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Hepatitis B virus infection in allogeneic bone marrow transplantation

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Summary:

Fourty-four patients who underwent allogeneic bone marrow transplantation (alloBMT) were studied for hepatitis B virus (HBV)-related complications. The mean follow-up period was 15.3 months. Positivity for HBV surface antigen (HBsAg) was observed in 10 patients (22.7%) throughout the study. Four of the 10 patients were HBsAg carriers before alloBMT, while the remaining six became HBsAg(+) after alloBMT. During the follow-up period (from 6 months to 45 months), an elevation in serum ALT activity was observed in the four carriers when immunosuppression was reduced or withdrawn. All of the four HBsAg carriers developed hepatitis, but none of them died of liver failure due to HBV. Only one death due to GVHD and diabetic ketoacidosis was observed in this group. Two of the four carriers received marrow from anti-HBs positive donors and one of them cleared HBsAg from his serum via adoptive immunity 8 months after transplantation. The remaining six patients acquired HBV after alloBMT, but we were unable to demonstrate the source of HBV. Five of them had a moderate increase in serum ALT activity while the other patient had a normal ALT. Two patients seroconverted to anti-HBs spontaneously. Two patients died during the follow-up, one due to intracranial hemorrhage and the other due to GVHD and accompanying pulmonary infection. The rest of the study group (34 patients) remained HBsAg(–) throughout the study. Two of them had an HBsAg(+) donor, but neither developed HBV infection in their follow-up period. The acquisition rate of HBV infection was relatively low in recipients who were positive for anti-HBs compared to those who were negative for anti-HBs (8 *vs* 19%). Anti-HBs positivity remained for a longer period in recipients who received marrow from anti-HBs positive donors compared to those recipients who had anti-HBs negative donors (median 12 *vs* 3 months). We think that HBV is a frequent cause of liver dysfunction in alloBMT patients where HBV infection is endemic. Whether the disease is in the form of reactivation of HBsAg-positive recipients, or is acquired from unknown sources in recipients who never had contact with the virus, the course of the disease is not fatal. Silent serologic changes can be demonstrated if viral serologic markers are sought serially. Among them, the disappearance of serum anti-HBs may be important as it increases the risk of HBV contamination in recipients.

Keywords: allogeneic bone marrow transplantation; hepatitis B virus

The liver is one of the organs most affected by allogeneic bone marrow transplantation (alloBMT).[1–3] Hepatitis B virus (HBV) infection is an important liver complication in the transplantation process. The pretransplant median prevalence of HBsAg positive patients has been reported as 3.5% (0–15%) in European recipients.[4] Host immunity is the most important determinant of the outcome of HBV infection;[5] therefore, alloBMT may be disadvantageous in several ways in the presence of HBV infection. The procedure suppresses the host's immune system via the conditioning regimen, graft-versus-host disease (GVHD) itself and GVHD prophylaxis/treatment.[6–8] Recipients are immunodeficient for months to years post-transplant; nonspecific components of the immune system recover within 2–6 months post-transplant while T cell and B cell functional reconstitution generally take 1–2 years. Immunosuppression may cause reactivation of HBV in the host.[9–14] HBsAg carrier recipients may develop fulminant hepatitis after transplantation during immune reconstitution.[9,10] However, some reports suggest that being a carrier of HBsAg is not a contraindication for transplantation.[15–17] Some recipients, who have an HBsAg carrier donor, may be infected with the virus after alloBMT. A case illustrating this has been reported[18] in which the patient developed fulminant hepatitis B infection and died of liver failure after transplantation. On the other hand, alloBMT may be advantageous for the outcome of HBV infection. If transplantation takes place with a donor who is immune to HBV, the recipient may clear the virus (adoptive immune response).[15,19] Our patients were examined to determine the frequency of HBV infection and the effects of alloBMT during the course of HBV infection. Our goal was to observe and define events related to HBV in recipients and answer the question of whether or not carrying HBV in recipients and/or donors is a contra-indication to alloBMT.

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Materials and methods

Patients

A total of 44 consecutive patients who underwent allogeneic bone marrow transplantation in the Ibn-i Sina Hospital, Dept of Hematology between 1989 and 1995 were included in the study. Twenty-two patients had acute nonlymphocytic leukemia (ANLL), 16 chronic myeloid leukemia (CML), three acute lymphocytic leukemia (ALL), one severe aplastic anemia (SAA), one myelodysplastic syndrome-refractory anemia (MDS-RA) and one had adrenoleukodystrophy. Twenty-seven were male and 17 female. The mean age at transplantation was 22 years (range 15–41 years). The mean follow-up after alloBMT was 15.3 months (range 12 days to 52 months).

Conditioning regimen and GVHD prophylaxis

Patients with leukemia were conditioned with a combination of busulfan, 4 mg/kg for 4 consecutive days, followed by cyclophosphamide 60 mg/kg/day for 2 days. 1250 cGy total body irradiation was used in 12 patients. Patients with SAA received anti-thymocyte/lymphocyte globulin, 30 mg/kg/day for 3 days and cyclophosphamide 50 mg/kg/day for 4 days. GVHD prophylaxis was with cyclosporine and methotrexate.

Virology

Hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), hepatitis B 'e' antigen (HBeAg), hepatitis B 'e' antibody (anti-HBe), hepatitis B core antibodies (anti-HBc IgM/G), hepatitis delta antibody (anti-D) of sera were all assayed with second generation enzyme-linked immunosorbent assay (ELISA) (Abbott Laboratories, North Chicago, IL, USA). These viral markers were tested in all patients before transplantation and in those who had liver dysfunction after transplantation. In 44 patients, HBV-related serologic markers were assayed at intervals of 3 months during the first 6 months, then at 6-month intervals throughout the study. In some patients the polymerase chain reaction (PCR) technique was used for the detection of HCV-RNA and HBV-DNA.

Liver function

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (AP) and bilirubin were measured on a 24 factor automated chemical analyser using standard reagents. The normal range is below 40 U/l for AST, ALT and GGT, 160 U/l for AP, 280 U/l for LDH, 1.4 mg/dl for bilirubin. The tests were carried out at 3-day intervals during the first month after bone marrow transplantation (BMT), and then every 2 weeks for the next 3 months. Patients were subsequently evaluated every 6 months.

Histological techniques

Twenty-two liver specimens were obtained from 19 patients by percutaneous liver biopsy. All liver tissue samples were fixed in 10% buffered formalin and embedded in paraffin. Sections were routinely stained with hematoxylin–eosin. The periodic-acid Schiff (PAS) stain was used to evaluate bile duct damage. Masson's trichrome was performed to show fibroelastic activity. Hepatitis B virus surface and core antigens were detected by immunohistochemical (IHC) examination (Biogenex, Dublin, Eire). IHC examination for HCV and CMV was also carried out on liver specimens.

Results

In recipients, the mean pre-transplant frequency of HBsAg positivity and anti-HBs positivity was 9% (4/44) and 31.8% (14/44), respectively. The rate of HBsAg positivity and anti-HBs positivity in donors was 4.5% (2/44) and 31.8% (14/44), respectively. Four patients were HBsAg-positive before alloBMT and six became HBsAg positive after alloBMT making a total of 10 (22.7%) HBsAg-positive patients. Two main types of hepatitis B virus (HBV)-related events were noted in recipients. The first type (type 1) of event, which occurred in 10 patients, was serum HBsAg positivity with an accompanying increase in serum ALT activity and other changes in HBV-related serologic markers (four patients in group 1, one patient in group 4, two patients in group 5 and three patients in group 6). The second type (type 2) of event occurred in 13 (29.5%) patients and consisted of changes only in HBV-related serologic markers. Type 2 events were all asymptomatic, without an increase in serum ALT activity, and featured only as changes in serum anti-HBs and anti-HBe positivity.

In those 10 patients with serum HBsAg positivity and an increase in serum ALT activity other causes of necroinflammatory reaction within the liver were sought. Superinfection or coinfection were not detected in any of the HBsAg(+) patients. Serum anti-HCV, HCV-RNA, CMV-IgM, EB-IgM, anti-HIV were all sought and found to be negative in these patients. Liver biopsies and IHC examination for HBsAg, HBcAg, HCV (all negative) and CMV (all negative) were performed in only eight patients and the details were given in the following section. The alloBMT patients were classified into six groups according to HBV-related serologic markers of recipients and donors.

Group 1: HBsAg carrier recipients (n = 4)

Four patients developed a HBV-related type 1 event in this group. Before alloBMT all patients had normal serum ALT values and serum HBeAg positivity was found in only one patient, while serum anti-HBe positivity was found in the remaining three patients (Table 1). Within the 4 to 5 months following transplantation, HBcIgM appeared in the serum of two patients paralleling the increase in serum ALT activity. When the dose of cyclosporine was reduced 5 months after alloBMT, an elevation in the serum transaminase activities was detected in all of the patients. The rise

Table 1 HBV-related events in four HBsAg positive recipients who underwent alloBMT

Patient number	*1*	*2*	*3*	*4*
Age/gender/underlying disease before BMT	17/male/MDS-RA	32/male/CML	41/male/ANLL	32/male/CML
Donor HBV serology (HBsAg/anti-HBs)	–/+	–/+	–/–	–/–
Recipient serum ALT (IU/l) before BMT	25	22	21	34
Recipient serum HBeAg/anti-HBe/anti-HBc-IgM before BMT	+/–/–	–/+/–	–/+/–	–/+/–
Time of event (months after BMT)	15	4	4	3
Serum ALT (IU/l) and T bilirubin during the event	109/1.1 mg/dl	144/0.8 mg/dl	254/2.2 mg/dl	1440/22 mg/dl
Serum HBV-DNA (hybridization) during the event	55 pg/ml	225 pg/ml	12 pg/ml	Not done
Serum HBeAg/anti-HBe/anti-HBc-IgM during the event	+/–/–	–/+/+	–/+/+	–/+/–
Interferon treatment	No	Yes	No	No
Serologic HBsAg clearance	No	Yes	No	No
Seroconversion to anti-HBs	No	Yes	Yes, but temporary	No
Time of biopsy (in months) after BMT	+15	+4	+4	+5
Histopathological diagnosis of liver specimens	CAH	CLH	CAH	CAH
Necroinflammatory activity/fibrosis score	7/1	10/1	13/2	9/1
Immunohistochemical staining in liver specimens				
Cytoplasmic HBsAg/nuclear HBcAg	+/+	+/+	+/–	Not done
Latest ALT value	39	23	22	90
Latest clinical status	+45th month/alive	+28th month/alive	+35th month/alive	+6th month/dead

CAH = chronic active hepatitis; CLH = chronic lobular hepatitis.

in serum ALT levels was moderate (less than 10 times the upper limit of the normal (ULN) range) in three patients and severe (10 times above the ULN range) in one patient. Jaundice occurred in the patient who had severely increased serum ALT activity, while in the remaining three, jaundice never developed. HBV-DNA was positive in this period in three patients and not studied in the other patient. Histological diagnosis of CH was confirmed in all four patients. The necro-inflammatory reaction in the liver was severe (grade 7–13) with a moderate fibrosis score (stage 2 in two patients and stage 1 in the other two patients) in all patients. IHC staining was carried out in three of the four liver samples. Cytoplasmic HBsAg positive staining was detected in all three patients (Nos 1, 2 and 3), but HBcAg-positive staining was detected in only two (Nos 1 and 2).

Two of the four patients had anti-HBs-positive donors. The disappearance of HBsAg and appearance of anti-HBs was detected 8 months after alloBMT in one. Interferon alpha-2b, 3 MU three times a week was used in this patient, for 2 months (from +5th month to +7th month) for the treatment of CH. It was discontinued due to pneumonitis.

In one of the two patients who had had an anti-HBs-negative donor, pulmonary tuberculosis developed when the HBsAg titer decreased, and the anti-HBs appeared in low titer in his serum. Anti-HBs disappeared after the development of pulmonary tuberculosis. The serum transaminase activity gradually decreased to normal in three patients. Three of four patients are still alive without liver dysfunction. The other patient died of diabetic ketoacidosis, deterioration of liver function and GVHD at 6 months following alloBMT.

Group 2: HBsAg carrier donors (n = 2)

Both patients underwent alloBMT for CML. Neither had any change in HBV serologic markers throughout the post-transplant follow-up periods (Table 2). One of the two patients had liver dysfunction starting from the +2nd month and lasting up to +12th month after transplantation. However, the cause of the liver damage could not be determined. The patient is still alive with normal liver function in the +34th month after alloBMT, but the other patient died of intracranial haemorrhage within 3 months of alloBMT.

Group 3: Recipients and donors both HBsAg-negative and anti-HBs-positive (n = 5)

HBV related type-1 events did not occur in any patient in this group (Table 3). Anti-HBs positivity remained in all patients for a median of 12 months after alloBMT but in the first patient it disappeared from the serum 14 months after alloBMT. Four (Nos 2, 3, 4 and 5) still had anti-HBs at the time of their last visit. However, anti-HBe positivity became negative in three patients (Nos 1, 2 and 3).

Table 2 HBV-related serologic events in group 2 (HBs Ag(–) and anti-HBs(+) recipients, HBs (Ag(+) and anti-HBs(–) donors)

Patient No.	*Pretransplant period*	*Post-transplant period (month)*						
		1st	*2nd*	*3rd*	*6th*	*12th*	*24th*	*34th*
1	–/+/–/+/–/+	–/+/–/+/–/+	–/+/–/+/–/+	–/+/–/+/–/+	–/+/–/+/–/+	–/+/–/+/–/+	–/+/–/+/–/+	–/+/ND/ND/–/+
2	–/+/–/+/–/+		–/+/–/+/–/+					

ND = not done.

Table 3 HBV-related serologic events in group 3 (Both recipients and donors HBs Ag negative, anti-HBs positive)

Patient No.	*Pretransplant period*	*Post-transplant period (month)*							
		1st	*3rd*	*6th*	*12th*	*14th*	*18th*	*24th*	*48th*
1	–/+/–/+/–/+	–/+/–/+/–/+	–/+/–/+/–/+	–/+/–/+/–/+	–/+/–/–/–/+	–/–/–/–/–/+	–/–/–/–/–/+		
2	–/+/–/+/–/+	–/+/–/+/–/+	–/+/–/–/–/+						
3	–/+/–/+/–/+	–/+/–/–/–/+	–/+/–/–/–/+	–/+/–/–/–/+	–/+/–/–/–/+				
4	–/+/–/ND/–/+	–/+/–/ND/–/+		–/+/–/ND/–/+	–/+/–/–/–/+			–/+/–/–/–/+	
5	–/+/–/+/–/+	–/+/–/+/–/+	–/+/–/ND/–/+	–/+/–/ND/–/+	–/+/–/ND/–/+				–/+/–/ND/–/+

ND = not done.

Group 4: HBsAg-negative, anti-HBs-positive recipients, HBsAg and anti-HBs-negative donors (n = 7)

Only one (No. 2) developed a HBV-related type 1 event in the +39th month (Table 4). At that time the patient's serum HBsAg, HBeAg and HbcIgM was positive with a moderate increase in serum ALT activity and normal bilirubin levels. HBV-related markers were all negative up to the +39th month. Clinical signs of GVHD with consistent liver changes developed in the +24th month. Liver biopsy revealed biliary stasis and focal necroinflammatory changes, and negative staining for HBsAg and HBcAg. Anti-HBs seropositivity disappeared after alloBMT in four patients (Nos 1, 3, 5 and 7) in the 3rd, 3rd, 9th and 12th months, respectively. Anti-HBe positivity, which was detected in only one patient, No. 2, before transplantation, disappeared in the +24th month after alloBMT with the development of GVHD.

Group 5: HBsAg and anti-HBs negative recipients, HBsAg(–) and anti-HBs(+) donors (n = 7)

HBV-related type-1 events occurred in two patients (Nos 2 and 4) (Table 5). Serum HBsAg was positive 6 months after alloBMT in one (No. 2) of the two. Serum HBeAg and HBcIgM positivity accompanied this change with a moderate increase in serum ALT activity. One year later, the patient cleared the HBsAg and anti-HBs and anti-HBe developed, with spontaneous resolution of the hepatitis. Notably, the pre-transplant HBcIgG was positive in the serum. In the other patient serum HBsAg, HBeAg and HBV-DNA (95 pg/ml) were all demonstrated to be positive at 1 month after alloBMT. Pre-transplant serum HBcIgG was not known in this patient, but 20 days after alloBMT it was found to be positive. Serum ALT activity was slightly increased at that time but the patient died of septicaemia while his liver function tests were impaired. In the 18

Table 4 HBV-related serologic events in group 4 (HBs Ag(–), anti-HBs(+) recipients, HBs Ag(–) and anti-HBs(–) donors

Patient No.	*Pretransplant period*	*Post-transplant period (month)*							
		1st	*3rd*	*6th*	*9th*	*12th*	*24th*	*35th*	*39th*
1	–/+/–/ND/ND/ND	–/+/ND/ND/ND/ND	–/–/–/–/–/–	–/–/–/–/–/–			–/–/–/–/–/–		
2	–/+/–/+/–/+	–/+/–/+/–/+		–/+/–/ND/–/+			–/+/–/–/–/+	–/+/–/–/–/+	+/–/+/–/+/+
3	–/+/–/–/–/+	–/+/–/ND/–/+	–/–/–/–/–/–	–/–/–/–/–/–		–/–/–/–/–/–			
4	–/+/–/–/–/+	–/+/–/–/–/+	–/+/–/–/–/+						
5	–/+/–/–/–/+	–/+/–/–/–/+	–/+/–/–/–/+		–/–/–/–/–/–		–/–/–/–/–/–		
6	–/+/–/–/–/+	–/+/–/–/–/ND			–/+/–/–/–/–				
7	–/+/–/–/–/–	–/+/–/–/–/–	–/+/–/–/–/–/–	–/+/–/–/–/–		–/–/–/–/–/–			

ND = not done.

Table 5 HBV-related serologic events in group 5 (HBs Ag and anti HBs(–) recipients, HBs Ag(–) and anti-HBs(+) donors)

Patient No.	*Pretransplant period*	*Post-transplant period (month)*								
		1st	*3rd*	*6th*	*12th*	*16th*	*22nd*	*27th*	*46th*	*52nd*
1	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–			–/–/–/–/–/–			
2	–/–/–/–/–/–	–/–/–/–/–/–		+/–/+/–/+/+						–/+/–/+/–/+
3	–/–/–/+/+/+	–/–/–/+/+/+	–/–/–/+/+/+		–/–/–/ND/–/+	–/+/–/ND/–/+			–/+/–/–/–/+	
4	–/–/ND/ND/ND/ND	+/–/+/–/–/+								
5	–/–/–/–/–/–	–/+/–/+/–/+	–/+/–/+/–/+	–/+/–/+/–/+	–/+/–/–/–/–	–/+/–/–/–/–				
6	–/–/–/–/ND/ND	–/–/–/–/ND/ND			–/+/–/ND/–/–		–/+/–/–/–/–	–/+/–/–/–/–		
7	–/–/–/–/–/–/–		–/+/ND/ND/ND/ND							

ND = not done.

months after alloBMT anti-HBs appeared in four patients (Nos 3, 5, 6, and 7) (at the 16th, 1st, 12th and 3rd months, respectively) whose pre-transplant anti-HBs levels had been negative.

Group 6: Both recipients and donors negative for HBsAg and anti-HBs (n = 19)

Among these, three patients developed HBV-related type 1 event (Nos 17, 18 and 19) (Table 6).

Patient 17: Serum HBsAg and HBeAg appeared to be positive at 1 month after alloBMT without serum HBcIgM positivity. Serum ALT activity remained within normal limits. Liver biopsy at that time revealed minimal hepatitis, steatosis and hemosiderosis. HBsAg and HBcAg were not apparent on IHC examination. When this patient was followed, serum HBcIgM positivity appeared at 6 months parallel to the moderate increase in serum ALT activity while the dose of cyclosporine was reduced. He remained HbeAg-positive and serum ALT activity returned to normal limits at 12 months spontaneously.

Patient 18: Serum HBsAg appeared at 3 months after alloBMT with accompanying anti-HBcIgM and HBV-DNA (70 pg/ml) positivity. There was a moderate and transient increase in serum ALT activity without jaundice. Liver biopsy was performed 3 months after alloBMT and revealed mild hepatitis (necro-inflammatory grade: 5) with positive staining for HBsAg and HBcAg. Serum HBeAg was never demonstrated but anti-HBe developed at 6 months. Immunosuppressive therapy ended at 9 months and following 1 month of severe hepatitis, seroconversion to anti-HBs occurred spontaneously with complete resolution of the hepatitis.

Patient 19: Serum HBsAg appeared at 3 months after alloBMT with accompanying HBcIgM and HBeAg positivity which remained throughout the observation period (20 months). Serum HBV-DNA was undetectable. Serum ALT activity was normal at 3 months but increased moderately at 6 and 9 months after alloBMT. Initial liver biopsy which was performed in the +3rd month was not diagnostic but subsequent liver biopsies, performed in the +6th month and the +8th month, were consistent with GVHD and CH

Table 6 HBV-related serologic events in group 6 (both recipients and donors negative for HBs Ag and anti-HBs)

Patient No.	*Pretransplant period*	*Post-transplant period (month)*							
		1st	*3rd*	*6th*	*9th*	*12th*	*20th*	*32nd*	*41st*
1	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–		–/–/–/–/–/–			
2	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–					
3	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–					
4	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–						
5	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–			
6	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–				
7	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–						
8	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–						
9	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–				
10	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–				–/–/–/–/–/–	
11	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–						
12	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–		–/–/–/–/–/–				
13	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–				–/–/–/–/–/–	–/–/–/–/–/–
14	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–					
15	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–						
16	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–	–/–/–/–/–/–					
17	–/–/–/–/–/–	+/–/+/–/–/–	+/–/+/–/–/+	+/–/+/–/+/+	+/–/+/–/+/+	+/–/+/–/–/+			
18	–/–/–/–/–/–	–/–/–/–/–/–	+/–/–/–/+/+	+/–/–/+/y/+	+/–/–/+/+/+	–/+/–/+/–/+			
19	–/–/–/–/–/–	–/–/–/–/–/–	+/–/+/–/+/+	+/–/+/–/+/+		+/–/+/+/+/+	+/–/+/+/+/+		

(necroinflammatory grade 5, fibroblastic stage 1). Cytoplasmic HBsAg positivity was detected in the last specimen, but it was not found in the first and second specimens. Nuclear HBcAg staining was negative in all specimens. The patient died of GVHD and pulmonary infection 20 months after alloBMT.

Two patients were alive with normal serum ALT activity, but in one of them HBeAg was positive. There were no changes in the HBV-related serologic markers in the remaining patients.

Discussion

The liver is one of the organs most effected by alloBMT.[1] The effects of alloBMT on the liver vary greatly. Besides GVHD of the liver and veno-occlusive disease, viral infections can cause hepatitis of variable severity after BMT. Impairment of immune response in BMT patients can effect the clinical and serologic outcome of HBV infection as the severity of the disease depends mainly on the immune system's ability to attack infected hepatocytes. It has been suggested that HBV-related liver disease after BMT may be more severe in this setting and, indeed, fulminant liver failure was seen more frequently in patients with HBsAg-positive donors when compared with their negative counterparts.

In Europe, the median prevalence of HBsAg-positive BMT candidates has been reported as 3.5%, with a range of 0 to 15%.[4] In Turkey, nearly 33% of the population acquire HBV and the median prevalence of HBsAg positivity in the community is 5%. The rate of serum HBsAg positivity was 9% (four BMT candidates) and 4.5% (two recipients) before transplantation and six (13.7%) patients acquired the disease after alloBMT. Therefore, the rate of HBV infection (22.7%) was higher than expected, as six more patients acquired the disease during the study, and the source is unknown. The high incidence of HBV infection is probably related both to immunosuppression and frequent transfusions of blood and blood products which were inadequately screened.

Four patients (group 1) were HBsAg carriers at the time of transplantation, with normal serum ALT activity, suggesting immune tolerance. Only one patient was serum HBeAg-positive, indicating active viral replication. Within the first months after alloBMT, serum ALT activity did not change as a result of profound immunosuppression, but silent viral replication was occurring. Later, 4–5 months after alloBMT, when immunosuppressive drugs were decreased, immune activity was restored as was evident in the increase in serum ALT activity which was moderate in three patients and severe in one patient. This information is consistent with the findings that liver damage caused by HBV infection occurs via the immune system, rather than as a direct effect of the virus.[5] We did not look for the presence of serum HBV-DNA before alloBMT, but this is important in anti-HBe-positive patients who may harbour an HBV strain with a mutation in the precore region of the genome that blocks synthesis of HBeAg, but who still have replicating HBV.[20] On the other hand, serum HBeAg/anti-HBe status was identical in all patients before and during the event and serum HBV-DNA was positive during exacerbations of the disease, indicating the possibility of this type of mutation.

After alloBMT, serum HBV-DNA was assayed in three patients and found to be positive in all, indicating active viral replication. These findings are consistent with the suggestion of some reports that immunosuppression causes viral replications.[9–14]

Another specific serologic marker of acute disease, anti-HBc-IgM, was found to be positive in two patients during the event. This is not always positive in patients with chronic hepatitis B, and acute exacerbation of the disease though this was the cause in our patients. Immunohistochemical testing is crucial in evaluation of necro-inflammation found in chronic hepatitis B as it may be due to (1) conversion of chronic HBV infection from the replicative to the nonreplicative phase; (2) spontaneous reactivation of HBV replication during the nonreplicative phase; (3) superinfection of chronic hepatitis B by other viruses; (4) reactivation of HBV replication following withdrawal of immunosuppressive therapy. The expected expression pattern of hepatitis markers in this latter group is positive nuclear HBcAg and cytoplasmic HBsAg in liver tissue.[21] We have seen a similar staining pattern in all cases, except for negative nuclear HBcAg staining in only one case, reinforcing the diagnosis of reactivation of HBV replication rather than reinfection with HBV.[15,16] Anti-HBs positivity was observed 8 months after alloBMT in one patient who received marrow from an anti-HBs-positive donor. At least 1 year after transplantation was required for the antibody response to recall antigens.[6–8] It is logical to explain the appearance of anti-HBs as an adoptive immune response.[15,19] The use of interferon might also have played a role in the clearance of the HBsAg from the serum of the patient, but the total dose and duration of interferon treatment for this patient was not adequate as it was discontinued prematurely due to a complication. In addition, interferon was not used in all the patients in our study because an increased risk of graft rejection with interferon administration has been reported.[22] Fulminant hepatitis did not develop in any of the four patients. Some studies have contradicted this finding.[9,10] None of the four died from hepatic failure due to HBV in the follow-up period of 6 to 45 months. Three of these patients are still alive with normal serum ALT activity. Our data is comparable with the suggestion that HBV carriage is not an absolute contraindication for BMT.[15–17]

It has been reported that hepatitis B may be acquired from HBsAg-positive donors.[18] We have only two donors with HBsAg positivity and their recipients (group 2) did not acquire the disease throughout the study period. This may be due to the presence of protective anti-HBs, and to the small number of our recipients.

The recipients in groups 3 and 4 both had serum anti-HBs positivity before alloBMT, but the donor serologic markers (anti-HBs(+) in group 3, and the anti-HBs(−) in group 4) were different. This difference appeared to be important after transplantation as serum anti-HBs positivity disappeared between 3 to 12 months in four patients in group 4, while only one patient was anti-HBs negative after 14 months in group 3. These patients are at risk of acquiring

a fresh hepatitis B infection especially in endemic areas, and vaccination against HBV should be considered for them.

HBV infection developed in only one of the 12 patients (8%) who had anti-HBs antibody before transplantation (groups 3 and 4), but it developed in five of the 26 patients (19%) who did not have anti-HBs before transplantation (groups 5 and 6). The source of HBV infection could not be determined in the patient who was in group 4, but negative staining for HBV-related antigens in the liver tissue suggests that it was not a reactivation but a freshly acquired infection. Similarly HBV may be acquired from improperly checked transfusions in group 5 as they have a history of multiple transfusions, but the presence of anti-HBcIgG before transplantation raised the possibility of reactivation of the disease under immunosuppression. Unfortunately IHC was not performed in these patients for this discrimination. One patient in group 5 spontaneously seroconverted to anti-HBs after 1 year. It would be reasonable to assume that the immunity against HBV from the donor side may act as a component of the recipient's immune system; it then intensifies after 1 year and provides immunity against HBV. These findings suggest that anti-HBs positivity in the pretransplant period may be protective for HBV infection after transplantation. This is a controversial issue in the literature. One study reports that pretransplant anti-HBs positivity may be protective for recipients who receive marrow from HBsAg-positive donors.[23] Other studies suggest that HBsAg reappeared when anti-HBs titers fell with continuing immunosuppression.[14,15]

It was shown that donor B lymphocytes could retain their antibody production ability in the recipient's body for nearly 50 days. The demonstration of anti-HBs positivity in two of the seven anti-HBs negative patients (group 5) who received marrow from anti-HBs(+) donors within 1 to 3 months after alloBMT can be explained in this way. Two additional patients developed anti-HBs positivity 12 and 16 months after transplantation. The appearance of anti-HBs in all patients was not temporary and can be detected throughout the study, indicating that the donor's immune system became a component of the recipient's immune system. None of them received IVIG therapy during the follow-up which could be the source of serum anti-HBs positivity. In the remaining two patients the development of hepatitis B again displayed the importance of the presence of anti-HBs as a protective antibody. Pretransplant immunization of the donor may facilitate the transfer of functioning B cells and helper T cells which are required for antibody production by B cells.[6–8] However, even if T cell-depleted bone marrow is given to recipients, the functioning humoral immune system can be transferred.[24] Activated natural killer cells may play a significant role in stimulating antibody production by B cells.[24] When either the donor or the recipient is exclusively immunized with HBV vaccine, then the rise in the antibody of the recipient is of low titer and shorter duration than that observed when both donor and recipient are immunized.[8,24] The presence of retained antigen in the recipient after transplantation, presumably in dentritic cells in lymph nodes, can stimulate donor B cells to produce antibody.[8,24,25]

Both donors and recipients were HBsAg and anti-HBs negative in group 6 and we did not expect any HBV-related event in these patients. To our surprise, three hepatitis B cases were identified during follow-up. The source of infection was unknown in all cases but multiple blood transfusions might be the source of it as only HBsAg positivity was sought in blood donors in some centers. Although all of them were fresh hepatitis cases, serum ALT activities were normal or slightly increased when HBsAg was found to be positive due to the immunosuppressive therapy, but as soon as the dose of therapy was decreased or following withdrawal of therapy, serum ALT activity increased and one patient even seroconverted to anti-HBs at that time. The outcome of this kind of immune attack is unpredictable. Few fatal reactivations have been reported even in patients treated with single immunosuppressive agents.[26] On the other hand some studies have suggested that antiviral effects of interferon may be enhanced by a short priming course of steroids.[27]

During the course of chronic hepatitis B infection abundant HBcAg with little HBsAg was found within the liver tissue in immunosuppressed patients, which was demonstrated in one of the three patients.[21] On the other hand, the chance of detecting viral particles in liver tissue is low in acute hepatitis especially in the early incubation period, which was the case in our two patients in group 6. The late appearance of HBsAg staining in one patient in group 6 can be interpreted as a sign of chronicity, and the finding of persistent serum HBeAg positivity during the follow-up period supports this statement. All three patients in this group survived the hepatitis period without clinical side-effects and two of them are still alive; one patient died of GVHD and accompanying pulmonary infection.

In conclusion, we suggest that HBV infection is frequent in alloBMT patients, especially where HBV infection is endemic. It may cause morbidity, but mortality related to HBV infection in alloBMT patients is unlikely. While investigating the cause of liver dysfunction in alloBMT patients, a detailed search for the presence of HBV should be carried out with modern techniques as the clinical and laboratory presentation may be unusual. Consequently, serial serologic, virologic and pathologic investigations are mandatory. The presence of serum HBsAg positivity is not a contraindication for alloBMT patients. Although the disease exacerbates during withdrawal of immunosuppressive therapy the outcome is benign. If possible, the selection of anti-HBs positive donors is worthwhile especially in areas where HBV infection is highly endemic as unexpected hepatitis B cases can be seen. Disappearance of serum anti-HBs positivity can be observed in recipients who have serum anti-HBs negative donors and the risk of HBV infection is higher in these patients.

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