Significance of detectable HBV DNA in liver allograft tissues in long-term follow-up of liver transplant recipients

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ABSTRACT

Background & Aims: We aimed to evaluate the long-term presence of hepatitis B virus (HBV) DNA in liver grafts of liver transplant patients who received hepatitis B immunoglobulin (HBIg) plus oral antiviral hepatitis B virus prophylaxis and had negative serum markers of HBV.

Materials and Methods: Patients aged 18 years or older who underwent liver transplantation for HBV-related liver disease, had negative serum viral markers and had a liver biopsy at least

3 years after liver transplantation were eligible for this study. Clinical, serological and pathological data were retrospectively obtained from medical records. HBV DNA in liver biopsy specimens was assessed using the polymerase chain reaction technique.

Results: A total of 150 patients were included. HBV DNA was positive in 18 (12%) of the liver biopsies. The presence of intrahepatic HBV DNA was not associated with pre-transplantation serum viral markers, types of pre- and post-transplantation antiviral treatment or post-transplantation immunosuppressive treatment.

Conclusion: Our findings suggest that after treatment with HBIg plus oral antiviral as post-transplantation HBV prophylaxis, a considerable percentage of patients may have persistent HBV DNA in their grafts; however, the presence of HBV DNA in liver grafts may not be related to clinical HBV recurrence.

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Introduction

Hepatitis B virus (HBV) infection is an important public health problem. Chronic HBV infection affects more than 250 million people globally and is a major etiology for cirrhosis and hepatocellular carcinoma.[1] Antiviral drugs can reduce the long-term complications of chronic HBV infection; however, liver transplantation remains the mainstay of treatment for patients with end-stage liver disease.

HBV reinfection is a significant cause of impaired prognosis after liver transplantation. It is defined as the reappearance of HBsAg and/or HBV DNA in the serum. Immunosuppressive treatment administered after liver transplantation may increase the risk of HBV reinfection.[2-5] The risk of reinfection increases in patients with pre-transplantation HBeAg positivity or high serum HBV DNA levels and who have resistance to antiviral drugs before transplantation or have hepatocellular carcinoma at the time of liver transplantation.[6-8] Additionally, extrahepatic reservoirs of HBV such as peripheral blood mononuclear cells and the spleen may contribute to graft reinfection.[9-13]

On the other hand, having negative serum HBsAg and HBV DNA after liver transplantation may not indicate eradication of HBV. HBV DNA remains detectable in liver tissue in a subset of patients after transplantation.[14-19] The prognostic significance of detecting HBV DNA in graft tissue with no serologic evidence of HBV reinfection remains to be determined.[20]

In this study, we assessed the presence of HBV DNA in the liver tissue of patients who underwent liver transplantation for HBV-related liver disease and achieved negative serologic markers of HBV infection. Additionally, we evaluated the association of the presence of intrahepatic HBV DNA with pre-transplantation serum viral markers, types of pre- and post-transplantation antiviral treatment and post-transplantation immunosuppressive treatment.

Materials and Methods

Patients

In our clinical practice, all patients with HBV-related liver disease are offered liver biopsy three years after liver transplantation to histologically assess the liver and tissue HBV markers. Patients aged 18 years or older who underwent liver transplantation for HBV-related acute or chronic liver disease between 1998 and 2009, had a minimum post-transplantation follow-up duration of 3 years, had negative serologic markers of HBV infection (serum HBsAg and HBV DNA) during the follow-up after transplantation, and underwent liver biopsy, were eligible for this study. Written informed consent was obtained from all patients before liver biopsy. This study was conducted in accordance with the Declaration of Helsinki and was approved by the ethical committee of XXXXX University. (#14-4.1/5).

Antiviral prophylaxis after transplantation

In the anhepatic phase of transplantation, 4000 IU HBIg (2000 IU intramuscular and 2000 IU intravenous) was given. On the following 5-20 days, daily 800-1600 IU HBIg intramuscular or 1500 IU HBIg intravenous was given until serum HBsAg became negative and serum anti-HBs were over 200 IU/L. Anti-HBs level was regularly monitored, and 200-1000 IU HBIg intramuscular was given every 1-4 weeks in order to maintain the level of serum anti-HBs over 50 IU/L.

Oral antiviral treatment commenced before liver transplantation was continued after transplantation. In patients who did not receive oral antiviral treatment lamivudine was started in addition to HBIg after the liver transplantation.

Immunosuppressive treatment after transplantation

In the absence of contraindications (e.g., renal failure), a prednisolone and calcineurin inhibitor (cyclosporin or tacrolimus) combination was administered. Otherwise, everolimus, rapamycin, mycophenolate mofetil, mycophenolic acid, azothioprine, basiliximab were given in different combinations. Steroid treatment was completely stopped after 6-12 months of treatment. Target serum level of tacrolimus was 5-10 ng/ml, cyclosporin was 100-200 ng/ml and rapamycin was 5-15 ng/ml.

Data collection

This was a retrospective cohort study. Clinical, serological and pathological data were retrospectively obtained from medical records. The date of liver transplantation, age of the patient at the time of transplantation, status of cadaveric vs live donor liver transplantation, pre-transplantation antiviral treatment, and pre-transplantation serologic markers (HBsAg, anti-HBs, HBeAg, anti-HBe, HBV DNA, anti-delta Ab, delta RNA) were recorded. Details of the methods for serological tests, serum and liver HBV DNA measurements are provided in Supplement 1. 'Child-Turcotte-Pugh' and 'Model for End Stage Liver Disease' (MELD) scores were calculated. The data regarding post-transplantation immunosuppressive and antiviral medications, histopathological findings of the liver explant, date of liver biopsy, presence of HBV DNA in the liver graft biopsy, serum HBsAg, HBV DNA and anti-HBs levels at the time of liver biopsy were also recorded. Long-term follow-up data for serum HBsAg and HBV DNA were collected.

The details of the serological tests, serum and liver HBV DNA measurements

Serologic tests were performed with Abbott A*SYM System, Abbott Diagnostics (Chicago, IL, USA) immunoassay. Serum HBV DNA was performed with different methods at different periods depending on availability: Digene Hybrid Capture Assay (Digene Diagnostics, USA; detection limit 5 pg/ml), Versant HBV DNA (bDNA; Bayer Diagnostics; detection limit 2000

IU/ml), COBAS Ampliprep TaqMan (real time PCR; Roche Molecular Diagnostics; detection limit 20 IU/ml), Abbott m2000 sp, m2000rt (real time PCR; Abbott Molecular Diagnostics; detection limit 10 IU/ml). HBV DNA in liver tissue was evaluated with polymerase chain reaction (PCR) (HBV S primers, HBV P7-P8 primers; LIPA primers) and gel electrophoresis (Innogenetics N. V. Ghent, Belgium). Anti-delta antibodies were assessed using microenzyme immunoassay (Murex Biotech, Dartford, UK).

Statistical analysis

Statistical analyses were performed with SPSS software (version 20.0; SPSS, Chicago, IL). Continuous variables are expressed as median and range. Categorical variables are summarized as count and percentage. Between group comparisons were performed using Mann-Whitney U test for continuous variables and chi-square test (or Fisher's exact test where appropriate) for categorical variables. A p value less than 0.05 was considered to indicate statistical significance. All statistical tests were 2-sided.

Results

Between January 1998 and November 2009, a total of 892 patients underwent liver transplantation, 745 of whom were adults. Of these, 421 (57%) had liver transplantation because of HBV-related liver disease. Liver biopsy after liver transplantation was performed in 152 patients. Two patients were excluded because of insufficient liver biopsy sample. A total of 150 patients were included in this study (Fig. 1).

Patient characteristics

Median age at liver transplantation was 47 (range 18-64) years. All patients had HBV-related cirrhosis. Thirty-four (22.7%) of the patients were female, and 116 (77.3%) patients were male. The median MELD score before transplantation was 16.5 (range 6-39). Pre-

transplantation HBeAg was positive in 11 of 121 (9.1%) patients. Pre-transplantation HBV DNA was positive in 49 of 122 (40.2%) patients. Pre-transplantation anti-delta was positive in 64 of 113 (56.6%) patients. Liver grafts were obtained from live donors for 108 (72%) patients, and cadaveric donors for 42 (28%) patients (Table 1).

In the pre-transplantation period, the following drugs were used (alone or sequentially): 57 (38%) patients received lamivudine, 13 (8.7%) patients received adefovir dipivoxil, 4 (2.7%) patients received entecavir, and 8 patients received interferon (5.3%). Overall, 21 (14%) patients did not receive any pre-transplantation antiviral treatment, and no data were available for 65 (43.3%) patients.

Presence of intrahepatic HBV DNA and associated factors

Median duration between liver transplantation and liver biopsy was 4.4 years (range 3-12.4 years). Intrahepatic HBV DNA was positive in the liver biopsies of 18 (12%) patients. Presence of liver HBV DNA was not associated with gender (p=0.962) or type of donor (live or cadaveric) (p=0.089). Pre-transplantation serum HBeAg (p=0.611), HBV DNA (p=0.167) and anti-delta (p=0.592) status were not associated with post-transplantation liver HBV DNA positivity (Table 1). Type of pre-transplantation antiviral treatment was not related to presence of post-transplantation hepatic HBV DNA (Table 2). Type of post-transplantation antiviral drugs and immunosuppressive treatments were not related to the presence of intrahepatic HBV DNA (Table 2 and table 3). At the time of liver biopsy, 19 patients had no measurable anti-HBs in serum and two had positive hepatic HBV DNA. Absence of serum anti-HBs was not associated with the presence of hepatic HBV DNA (p=1.000).

Long-term serological outcomes

After a median post-transplant follow-up duration of 7.5 years (range 4 – 15.2 years), four patients (2.7%) had positive serum HBsAg levels. Only one of these four patients was found to have positive intrahepatic HBV DNA; there was no association between intrahepatic HBV DNA and long-term HBsAg positivity (p=0.417). All of these four patients had negative serum HBV DNA.

Discussion

We investigated the presence of intrahepatic HBV DNA in a cohort of long-term survivors who underwent liver transplantation for HBV-related liver disease. To the best of our knowledge, this study describes the largest cohort of liver transplant recipients on this subject and includes long-term follow-up results. The main conclusion of our study is that after a median post-transplantation follow-up of 4.4 years, the majority of patients who had combination prophylaxis with HBIg and an oral antiviral did not have intrahepatic HBV DNA. In the long-term follow-up, a positive intrahepatic HBV DNA was not associated with clinical HBV recurrence.

The presence of intrahepatic HBV DNA in patients who underwent liver transplantation for hepatitis B-associated liver disease was investigated in previous studies. Hussain et al. reported a higher rate of positive hepatic HBV DNA in post-transplantation liver biopsies (total HBV DNA was positive in 83% of the 47 liver biopsy samples from 25 patients).[21] The differences between the two studies are the pre-transplantation HBV DNA levels, and the follow-up duration after liver transplantation. Most of the patients included in our study were HBeAg-negative, and had undetectable pre-transplantation serum HBV DNA. Additionally, the post-transplantation follow-up duration in our study (median 4.4 years, range 3 to 12.5 years) was longer than the study of Hussain et al. (range 7 days to 48 months). In their study, they proposed that intrahepatic HBV DNA has no predictive value for HBV recurrence, but

there were two HBV recurrences in 25 patients, which can be considered as a high recurrence rate and can be related to the high rate of liver HBV DNA positivity.[21] Our patients belonged to a low risk group with regard to HBV recurrence and represented the prevalent hepatitis B patient population being transplanted at most centers. The antiviral prophylaxis regimen consisted mainly of HBIg and lamivudine, which was similar to our study.

Another study reported that among 18 patients with positive serum HBV DNA, 9 of them were found to have positive intrahepatic HBV DNA.[17] The proportion of liver HBV DNA positivity was considerably higher than our study, most probably because all patients in our study had undetectable serum HBV DNA levels. Furthermore, the antiviral prophylaxis regimen consisted of only HBIg rather than a combination of HBIg and oral antiviral treatment in most patients, which can be considered as a less effective treatment compared to the regimen used in our study.

In accordance with our findings, Lenci et al. reported that about 7% of post-transplantation patients with negative serum HBV DNA had positive liver HBV DNA detected within a mean follow-up period of 88.3 months. One patient had HBV recurrence in the follow-up period and his liver HBV DNA was positive.[22]

The mechanism of post-transplantation HBV recurrence is not clearly understood, but the immunosuppressive treatment administered after the transplantation may play a role.[2] In the previous studies, there were not enough data on the effects of different immunosuppressive medications on HBV recurrence, most probably because the number of patients included in these studies was too low. Our results suggest that the type of immunosuppressive regimen has no effect on post-transplantation HBV recurrence.

This study has limitations. Due to its retrospective design, there were missing data for various parameters, including baseline characteristics, medications and treatment durations. We were

not able to obtain data about the histopathological findings in the liver biopsy specimens. Additionally, the data was reflecting patients treated over a long-time frame, and during that time different measurement methods of serum HBV DNA with different thresholds were used.

In conclusion, intrahepatic HBV DNA is rarely detectable in long-term survivors among liver transplant recipients who had combination prophylaxis with HBIg and an oral antiviral drug after transplantation, especially in those patients with low risk of HBV recurrence. The presence of intrahepatic HBV DNA was not associated with pre-transplantation serum viral markers, types of pre- and post-transplantation antiviral treatment or type of immunosuppressive treatment. Our results suggest that presence of intrahepatic HBV DNA is not a reliable indicator of HBV recurrence after liver transplantation. Presence of HBV DNA in liver grafts may not indicate clinical recurrence. Further studies are needed to assess the prognostic value of detection of intrahepatic HBV DNA and its role in guiding further treatment options.

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Tables

Table 1. Patient characteristics (n=150)

	All patients*	Intrahepatic HBV DNA		*p*
		Positive	Negative	
Age, median (range), years	47 (18-64)	48.5 (39-53)	47 (39-51)	0.670
Gender, n (%)				
Female	34 (22.7)	4 (22.2)	30 (22.7)	0.962
Male	116 (77.3)	14 (77.8)	102 (77.3)	
Child-Turcotte-Pugh score, n (%)				
A	9 (6.0)	0 (0.0)	9 (6.8)	0.324
B	24 (16.0)	3 (16.7)	21 (15.9)	
C	48 (32.0)	9 (50.0)	39 (29.5)	
Unknown	69 (46.0)	6 (33.3)	63 (47.7)	
MELD score, median (range)	16.5 (6-39)	19 (14-23)	16 (13-22)	0.392
Pre-transplantation serum HBV DNA, n (%)				
Positive	49 (32.7)	9 (50.0)	40 (30.3)	0.167
Negative	73 (48.7)	7 (38.9)	66 (50.0)	
Unknown	28 (18.7)	2 (11.1)	26 (19.7)	
Pre-transplantation serum HBeAg, n (%)				
Positive	11 (7.3)	2 (11.1)	9 (6.8)	0.611
Negative	110 (73.3)	14 (77.8)	96 (72.7)	
Unknown	29 (19.3)	2 (11.1)	27 (20.5)	
Pre-transplantation serum Anti-delta, n (%)				
Positive	64 (42.7)	7 (38.9)	57 (43.2)	0.592
Negative	49 (32.7)	7 (38.9)	42 (31.8)	
Unknown	37 (24.7)	4 (22.2)	33 (25.0)	
Donor type, n (%)				
Live	108 (72)	16 (88.9)	92 (69.7)	0.089
Cadaveric	42 (28)	2 (11.1)	40 (30.3)	
Histopathology of explant liver, n (%)				
Liver cirrhosis	59 (39.3)	10 (55.6)	49 (37.1)	0.400
Hepatocellular carcinoma†	41 (27.3)	4 (22.2)	37 (28.0)	
Dysplastic nodules†	35 (23.3)	3 (16.7)	32 (24.2)	
Unknown	15 (10.0)	1 (5.6)	14 (10.6)	

Abbreviation: MELD, model for end stage liver disease.
p values are calculated using Mann-Whitney U test for continuous variables and chi-square test for categorical variables. *p* values less than 0.05 are considered statistically significant.
*Percentages may not sum up to 100 because of rounding.
†Findings in addition to liver cirrhosis.

Table 2. Association between pre- and post-transplantation antiviral use and post-transplantation intrahepatic HBV DNA status.

	Liver HBV DNA (-)		Liver HBV DNA (+)		Total*	$p^{\dagger}$
	n	**(%)**	**n**	**(%)**		
Pre-transplantation antiviral use						
Interferon	7	(87.5)	1	(12.5)	8	1.000
Lamivudine	47	(82.5)	10	(17.5)	57	0.323
Adefovir	13	(100)	0	(0.0)	13	0.198
Entecavir	4	(100)	0	(0.0)	4	1.000
None	19	(90.5)	2	(9.5)	21	
Unknown	58	(89.2)	7	(10.8)	65	
Post-transplantation antiviral use						
Lamivudine	126	(88.1)	17	(11.9)	143	0.545
Adefovir	11	(78.6)	3	(21.4)	14	0.379
Telbivudine	14	(87.5)	2	(12.5)	16	1.000
Entecavir	12	(92.3)	1	(7.7)	13	1.000
Tenofovir	27	(87.1)	4	(12.9)	31	1.000

*Some patients received more than one type of antiviral drug; therefore the total count exceeds the total number of patients.

† *p* values calculated by chi-square test based on contingency tables including number of patients who were or were not treated for each drug. *p* values less than 0.05 are considered statistically significant.

Table 3. Association between type of post-transplantation immunosuppressive drugs and the presence of post-transplantation intrahepatic HBV DNA.

	Liver HBV DNA (-)		Liver HBV DNA (+)		Total	$p^{\dagger}$
	n	(%)*	n	(%)*		
Methylprednisolone	130	(88.4)	17	(11.6)	147	0.228
Tacrolimus	110	(87.3)	16	(12.7)	126	0.741
Cyclosporin A	24	(92.3)	2	(7.7)	26	0.740
Rapamycin	35	(85.4)	6	(14.6)	41	0.579
Everolimus	9	(69.2)	4	(30.8)	13	0.054
Basiliximab	9	(90.0)	1	(10.0)	10	1.000
Mycophenolat Mofetil	46	(86.8)	7	(13.2)	53	0.754
Mycophenolic Acid	9	(100)	0	(0.0)	9	0.601
Azathioprine	2	(100)	0	(0.0)	2	1.000
Unknown	1		0		1	

*Row percentages are given.

† *p* values calculated by chi-square test based on contingency tables including number of patients who were or were not treated for each drug. *p* values less than 0.05 are considered statistically significant.

Fig. 1. Flowchart of the study population