



Occult Hepatitis B Reactivation after Liver Transplant: The Role of a Novel Mutation in the Surface Antigen

Harjot K. Bedi¹, Daljeet Chahal^{1,2}, Christopher F. Lowe³, Gordon Ritchie³,
Trana Hussaini⁴, Vladimir Marquez^{1,2} and Eric M. Yoshida^{*1,2}

¹Department of Medicine, University of British Columbia, Vancouver, BC, Canada; ²Division of Gastroenterology, University of British Columbia, Vancouver, BC, Canada; ³Department of Pathology & Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; ⁴Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada

Abstract

Occult hepatitis B infection is characterized by loss of hepatitis B surface antigen (HBsAg) and persistence of low levels of hepatitis B virus (HBV) replication that may or may not be detectable in plasma/serum. We present a case of HBV reactivation in a male patient who underwent orthotopic liver transplant for hepatocellular carcinoma secondary to active hepatitis C (HCV) infection. Pre-transplant, he was HBsAg-negative and hepatitis B core antibody-positive, with an undetectable HBV viral load that was incidentally found to be positive at a very low HBV viral load on the day of transplant. Post-transplant, his HBsAg remained undetectable, with an undetectable HBV viral load, until eradication of his HCV infection with direct acting antiviral agents. After eradication of HCV, there was reactivation of HBV, with a high viral load and emergence of serum HBsAg. A deep sequencing genetic analysis of his HBV both pre- and post-transplant revealed the presence of a mutation in the "a" determinant of the HBV surface antigen. The role of HBV genotype 'a' determinant mutation in HBV reactivation post-transplant is unknown and needs further examination. Our experience suggests a possible role for antiviral prophylaxis in these patients or monitoring of HBV viral loads post-transplant.

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Introduction

Hepatitis B virus (HBV) is a significant public health concern. Clinical manifestations depend on patients' age and

immune status. Less than 5% of adult patients with acute horizontally-transmitted HBV develop chronic hepatitis, which is characterized by a persistent hepatitis B surface antigen (HBsAg) that indicates active infection. However, 90–95% of those who are infected at birth via vertical transmission will become chronic carriers.¹ Despite the ability of patients to clear HBsAg (*i.e.* revert to HBsAg seronegativity), HBV genomes can persist in liver tissue.² This state is known as occult hepatitis B infection (OHBI) and is characterized by loss of HBsAg and persistence of a very low level of HBV DNA, that may or may not be detectable in plasma/serum.³

OHBI may be the consequence of mutations that occur during viral replication. HBV replicates via an error-prone reverse transcriptase.⁴ Accumulation of mutations during this process results in a large pool of genetically distinct variants termed "quasispecies". The 'a' determinant is a region of the HBsAg surface protein where most antigenic epitopes are located, and is the antigenic target of commercial hepatitis B vaccines.⁵ Mutations in this region can result in immune and vaccine escape variants of HBV. These variants can remain dormant not only in liver tissue but also in extrahepatic tissue, such as bone marrow and lymphatic tissues.⁶ These variants may not be detectable by routine immunoassays available in clinical diagnostic laboratories, necessitating advanced analysis, such as DNA sequencing.⁷

In addition to viral mutations, immune-biology and co-infections appear to facilitate OHBI.³ Up to 15% of patients with hepatitis C virus (HCV) may be co-infected with HBV.⁸ HCV often predominates, suppressing HBV replication and transcription.⁸ Several cases have demonstrated that clearance of HCV with direct acting antivirals allows HBV to resume replication and become HBsAg reactive.^{9,10} Other factors that appear to increase the risk of HBV reactivation include immune-suppressive medications.¹¹ Various interventional therapies for hepatocellular carcinoma (HCC), including surgical resection and locoregional modalities, may also increase risk of reactivation.¹²

Liver transplantation necessitates the use of post-operative immune-suppressive medications (*i.e.* "anti-rejection drugs"). Even in the absence of prophylaxis, the risk of seroconversion to HBsAg positivity after transplantation, in pre-transplant HBsAg-negative, hepatitis core antibody (anti-HBc)-positive recipients is thought to be rare, as long as the allograft is from an HBV naïve donor. A French study reported an estimated risk of 1.5%,¹³ whereas in retrospective studies, both British and American centers have reported no HBV reactivations in their patients.^{14,15} There are no guideline recommendations that discuss HBV prophylaxis before or after liver transplantation in this specific group of patients. With these points in mind, we present a case of HBV reactivation after liver transplantation for

Keywords: Hepatitis; Hepatitis B; Reactivation; Seroconversion; Liver transplant; Transplant.

Abbreviations: CASL, Canadian Association for the Study of the Liver; DNA, deoxyribonucleic acid; HBsAg, hepatitis B surface antigen; HBV, hepatitis B Virus; HCC, hepatocellular carcinoma; HCV, hepatitis C Virus; OHBI, occult hepatitis B infection; OLT, orthotopic liver transplant; RFA, radiofrequency ablation; TACE, transarterial chemoembolization.

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*Correspondence to: Eric M. Yoshida, Vancouver General Hospital, University of British Columbia, 5th Floor – 2775 Laurel Street, Vancouver, BC, Canada. Tel: +1-604-875-5244, Fax: +1-604-875-5447, E-mail: eric.yoshida@vch.ca

HCC secondary to HCV cirrhosis in a pre-transplant HBsAg-negative, anti-HBc antibody-positive patient after post-transplant HCV therapy. Genotypic analysis subsequently identified a mutation in the 'a' determinant within the surface antigen.

Case report

A Caucasian 60-year old male presented for orthotopic liver transplant (OLT) workup after diagnosis of HCC secondary to HCV infection. His comorbidities included Crohn's disease, for which he received monthly infusions of infliximab. He had been diagnosed with HCV infection on routine screening and developed cirrhosis 15 years after diagnosis. He carried HCV genotype 3a, with RNA level of 459,195 IU/mL. At that time, HBsAg and hepatitis B surface antibody were non-reactive. His anti-HBc was positive but HBV DNA level was undetectable (<20 IU/mL) (COBAS® Ampliprep/COBAS® Taqman® HBV v.2.0; Roche Diagnostics). He was, therefore, thought to have past HBV infection that had cleared.

Six years after developing cirrhosis, he was diagnosed with HCC and serial loco-regional therapies including transarterial chemoembolization (commonly known as TACE) and radiofrequency ablation (commonly known as RFA) were performed. Despite this, residual HCC persisted, and he received an OLT without complications. His immunosuppressants included tacrolimus, mycophenolate mofetil, and prednisone. Infliximab was held-off for the month after transplant and resumed after that. Crohn's disease remained in remission.

The patient did not receive antiviral treatment for HCV prior to the transplant. A few days post-transplant, his HCV RNA level was found to be 3,617 IU/mL. His plasma HBV DNA on the hospital admission day immediately pre-transplant was detectable for the first time at 645 IU/mL (Cobas® HBV; Roche Diagnostics) and was considered to be a contaminant, with a negative HBsAg finding. The donor liver was anti-HBc-negative, and a repeat HBV DNA determination post-transplant was undetectable (lower limit of detection, 25 IU/mL); no antiviral treatment was initiated. Two months after transplant, his HCV viral load increased to 2,621,255 IU/mL. Treatment was initiated with sofosbuvir/velpatasir (Epclusa®; Gilead Sciences) after completion of prednisone. A 12-week antiviral treatment with sofosbuvir/velpatasir was completed with achievement of a sustained viral response.

Despite previous undetectable levels of HBV DNA, the HBV DNA levels increased to 14,100,000 IU/mL 6-weeks after initiation of direct acting antiviral therapy for HCV. Additionally, there was seroconversion of HBsAg from negative to positive. Amplicon-based next-generation sequencing was utilized to investigate the presence of HBsAg mutations. Briefly, plasma samples were extracted on the MagNA Pure 24 (Roche) and eluted in 50 µL elution buffer. PCR was performed using 0.2 µM ILF (CGTGGTGGACTTCTCAATTTTC) and ILR (AGAAAGGCCTTGAAGTTGGCGA) using the Kapa HiFi enzyme on the LightCycler® 480 (Roche). A 50 ng aliquot of Agencourt bead purified PCR product was processed by 1D Native barcoding with EXP-NBD114 and SQK-LSK109 (Oxford Nanopore Technologies) and sequenced on flow cell FLO-MIN106D. FAST5 files were basecalled with Guppy V3.6.1. FASTQ files were analyzed and consensus sequence generated using Geneious V10.2.6, with 15,000 read coverage. Codons associated with immune escape were analyzed from codon 42 to the stop codon of the S gene. Genetic sequencing of HBV DNA demonstrated that a mutation in the 'a' determinant of the surface antigen, F/Y134H, was present in both pre-transplant and post-transplant plasma.

The patient currently remains HBV viral load undetectable, HBsAg detectable, with stable allograft function, while on tenofovir antiviral therapy.

Discussion

We have presented a case of HBV reactivation after liver transplantation and subsequent HCV antiviral therapy. Undetectable HBV DNA, negativity for HBsAg, and positivity for anti-HBc pre-transplant in addition to reactivation post-transplant suggests that this patient had OHBI. DNA sequencing performed on samples acquired before and after the transplant demonstrated the presence of mutations in the 'a' determinant of HBsAg, further supporting this hypothesis. This patient did not reactivate pre-transplant, despite treatment for HCC and regular infliximab therapy. He also did not suffer sustained reactivation, aside from a transient virologic breakthrough pre-transplant until eradication of his HCV infection when his HBsAg appeared for the first time with a sustained high HBV viral load. The appearance of HBsAg for the first time probably reflects the virologic heterogeneity of HBV within a given patient: the wild-type virus producing surface antigen was most likely silent prior to reactivation, but reactivation with significant viremia allowed it to re-emerge enough for surface antigen to become detectable.

In the absence of prophylaxis, the risk of seroconversion to HBsAg positivity in naïve liver donors or isolated anti-HBc positive recipients is reported to be very rare.¹³⁻¹⁵ Studies of positive anti-HBc, HBsAg-negative patients receiving kidney transplants, which require higher level of immunosuppression, also appear to have less than a 1% risk of post-operative reactivation.¹⁶ These studies collectively appear to conclude that there is a low risk of post-transplant reactivation. The liver itself is the largest reservoir of HBV DNA, and its removal during transplantation may be a simple explanation for the low risk of post-liver transplant reactivation. However, it should be noted that these studies did not examine for 'a' determinant mutation. Although unknown at the current time, the presence of such mutations may confer a higher risk of reactivation.

Despite removal of the HBV reservoir in the liver, extrahepatic sources of HBV DNA, such as bone marrow or lymphatic tissue, may have been the source for HBV reactivation. In this case, the precipitating factors for HBV reactivation were multifactorial. Co-infection with, and therapy for, HCV likely contributed to post-transplant HBV reactivation in our patient. HCV usually predominates over HBV in co-infected patients and eradication of HCV can result in HBV reactivation.⁸ In a systematic review, Mucke *et al.*¹⁷ demonstrated that 24% of patients with positivity for HBsAg experienced HBV reactivation during direct antiviral therapy for HCV, compared to only 1.4% of patients who were HBsAg-negative. Local therapy for HCC, history of Crohn's disease maintained on infliximab, corticosteroids, and solid organ transplantation are considered to represent moderate to higher risk. Lazarevic *et al.*¹⁸ found that beside immunosuppressive factors (*i.e.* transplantation, corticosteroid use, direct antiviral therapy for HCC, and age-related immunosuppression), mutation in the 'a' determinant of HBsAg was essential for HBV reactivation in those receiving anti-CD20 monoclonal antibodies (*e.g.* rituximab). Therefore, the 'a' determinant mutation plays an important role in HBV reactivation and needs further investigation.

Multiple guidelines distinguish between chronic HBV infection and resolved HBV infection when making recommendations regarding prophylactic therapy.¹⁹ Guidelines by the Canadian Association for the Study of the Liver (commonly

referred to as the CASL) advocate for clinical monitoring without antiviral treatment in patients with normal alanine aminotransferase and HBV DNA levels <2,000 IU/mL.¹ Although patients with anti-HBc positivity are considered to be at higher risk of HBV reactivation, such categorizations are based on studies of non-transplant patients with their native liver. There are no guidelines that comment on post-transplant prophylaxis when the donor graft is HBV naïve. There is also a paucity of studies that examine the clinical impact of 'a' determinant mutation and how it may affect reactivation.²⁰

Pre- and post-transplant screening with HBV DNA from serum/plasma can be utilized in the assessment of OHBI. Although OHBI can be diagnosed through HBV DNA detection in liver biopsies, validated assays are not routinely available to be incorporated into standard pre-transplant OHBI assessments.²⁰ Our case highlights the need to consider possible post-transplant prophylaxis in OHBI, but certainly post-transplant monitoring with nucleic acid testing, particularly in the context of multiple risk factors for reactivation. The identification of a surface antigen mutant may have contributed to reactivation in this case. Further studies are required to determine the relative risk of reactivation for 'a' determinant mutations post-OLT.

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Conflict of interest

Trana Hussaini is recipient of unrestricted research grants from Paladin Inc. Eric Yoshida is an investigator of clinical trials sponsored by Gilead Sciences, AbbVie, Merck, Intercept, Madrigal, Pfizer, Novartis, Allegan and Genfit, and is recipient of unrestricted research grants from Paladin Inc. and honoraria for CME/Ad Board lectures from Gilead Canada, Abbvie Canada, Merck Canada, Intercept Canada and Celgene Canada. The other authors have no conflict of interests related to this publication.

Author contributions

Literature review and drafting of the manuscript (HKB, DC), editing of the manuscript (DC, TH, EMY, CFL, and GR), provision of resources (TH, EMY, CFL, GR), laboratory investigations (CFL, GR), review for important intellectual content (DC, EMY, CFL, GR).

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