Hepatitis B Reactivation in Liver Transplant Recipients With Hepatitis B Virus Core Antibody Positive Grafts: a Retrospective Study

Benjamine Khiangte, Sunil R. Kothakota, Madhu Sasidharan, Harish Kareem, Ajith K. Nair, Vijosh V. Kumar, Jagadeswara R. Kanala, Praveen C. Kumar

Department of Gastroenterology, KIMS Hospital, Trivandrum, Kerala, India

Background: Liver grafts from hepatitis B core antibody (anti-HBc) positive donors increase the risk of hepatitis B virus (HBV) reactivation in recipients due to posttransplant immunosuppressive therapy. **Aim and objective:** to study the HBV reactivation in liver transplant recipients with anti-HBc-positive donors. **Methods:** This was a retrospective study. Liver transplant recipients who received grafts from anti-HBc-positive donors between January 2013 and December 2017 were included in analysis. Hospital records of all subjects for a 2-year posttransplantation period were studied to observe reactivation of hepatitis B. As per our institute protocol, prophylaxis for HBV was given to subjects with either positive hepatitis B surface antigens or hepatitis B surface antibody (anti-HBs) titre <100 mIU/ml, after transplantation with anti-HBc-positive donor grafts. Recipients with anti-HBs titre >100 mIU/mL were exempted from prophylaxis and kept on regular monitoring for HBV markers. **Results:** Of 85 liver transplant recipients, 20 subjects who received anti-HBc-positive grafts were included in analysis. The mean age of the study population was 46 years (range 2–68 years). The most common aetiology of cirrhosis in our study population was cryptogenic followed by ethanol. Among the study population, 16 (80%) transplant recipients had anti-HBs titre less than 100 mu/ml and 4 (20%) subjects had anti-HBs > 100 mIU/ml. HBV reactivation occurred in 6 (30%) subjects. Reactivation was seen even in those who received HBV prophylaxis, while none of the subjects with anti-HBs titre >100 mIU/ml developed HBV reactivation despite absence of prophylaxis. **Conclusion:** HBV reactivation can occur even in the presence of target anti-HBs titre (i.e. >10 mIU/ml) and HBV prophylaxis during postliver transplantation. However, HBV reactivation is not seen in recipients with anti-HBs titre of >100 mIU/ml. (J CLIN EXP HEPATOL 2020;10:548–554)

Chronic hepatitis B virus (HBV) infection is a major global health problem. According to an estimate, in 2010, about 248 million individuals globally were infected with HBV, approximately 15–40% will develop life-threatening liver manifestations and resulting in 600,000 to 1.2 million deaths per year.¹ Neither seroconversion of hepatitis B surface antigens (HBsAg) in patients with chronic hepatitis B nor recovery from a self-limiting acute hepatitis B guarantees protection from reactivation. Occult hepatitis B infection (OBI) is a state of undetectable

serum HBsAg and detectable serum and/or intrahepatic HBV DNA. Its prevalence in cryptogenic cirrhosis or advanced liver fibrosis ranges from 4 to 38%.^{2–4} A potentially life-threatening condition would be hepatitis B reactivation in patients during immunosuppression therapy, especially in the setting of post-transplant period, which may lead to graft failure and death.⁵

Occult transmission of hepatitis B from HBsAg-negative and hepatitis B core antibody (anti-HBc)-positive liver donors is possible, especially when the recipient is negative for all markers of HBV infection.⁶ De novo HBV infection is around 78% in liver transplant recipients from anti-HBc-positive donors, whereas it is only 0.5% in recipients from anti-HBc-negative donors.⁷ To prevent OBI in organ transplant recipients, screening of serum HBV DNA in both core positive donors and organ transplant recipients along with other parameters is required.⁸ Organ shortages in liver transplantation have led to increase in usage of marginal donors, including steatosis donors and donors with positive serologies for viral hepatitis. The identification of anti-HBc on donor serologies was once considered as a contraindication for transplant in patients without prior exposure to HBV.^{9,10} About two decades ago, studies from four transplant centres reported that donation

Keywords: anti-HBc positive grafts, HBV reactivation, HBV prophylaxis, posttransplant immunosuppression

Received: 18.2.2020; **Accepted:** 1.5.2020; **Available online** 8 May 2020

Address for correspondence: Sunil Raviraj K, Senior Resident, Department of Gastroenterology, KIMS hospital, Postal code/ P.O. Box: Anayara PO., Trivandrum, Kerala, 695029, India.

E-mail: ksrdnb@gmail.com

Abbreviations: Anti-HBc: Hepatitis B Core Antibody; Anti-HBs: Hepatitis B Surface Antibody; ALT: Alanine Transferase; DDLT: Death Donor Liver Transplant; EMR: Electronic Medical Record; HBV: Hepatitis B Virus; HBsAg: Hepatitis B Surface Antigen; LDLT: Live Donor Liver Transplant; OBI: Occult Hepatitis B Infection

<https://doi.org/10.1016/j.jceh.2020.05.001>

from anti-HBc-positive donors resulted in hepatitis B transmission in almost 80% of liver recipients.⁷ This might be due to the fact that this study was conducted before routine prophylaxis with lamivudine or Hepatitis B immunoglobulin (HBIG). Nowadays, numerous strategies are used to prevent HBV transmission, including passive immunization with HBIG and antiviral therapies.

Still it remains controversial whether the graft from HBsAg-negative and anti-HBc-positive donors in the regions where HBV is highly prevalent should be denied, as it would substantially compromise the availability of suitable organ donors.¹¹

AIM

The aim of the study was to study the reactivation of HBV in liver transplant recipients with anti-HBc-positive donors.

METHODS

We conducted a retrospective study at our tertiary care centre. The electronic medical record (EMR) facilitates the retrieval of clinical and laboratory data of all patients and provides good quality information to support retrospective clinical and management decisions. The study protocol was approved by the ethics committee of our institute. The requirement for informed consent was waived in view of the retrospective nature of this study.

Study Population

Among all individuals who underwent liver transplantation at our institute between January 2013 and December 2017, those who received grafts from HBsAg-negative and anti-HBc-positive donors were included in our study. Both live and deceased donors were considered for the study.

Data Collection

Data were retrieved from the well-equipped EMR system of the institute. Demographic data including gender, age and type of donors were captured. Details regarding aetiology of liver disease, recipient's hepatitis B surface antibody (anti-HBs) titre, anti-HBc status and duration of steroid therapy and details of immunosuppressants and HBV reactivation were studied. The standard immunosuppression protocol after liver transplantation in our centre consisted of tacrolimus, mycophenolate and corticosteroids. Within the first 3–6 months after transplant, corticosteroids and mycophenolate mofetil were tapered, and most patients were on tacrolimus monotherapy by the end of the first year.

DEFINITIONS

HBV Reactivation¹²

HBV reactivation is defined as follows:

- Elevated alanine transferase and HBV DNA >2000 IU/mL in a person known to have the inactive HBsAg carrier state or resolved hepatitis (or)
- Seroreversion from HBsAg negative to HBsAg positive (or)
- HBV DNA level of 10-fold or more from the baseline level or reappearance of HBV DNA in the serum

Hepatitis was defined and graded as per levels of alanine transferase, bilirubin and prothrombin time.

PROTOCOL FOR HBV PROPHYLAXIS

As per our institute protocol, nucleos(t)ide analogues (300 mg of tenofovir or 0.5 mg of entecavir) were administered to subjects with positive HBsAg or anti-HBs titre <100 miu/ml in recipients with anti-HBc-positive donor grafts. Preferably all qualified subjects with no contraindications were initiated on tenofovir, and renal parameters were monitored regularly. In subjects with contraindications for tenofovir and those who developed complications, it was replaced with entecavir. Hepatitis B immunoglobulin (HBIG 10000 IU) was administered intraoperatively during anhepatic phase in high-risk subjects (i.e. recipients with positive anti-HBc with detectable HBV DNA or positive HBsAg serology). Recipients with anti-HBs titre >100 miu/mL were exempted from prophylaxis and kept on regular monitoring for HBV markers. HBsAg, anti-HBs titre, quantitative HBV DNA and liver function tests were checked once in two weeks for one month, then once in a month for 3 months, then every 3 months for one year followed by once in 6 months for all subjects. Adherence for antiviral prophylaxis was assured.

STATISTICAL ANALYSIS

Numerical data were expressed as mean $\pm$ standard deviation and categorical data as frequencies. In univariate analysis, categorical variables were tested using the chi-square and Fisher's exact test. Data analysis was performed using R software [R version 3.6.2 (2019-12-12)]. All statistical tests were two sided. A *P* value < 0.05 was considered as statistically significant. Graphical representation was performed using Microsoft Excel.

RESULTS

The mean age of the study population (*n* = 20) was 46 years (range 2–68 years). Majority of them were men (90%). Thirteen (65%) of them received deceased donor grafts. The

Figure 1 Flow diagram of study.

most common aetiology of cirrhosis was cryptogenic followed by ethanol. The anti-HBs titre was less than 100 miu/ml in 16 (80%) subjects, despite receiving an accelerated dose of hepatitis B vaccine. Only four subjects had anti-HBs titre >100 mu/ml. Of 20 recipients, 8 of them were anti-HBc positive, and all were negative for HBV DNA at the time of liver transplant. One patient had positive HBsAg serology but was negative for HBV DNA at the time of transplant. Living donors who were anti-HBc positive underwent testing for HBV DNA, and all were negative. Deceased donors with positive anti-HBc were not screened for HBV DNA before transplant. Among the study population, recipients with anti-HBs titre less than 100 miu/ml were given HBV prophylaxis ($n = 15$). One child aged 2 years with anti-HBs titre 36 miu/ml was not given antiviral prophylaxis (Figure 1). Subjects who were started on tenofovir disoproxil fumarate had tolerated it well, and none of them required either entecavir or tenofovir alafenamide.

HBV reactivation was seen in 6 (30%) recipients (Table 1). It occurred in subjects with anti-HBc-positive ($n = 3/$

8; 37.5%) and negative ($n = 3/12$; 25%) recipients. Reactivation occurred despite HBV prophylaxis, but only one of them had acute hepatitis (rise in transaminases). Only one of those subjects with reactivation did not receive HBV antiviral prophylaxis, as he was 2 years old. Among six cases of HBV reactivation, only two of them had detectable HBV DNA. Later, all were continued on tenofovir and kept on monitoring. No mortality happened due to reactivation.

One recipient was HBsAg positive but HBV DNA negative at the time of transplant. His anti-HBs titre was 3 miu/ml before transplant surgery. He was given both intraoperative HBIG and postoperative tenofovir for prophylaxis. His HBsAg became negative within 2 months after transplant and remained negative, while continuing tenofovir for almost 18 months after seroconversion. However, over a period of 2 months after stopping antivirals, he developed HBsAg seroreversion (HBsAg became positive). His anti-HBs titre was 3 miu/ml before transplant and 135 miu/ml at the time of stopping antivirals. He was restarted on tenofovir.

Table 1 The Characteristics of Recipients With HBV Reactivation in Our Study.

S. No.	Characteristics							
	Age (years)	Sex	DDLT/LDLT	Anti-HBc	Anti-HBs (miu/ml)	HBV prophylaxis	Duration of steroid (months)	Time of HBV reactivation (months)
1	56	Male	DDLT	Negative	5	Yes	60	20
2	48	Male	DDLT	Positive	5	Yes	4	7
3	52	Male	DDLT	Positive	3	Yes	3	8
4	42	Male	LDLT	Positive	3	Yes	5	20
5	66	Male	DDLT	Negative	5	Yes	4	17
6	2	Male	LDLT	Negative	36	No	5	9

DDLT, death donor liver transplant; LDLT, live donor liver transplant; HBV, hepatitis B virus; anti-HBs, hepatitis B surface antibody; anti-HBc, hepatitis B core antibody.

Risk factors for reactivation were analysed. Effect of age, sex, type of transplant (live versus deceased donor), recipient anti-HB core status and duration of steroid was not significant (Table 2). Both live and deceased donor grafts had similar risk for hepatitis B reactivation ($P = .092$). No significant difference was noted either in duration of steroid use or combination of immunosuppressants between subjects with and without HBV reactivation ($P = 0.34$; Figure 2). Effect of aetiology of liver disease was also not significant on reactivation of HBV ($P = 0.75$; Figure 3).

DISCUSSION

In our study, we found that HBV reactivation occurred in about 30% ($n = 6$) of patients who received hepatitis B core-

positive grafts, despite HBV prophylaxis. Three of six recipients who developed HBV reactivation were anti-HBc negative and other 3 were positive. Earlier studies reported that HBV-naïve recipients without prophylaxis were at the highest risk (38%–100%) for developing de novo hepatitis B when they receive grafts from anti-HBc-positive donors.¹³ However, in our study, we noticed that 37.5% (3/8) of anti-HBc-positive recipients had HBV reactivation, while only 25% (3/12) had reactivation among anti-HBc-negative recipients.

Majority of the recipients (70%) in our analysis had low anti-HBs titre despite an accelerated dose of HBV vaccination. According to the available evidence, presence of adequate anti-HBs titre in the recipient protects from de novo HBV infection.^{14,15} In our observation, we also noticed that none of the subjects with anti-HBs titre

Table 2 Relation Between HBV Reactivation and Studied Parameters.

Parameters assessed		HBV reactivation				Total (n = 20)		P
		Yes (n = 6)		No (n = 14)		n	%	
		n	%	n	%			
Age (years)	≤50	3	30	7	70	10	100	1.00
	>50	3	30	7	70	10	100	
Sex	Male	6	33.3	12	66.7	18	100	0.33
	Female	0	0.0	2	100	2	100	
Type of transplant	DDLT	4	30.8	9	69.2	13	100	0.92
	LDLT	2	28.6	5	71.4	7	100	
Recipient anti-HBc status	Positive	3	37.5	5	62.5	8	100	0.55
	Negative	3	25	9	75	12	100	
HBV prophylaxis	Yes	5	33.3	10	66.7	15	100	0.57
	No	1	20	4	80	5	100	
Anti-HBs titre miu/ml	<100	6	37.5	10	62.5	16	100	0.94
	>100	0	0.0	4	100	4	100	
Steroid use (months)	<5 months	4	33.3	8	66.7	12	100	0.69
	≥5 months	2	25	6	75	8	100	

HBV, hepatitis B virus; DDLT, death donor liver transplant; LDLT, live donor liver transplant; anti-HBs, hepatitis B surface antibody; anti-HBc, hepatitis B core antibody.

Figure 2 Effect of various combinations of immunosuppressants on hepatitis B reactivation in liver transplant recipients with anti-HB core positive grafts ($P = 0.34$).

>100 miu/ml ($n = 4$) developed reactivation, irrespective of the recipient's anti-HBc status. None of them were given prophylaxis. However, literature recommends HBV prophylaxis to recipients with isolated anti-HBs or anti-HBc positive and whoever receives grafts from anti-HBc-positive donors, while not recommending prophylaxis in subjects with both markers positive.¹⁴ The administration of HBIG has been shown to be protective in HBV transmis-

sion, but it has several limitations including low levels of compliance and high costs.¹⁶

As per the recommendations, patients with positive HBsAg before transplant need indefinite antivirals after transplant surgery, but consensus is lacking in taking decision for patients who developed HBsAg seroconversion.⁶ In our study, one patient who had withdrawn prophylaxis (tenofovir) 18 months after HBsAg seroconversion with

Figure 3 Effect of aetiology of cirrhosis on HBV reactivation in liver transplant recipients with anti-HB core positive grafts ($P = 0.75$). HBV, hepatitis B virus.

adequate anti-HBs (135 miu/ml) titre during the post-transplant period developed reactivation of HBV infection within 2 months. This highlights the importance of life-long HBV treatment in HBsAg-positive recipients irrespective of seroconversion. HBV prophylaxis may reduce the rate of HBV reactivation but cannot completely prevent it. Among our study group, five of six patients had HBV reactivation despite continuing on antiviral prophylaxis Nucleotide Analogues (NAs) started from immediate post-operative period. Time taken for the reactivation was 7–20 months after transplant. This could be due to development of drug resistant mutants to NAs which carries a potential risk of prophylaxis failure.¹⁷

A recent American Gastroenterology Association technical review stated that prolonged courses (longer than 4 weeks) of moderate-to high-dose corticosteroids for >4 weeks may lead to HBV reactivation in 1%–10% of patients, whereas either short courses (short than 1 week) of high-dose corticosteroids or prolonged courses of low-dose corticosteroids only lead to reactivation in less than 1%.¹⁸ We could not demonstrate statistically significant relation between age, sex, aetiology of cirrhosis, type of donor, immunosuppressants, duration of steroid, recipient's anti-HBc status and anti-HBs titre and HBV reactivation. This could be due to the small sample size.

LIMITATIONS

It was a retrospective study, so incidence of hepatitis B reactivation could not be analysed. This study was from a single transplant centre, and hence, the number of patients included was relatively small. It is difficult to demonstrate either statistically or clinically significant differences between subgroups of anti-HBc-positive organ recipients due to the small sample size. Immunohistochemistry for HBV was not performed in explants with positive anti-HBc serology, which might help in predicting the risk of reactivation. Multicentric prospective studies may help to draw more conclusive results in this regard.

Most cirrhotics develop poor anti-HBs titre after vaccination. HBV reactivation can occur even in the presence of recommended target anti-HBs titre (i.e. >10 miu/ml) and HBV prophylaxis during postliver transplantation. No recipients with anti-HBs titre >100 miu/ml developed HBV reactivation. Hence, in this scenario, further large-scale trials are needed to define the protective level of anti-HBs titre. HBsAg-positive recipients may require life-long HBV prophylaxis after transplantation, irrespective of HBsAg clearance or anti-HBs titre level.

FUNDING

The authors received no funds from any organization.

CONFLICTS OF INTEREST

All authors have none to declare.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Benjamine Khiangte: Data curation, Software. **Sunil R. Kothakota:** Data curation, Formal analysis, Investigation, Visualization, Writing - original draft. **Madhu Sasidharan:** Conceptualization, Methodology, Validation, Writing - review & editing. **Harish Kareem:** Supervision. **Ajith K. Nair:** Funding acquisition, Project administration, Resources. **Vijosh V. Kumar:** Software. **Jagadeswara R. Kanala:** Writing - review & editing. **Praveen C. Kumar:** Visualization.

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