seroconversion.

Changes in HBV Serologic Markers

Pretransplantation surveillance of HBV infection showed that 12 patients (9%) had HBsAg. The num-

Table 1. *Baseline Characteristics of Patients Who Received Autologous HSCT*

Characteristics	No. of Patients (%)
Total	**141 (100)**
Age, median (range)	**45 (16~74)**
Male/female	**69 (49)/72 (51)**
Diagnosis	
Acute leukemia	**38 (27)**
Malignant lymphoma	**37 (26)**
Multiple myeloma	**53 (38)**
Solid tumors	**13 (9)**
Conditioning regimen	
BEAM	**32 (23)**
Bucy	**5 (3)**
VCT	**38 (27)**
Melphalan	**53 (38)**
Others	**13 (9)**

BEAM indicates combination therapy of carmustine, etoposide, cytarabine, and melphalan; BuCy, combination therapy of busulfan and cyclophosphamide; VCT, combination therapy of etoposide, cyclophosphamide, and TBI.

ber of patients with anti-HBs was 110 (78%). Eight patients (5%) had neither anti-HBs nor anti-HBc (Table 2).

All patients with pretransplantation HBsAg received prophylactic doses of lamivudine at 100 mg/d before HSCT. Liver function test results for these patients were normalized before the start of the conditioning regimen. At the time of HSCT, 4 patients (33% of 12 patients positive for HBsAg) had HBV DNA in the blood (median, 47.3 pg/mL; range, 9.8-240.0 pg/mL). After HSCT, we tested for HBV DNA with a quantitative assay every 3 mo. Two patients showed a loss of HBV DNA to nondetectable levels, and 1 patient still had HBV DNA without an increase in titer. HBV reactivation developed in 1 patient, in whom post-transplantation HBV DNA increased in titer within 3 mo from 47.3 to 317.8 pg/mL. Liver function in this case remained within the normal range during and after transplantation and the HBV DNA titer decreased to a nondetectable range by the next follow-up visit without additional treatment with lamivudine.

Among the 129 patients without pretransplantation HBsAg, 7 patients (5%) became HBsAg positive and anti-HBs negative after transplantation (Figure 1) and thus represented cases of reverse seroconversion. All of these patients had pretransplantation anti-HBs or anti-HBc. The median patient age in these cases was 55 yr (range, 44-74 yr), and 6 cases were multiple myeloma. Four were male and 3 were female. The median interval between HSCT and first HBV marker follow-up was 11 mo (range, 4-34 mo). Five of 7 developed acute hepatitis and 2 showed no abnormal liver function test results. Median time for acute hepatitis in 5 patients was 10 mo (range, 4-17 mo) after autologous HSCT and median HBV DNA titer of 7 patients in reverse seroconversion was 526.2 pg/mL (range, 58.4-654.1 pg/mL). All 7 patients had HBeAg when reverse seroconversion occurred and 1 patient also had anti-HBe. All patients with reverse seroconversion were treated by lamivudine 100 mg/d. Six patients had normalized liver function test results, anti-HBe without HBeAg, and undetectable HBV DNA titer after treatment with lamivudine therapy for 6 mo. One patient who had HBeAg without anti-HBe and high titer of HBV DNA despite lamivudine therapy continued lamivudine therapy for 16 mo.

In addition to reverse seroconversion, loss of anti-HBs, which is a protective antibody against HBV infection, was observed in 37 patients (29%). Twelve of 37 patients had multiple myeloma and 12 of 37 patients had malignant lymphoma. In addition, 9 patients had acute leukemia and 4 had solid tumor.

Analyses for risk factors for reverse seroconversion were performed, and older age (>45 yr) and multiple myeloma emerged as leading risk factors. Multiple myeloma achieved statistical significance as a risk factor for reverse seroconversion as determined through multivariate analysis by logistic regression. However, 3 of 19 patients with multiple myeloma who received maintenance therapy after autologous transplantation actually developed reverse seroconversion and this does not have statistical significance ($P = .691$; Table 3). However, no risk factor could be found for the loss of anti-HBs.

DISCUSSION

In the current analysis, the frequency of pretransplantation HBsAg positivity was determined to be 9% (95% confidence interval, 4.3%~13.7%). All but 1 patient positive for HBsAg before transplantation were able to undergo autologous HSCT without HBV reactivation while maintained on lamivudine prophylaxis. Seven of 129 patients (5%) developed reverse seroconversion, which presented as acute hepatitis in 5 patients. Moreover, a post-transplantation

Table 2. *Hepatitis B Markers before HSCT (n = 141 Patients)*

HBsAg	Anti-HBs	Anti-HBc	No. of Patients (%)	95% CI
Positive	**Negative**	**Positive**	**12 (9)**	**4.3-13.7**
Negative	**Positive**	**Positive**	**62 (44)**	
		Negative	**45 (32)**	
		Unknown	**3 (2)**	
		Subtotal	**110 (78)**	**71.2-84.8**
	Negative	**Positive**	**10 (7)**	
		Negative	**8 (5)**	
		Unknown	**1 (1)**	
		Subtotal	**19 (13)**	**7.4-18.6**

CI indicates confidence interval.

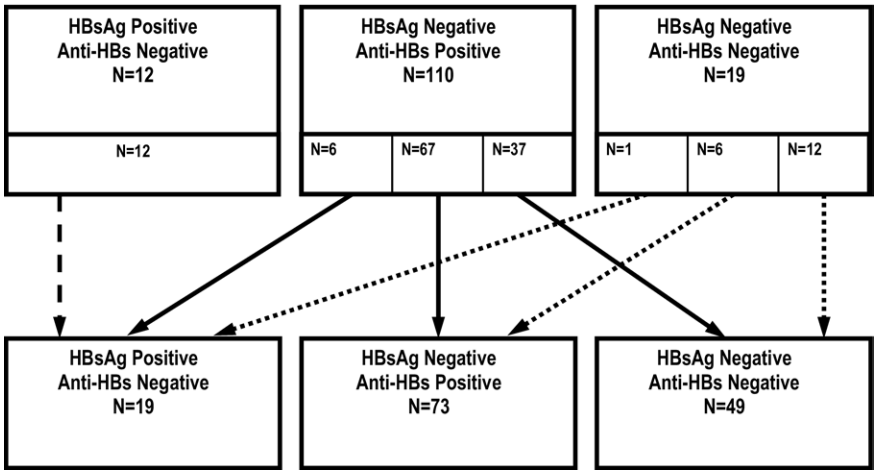

Figure 1. Changes in status of HBsAg and anti-HBs after autologous HSCT.

survey of HBV markers showed anti-HBs loss with high frequency (37 of 110 patients, 34%).

HBV infection is endemic to Korea, with 7%-9% seropositivity for HBsAg within the population in the 1980s [7]. Although HBsAg seropositivity was reported as only 5.8% in males and 4.4% in females in 1998, the prevalence of HBsAg in South Korea is still much higher than in the United States (0.1%-1%) [6,7].

In clinical practice, patients with various hematologic and oncologic diseases have often become candidates for HSCT, and patients with HBsAg have had an increased risk for hepatic complications such as HBV reactivation related to HSCT or immunosuppressive chemotherapy [2,3,5,8-10,14,15]. Therefore, HBV reactivation and reverse seroconversion of HBV may play important roles in the morbidity and mortality of HSCT-related hepatic complications in HBV prevalent areas [5,6,14,15].

There are currently 2 different methods for managing HBV reactivation in HBsAg-positive patients undergoing autologous HSCT: (i) deferred therapy, in which HBsAg-positive patients are treated with anti-HBV therapy only when there is evidence of HBV virologic reactivation or hepatitis due to HBV reactivation and (ii) preemptive therapy in which HBsAg-positive patients are started on anti-HBV therapy at the time of initiation of conditioning therapy [1,6]. Our center has treated all HbsAg-positive patients in a preemptive manner before autologous or allogeneic HSCT.

There are several reports describing effective lamivudine prophylaxis in HBsAg-positive patients receiving chemotherapy or HSCT [1,2,13,16-24]. Hsiao et al [13] reported 12% viral breakthrough during lamivudine therapy (median, 73 wk; range, 19-153 wk) administered to HBsAg-positive HSC transplant recipients. Although lamivudine-resistant mutations were detected in 10% of these patients, there was no HBV reactivation-related mortality or fulminant hepatitis [13]. In the present study, only 1 of 12 patients positive for HBsAg before transplantation developed HBV reactivation, and this was without acute hepatitis. Although the causes of viral breakthrough and spontaneous resolution were unclear, and there were no available data on mutations or lamivudine resistance; these results might also suggest an efficacy of lamivudine prophylaxis in HBsAg-positive patients undergoing HSCT.

Many investigators have reported reverse seroconversion, appearance of HBsAg, and disappearance of anti-HBs after allogeneic or autologous HSCT [3,5,8-11,25]. Onozawa et al [11] followed up on the anti-HBs titer of the 14 allogeneic HSC transplant recipients who had pretransplantation anti-HBs. They observed a decreased titer of anti-HBs in all patients

Table 3. *Risk Factors for Reverse Seroconversion after Autologous HSCT*

Risk Factor	Relative Risk	95% CI		*P*	
		Lower	Upper	Univariate	Multivariate
Age ≥45 yr	6.410	1.334	55.555	0.060	0.054
Male	1.527	0.327	7.124	0.437	0.588
Multiple myeloma	12.154	1.414	104.493	0.009	0.005
Pretransplantation anti-HBs	1.010	0.114	8.920	0.735	0.993
Pretransplantation anti-HBc	1.903	0.355	10.208	0.364	0.446

CI indicates confidence interval.

after transplantation and 5 cases of reverse seroconversion [11]. Dhedin et al [10] reported 4 cases of reverse seroconversion in 37 patients after allogeneic HSCT. Onset of hepatitis due to reverse seroconversion after HSCT has been reported to occur at 6-52 mo [8,11,25]. Goyama et al [15] reviewed 16 reverse seroconversion cases related to HSCT. Five of 16 cases developed in a median time of 13 mo (range, 6-22 mo) after autologous HSCT [5].

In the present analysis, reverse seroconversion developed in 7 of 129 patients who had pretransplantaiton anti-HBs or anti-HBc but not pretransplantation HBsAg. Four patients had anti-HBs and anti-HBc before HSCT and 2 patients had only pretransplantation anti-HBs. One patient with reverse seroconversion had pretransplantation anti-HBc without anti-HBs. Because of lack of information about previous vaccinations, we cannot exclude the possibility of new infection of HBV in the 2 patients with reverse seroconversion and pretransplantation anti-HBs.

Five of 7 patients with reverse seroconversion presented with acute hepatitis after autologous HSCT. In univariate analysis, multiple myeloma was revealed as a risk factor. Tur-Kaspa et al [26] considered the use of corticosteroid to be responsible for reverse seroconversion. However, there was no statistical correlation between reverse seroconversion and thalidomide-dexamethasone maintenance therapy in patients with multiple myeloma. Another plausible explanation that may account for risk of reverse seroconversion due to multiple myeloma is dysfunction of humoral immunity. In the case of allogeneic HSCT, reverse seroconversion hepatitis is caused by the loss of recipient-derived IgG and naive donor immunity against HBV [11,27]. However, further investigations are needed to elucidate the pathogenesis of reverse seroconversion after autologous HSCT.

In addition to reverse seroconversion, loss of anti-HBs was observed in 37 of 110 patients who had pretransplantation anti-HBs. This result is consistent with reports by Onozawa et al [11] that demonstrated progressively decreasing titers of anti-HBs after allogeneic HSCT. They also reported that disappearance of anti-HBs occurred 0-18 mo (median, 1 mo) before the occurrence of reverse seroconversion (range, 12-51 mo; median, 20 mo) [11]. However, it was not determined whether loss of anti-HBs occurs in advance of HBV-related acute hepatitis after HSCT [11].

In conclusion, reverse seroconversion of HBV is not a rare complication of autologous HSCT, and loss of anti-HBs is frequently observed after autologous HSCT. Therefore, it is critical to pay close attention to the twin possibilities of anti-HBs loss and reverse seroconversion in cases where patients have anti-HBs before autologous HSCT. Further, the sequence of events for HBV hepatitis and reverse seroconversion needs to be validated, and discussion is needed regarding the appropriate scheduling of follow-up testing for hepatic enzymes and HBV serology after HSCT.

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