Case Report

Long-term survival of occult hepatitis B associated hepatocellular carcinoma following surgery and antiviral therapy

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Abstract

Occult hepatitis B infection (OBI) is characterized by absent hepatitis B surface antigen (HBsAg), low or undetectable serum hepatitis B viral DNA (HBV-DNA), and detectable DNA in the liver. There is debate over whether OBI increases the risk of hepatocellular carcinoma (HCC). We present a patient with negative HBsAg and a large HCC tumor who underwent a large right hepatic lobectomy. Initially, the etiology of HCC was unknown, but through more sensitive molecular testing, it was believed to be due to OBI. In this case report, we discuss the patient’s clinical course, the effect of antiviral therapy, mechanism of carcinogenesis in OBI, and the need for more rigorous HBV DNA assay testing for the detection of OBI.

Keywords: Occult hepatitis B infection, OBI-associated HCC, HBsAg negative HCC

INTRODUCTION

Hepatitis B virus (HBV) has caused over 50 percent of hepatocellular carcinoma (HCC) cases worldwide. The prognosis for HCC is generally poor especially when patients present with multifocal disease. Radical liver resection is usually ineffective as new tumors can present in the remnant liver. High levels of HBV-DNA are believed to increase the risk for HCC and sensitive molecular testing has identified OBI as a risk factor in
the progression of cancer. OBI has been recognized as a possible phase of chronic hepatitis B infection and is characterized by absent HBsAg, low or undetectable serum HBV-DNA, and detectable DNA in the liver\textsuperscript{2}. While the clinical significance of OBI remains unknown, the frequency varies across populations, and with the specificity and sensitivity of routine laboratory assays. The prevalence is as high as 41%-90% in those with prior HBV exposure in high-prevalence areas, and 5%-20% in low-prevalence areas\textsuperscript{3,4}. Except for cases of replication-defective variants or S escape mutants that produce undetectable modified HBsAg, most OBI are capable of replicating but are suppressed in their activity by host defense mechanisms\textsuperscript{5}.

We present the case of a patient with negative HBsAg and a large, 10 cm hepatocellular carcinoma who underwent a right hepatic lobectomy. The patient subsequently required lung resection for metastasis 4 years later. After tumor recurrence, additional testing revealed the presence of OBI. The patient was started on anti-HBV therapy and has remained disease free for the past 16 years. The patient’s earlier course was published previously\textsuperscript{6}. This is a follow up of the patient’s 16-year course to date. In this paper, we describe the patient’s initial hepatic surgical resection, the subsequent lung resection complicated by postoperative infection, and the patient’s long-term management, functional outcome, and survival.

**CASE REPORT**

A 64-year-old woman presented with right shoulder pain for one month and was found to have a 9 cm mass on magnetic resonance imaging (MRI) scan in the right lobe of the liver [Figure 1]. Physical exam revealed a hard, non-tender mass in the right upper quadrant (RUQ) extending to the pelvis. Ultrasound-guided core biopsy of the mass was compatible with HCC. Family history was negative for known HBV or liver cancer. Her mother died from injuries sustained during a bomb explosion when the patient was 14. Her father died of pulmonary disease. Her three younger siblings (60F, 56F, 52M) were well without liver disease. The patient has three children. Her 43-year-old daughter was found to be HBsAg positive whilst her 2nd daughter, aged 37-year-old, was negative for HBsAg but positive for anti-HBc total, suggestive of past exposure. Data was unavailable on her youngest daughter. The patient has a history of depressive disorder and has been on Prozac for the past 12 years.

\*Figure 1. Contrast-enhanced magnetic resonance imaging showing a large liver mass. The axial T2-weighted fat-suppressed image (A) shows a large hyperintense mass replacing most of the right hepatic lobe. The corresponding T1-weighted fat-suppressed pre-contrast (B), arterial phase post-contrast (C) and delayed post-contrast (D) images demonstrate hypointensity with patchy arterial hyperenhancement and washout with capsule appearance of the periphery and central necrosis (asterisk)
On initial presentation, the patient was not in acute distress. Blood results revealed an AFP of 7,981 ng/mL, HBsAg (-), Anti-HBs (+), Anti-HBC total (+), Anti-HCV (-), HBV DNA (-), serum albumin 4.0 g/dL, total bilirubin 0.7 mg/dL, ALT 17, AST 95, alkaline phosphatase 98 U/L, WBC 5.4 K/µL, platelets 229 K/µL, serum creatinine 0.7 mg/dL and normal coagulation studies. HBsAg was determined with a quantitative HBsAg assay (AxSYM, Abbott Laboratories, IL, USA).

The patient was evaluated for potential curative resection. computed tomography (CT) and MRI staging studies did not reveal intrahepatic or distant metastatic disease. No regional adenopathy was identified. She was assessed to be medically fit for resection and her calculated remnant liver volumes were acceptable. In the operating room, a staging laparoscopy revealed no evidence of peritoneal metastases. There was no evidence of macro-nodular cirrhosis or portal hypertension. Intraoperative ultrasound of the liver confirmed a single large right hepatic lobe HCC without evidence of satellite lesions or additional tumors. Through abdominal exploration, there was suspicion of invasion of the right diaphragm at the bare area of the liver. A portion of the right diaphragm was resected with the right hepatic lobe to achieve grossly clean margins and the diaphragm was repaired primarily. The patient had an uneventful recovery and was discharged home. Final pathology revealed moderately differentiated HCC.

Follow up AFP decreased to 2.4 mg/mL at 4 months post-surgery. Four years later, the patient’s AFP increased to 25.5 ng/mL and peaked at 79.8 ng/mL 3 months later. Abdominal MRI showed a 3.2 cm mass behind the heart [Figure 2]. Chest CT confirmed a mass behind the right pulmonary vein and she underwent video-assisted thoracoscopic surgery (VATS) to remove the right lower lobe lung mass. Histologically, the lung mass was confirmed to be HCC. Her postoperative course was complicated by continued pleural effusions and empyema for which she underwent a right lower lobectomy and decortication via VATS [Figure 3A-C].

At this juncture, questions of whether the patient’s HCC could be attributed to HBV infection were raised. While she had remained HBV DNA negative by the commercial assay, her daughters’ positive HBV markers prompted consultation with a laboratory where more sensitive HBV DNA testing had been developed [6,7]. The analytical sensitivity was 15-20 copies/mL, while at the time of assay development, the sensitivity of the Roche Cobas HBV DNA assay was at ~150 copies/mL. Since HBV DNA tends to mutate, it is possible that our assay detected a HBV strain that the commercial assay could not due to rare mutation(s).

HCC tissue from the original liver tumor, the lung tumor, and the serum specimens collected both at the time of her HCC diagnosis and at the time of her lung metastasis were sent to the laboratory. DNA was extracted from formalin-fixed liver or lung tissue blocks using the DNeasy Blood & Tissue Kit from Qiagen. The extracted DNA was then subject to real time PCR[7].

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**Figure 2.** Magnetic resonance imagingand computed tomography (CT) showing a right lower lobe mass. The axial T1-weighted fat-suppressed postcontrast (A) and enhanced CT (B) images show an enhancing mass in the right lower lobe (arrow)
The liver tumor was positive for HBV DNA while the metastatic lung mass was negative for HBV DNA. Serum samples from the time of her diagnosis of HCC and subsequent lung metastasis (both negative by commercial assay) showed HBV DNA levels of 3,271 copies/mL and 52 copies/mL respectively.

Based on these findings, the patient was started on lamivudine 150 mg daily, 1 year after lung metastasis resection. At that time, her HBV profile by commercial assay showed HBsAg (-), Anti-HBs (+), Anti-HBc total (+), anti-HAV (+), and AFP 2.8 ng/mL. Seven years after lamivudine therapy, her anti-HBc total became negative, which was suggestive of possible decrease or elimination of the HBV covalently closed circular DNA (cccDNA) in her liver.

For the past 12 years, after resection of lung metastasis and antiviral therapy, she has had no evidence of recurrence of the HCC and has maintained undetectable HBV DNA levels. Imaging shows that her left hepatic lobe has hypertrophied [Figure 3D].

DISCUSSION

Well-established risk factors for the development of HCC in patients with chronic HBV infection are viral load and the presence of HBeAg and HBsAg[8-10]. However, studies have demonstrated a high rate of OBI in patients with HCC who are immunocompromised during chemotherapy for malignancy[11,12], as well as in patients with hepatitis C[13]. The 38%-73% of patients from endemic areas with cryptogenic HCC actually have underlying OBI[13-15]. Despite such evidence, the direct correlation between OBI and carcinogenesis remains controversial. While some studies have linked OBI to hepatocellular carcinoma[16,17], other studies have failed to show direct causality[15,18].

Occult HBV can persist in hepatocytes as both integrated DNA or as a free episome known as covalently closed circular DNA (cccDNA), while maintaining transcription activity and synthesizing proteins at low levels[13]. HBV can promote carcinogenesis through the integration of HBV sequences into the host genome, as well as through mild continuous micro-inflammation, contributing to chronic liver disease and cirrhosis[19].
With our patient, the etiology of the HCC was unclear given her negative HBsAg and undetectable serum viral load. Suspicion for HBV as the possible cause for her HCC was prompted by HBV positivity in her daughters. It is likely that she was infected with HBV during her reproductive period and vertically transmitted the virus to her daughters. Additionally, the presence of HBV DNA in the liver tumor and the more sensitive HBV serum DNA assay suggested a case of OBI. Indeed, the failure of commercial HBV assays to detect HBV DNA has been reported, particularly in patients harboring treatment-resistant mutations\[^20\]. This highlights the need for a more rigorous HBV DNA assay test and reinforces the need to target at least three different locations in the HBV genome\[^2\]. Furthermore, HBV DNA assays used in diagnosing OBI should be able to distinguish between the detection of integrated HBV DNA and replication competent HBV DNA, which encompasses both cccDNA and/or relaxed circular DNA (rcDNA), the direct product of transcriptionally active cccDNA.

The presence of integrated HBV in OBI-associated HCC has recently been reported at a high frequency (76\%)\[^21\] and in cccDNA-negative patients (88\%)\[^22\]. The presence of integrated HBV DNA in OBI-HCC can further complicate disease management and antiviral regimens. However, its detection can play a critical role in patients’ HCC management as new HBV-directed T cell immunotherapies emerge. Recently, the potential application of HBV-specific T cells in targeting HBV antigens derived from integrated HBV DNA has been shown to have antiviral and anti-tumor effects\[^23\].

In this report, we present a rare case of a patient with OBI with HCC who survived multiple resections, including resection of a pulmonary metastasis, and had no recurrence of disease or tumor burden after beginning antiviral therapy. This case is interesting for many reasons and offers several educational points. HBV DNA was negative in the commercial assay suggesting that the patient had reached a “functional cure” in the setting of negative HBsAg and positive anti-HBs. Numerous studies have shown that seroconversion of HBsAg is associated with improved clinical outcomes\[^24,25\]. Unfortunately, the association between her HCC and HBV profile was unclear at presentation. It was not clarified until later in her clinical course when a more sensitive assay found a viral load of 3,271 copies/mL in her serum. Therefore, it is important to realize that the diagnosis of OBI can be challenging given different serological presentations and the limitations of routine assays. Further, it has been reported that spontaneous HBsAg seroconversion does not mean complete elimination of HBV and patients can still have risk of developing HBV associated HCC\[^26\].

Even if the diagnosis of OBI is made, management of such patients can be difficult as there are no guidelines regarding the initiation of antivirals or screening for HCC. Additionally, the prognosis of OBI-associated HCC is unclear and outcome studies are limited. A prior study investigated surgical outcomes in patients with OBI and HCC. They found that patients with OBI were younger at the time of surgery but did not differ in disease free survival or overall survival compared to those with HCC attributed to other carcinogenetic factors such as alcohol abuse, NASH, and diabetes\[^27\]. Regarding the management of OBI related HCC, studies have shown that after the development of HCC, anti-HBV therapy can prevent recurrence or new HCC in the majority of cases\[^28-30\].

In summary, this case suggests a strong role for OBI in HCC development and indicates that OBI can be cured with anti-HBV treatment and complete surgical removal of HBV infected hepatocytes and other cells. Further studies are required to better define the role of OBI in carcinogenesis, and to determine the mechanisms by which it exerts pro-oncogenic activity.

**DECLARATIONS**

**Authors’ contributions**

Made substantial contributions to conception, design of the study and writing of the paper: Boortalary T, Hann HW
Made contributions to surgical aspects of the paper: Rosato E
Made contributions to radiological imaging: Roth C
Made contributions on providing details on HBV DNA assay: Ren XD
Made contributions to concept of OBI-HCC: Lin SY

Availability of data and materials
Not applicable.

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Conflicts of interest
Dr. Hie-Won Hann has received research grants from Gilead Sciences, Assembly Biosciences, Trio-Health and has served on the National Advisory Board of Gilead.

Ethical approval and consent to participate
Not applicable.

Consent for publication
Written informed consent for this study was obtained from all patients.

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REFERENCES
1. Maucort-Boulch D, de Martel C, Franceschi S, Plummer M. Fraction and incidence of liver cancer attributable to hepatitis B and C viruses worldwide. Int J Cancer 2018;142:2471-7.
2. Raimondo G, Locarnini S, Pollicino T, Levrero M, Zoulim F, Lok AS, et al. Update of the statements on biology and clinical impact of occult hepatitis B virus infection. J Hepatol 2019;71:397-408.
3. Conjeevaram HS, Lok AS. Occult hepatitis B virus infection: a hidden menace? Hepatology 2001;34:204-6.
4. Kim YS, Jang JY, Eun SH, Cheon YK, Kim YS, et al. Detection of intrahepatic HBV DNA in hbsag-negative liver diseases. Korean J Hepatol 2006;12:201-8.
5. Pollicino T, Raimondo G. Occult hepatitis B infection. J Hepatol 2014;61:688-9.
6. Wong SY, Ren XD, Hann HW. Development of hepatocellular carcinoma in patients with chronic hepatitis B long after achieving hbsag seroconversion: a need for an improved hepatitis B virus DNA assay. Clin Microbiol 2013;2:127.
7. Loeb KR, Jerome KR, Goddard J, Huang ML, Cent A, et al. High-throughput quantitative analysis of hepatitis B virus DNA in serum using the taqman fluorogenic detection system. Hepatology 2000;32:626-9.
8. Chen CJ, Yang HI, Su J, Jen CL, You SL, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65-73.
9. Chen CF, Lee WC, Yang HI, Chang HC, Jen CL, et al. Changes in serum levels of HBV DNA and alanine aminotransferase determine risk for hepatocellular carcinoma. Gastroenterology 2011;141:1240-8, 1248.e1-2.
10. Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, et al. High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load. Gastroenterology 2012;142:1140-9.e3; quiz e13-4.
11. Pitini V, Rizzo M, Arrigo C, Mondello P, Altavilla G. Fatal hepatocellular carcinoma in a patient with occult hepatitis B virus infection following the administration of R-chop for diffuse large B-cell lymphoma. Case Rep Hematol 2012;2012:803298.
12. Evens AM, Jovanovic BD, Su YC, Raisch DW, Ganger D, et al. Rituximab-associated hepatitis B virus (HBV) reactivation in lymphoproliferative diseases: Meta-analysis and examination of fda safety reports. Ann Oncol 2011;22:1170-80.
13. Wong DK, Huang FY, Lai CL, Poon RT, Seto WK, Fung J, et al. Occult hepatitis B infection and HBV replicative activity in patients with cryptogenic cause of hepatocellular carcinoma. Hepatology 2011;5:4:829-36.
14. Shim CW, Park JW, Kim SH, Kim JS, Kim BH, et al. Noncirrhotic hepatocellular carcinoma: Etiology and occult hepatitis B virus infection in a hepatitis B virus-endemic area. Therap Adv Gastroenterol 2017;10:529-36.
15. Muto J, Sugiyama M, Shirabe K, Mukaide M, Kirikae-Muto I, et al. Frequency and characteristics of occult hepatitis B infection among hepatocellular carcinoma patients in Japan. Ann Hepatol 2018;17:596-603.
16. Pollicino T, Squadrito G, Cerenzia G, Cacciola I, Raffa G, et al. Hepatitis B virus maintains its pro-oncogenic properties in the case of occult HBV infection. Gastroenterology 2004;126:102-10.
17. Squadrito G, Cacciola I, Alibrandi A, Pollicino T, Raimondo G. Impact of occult hepatitis B virus infection on the outcome of chronic hepatitis C. J Hepatol 2013;59:696-700.
18. Lok AS, Everhart JE, Di Bisceglie AM, Kim HY, Hussain M, et al. Occult and previous hepatitis B virus infection are not associated with hepatocellular carcinoma in united states patients with chronic hepatitis C. Hepatology 2011;54:434-42.
19. Torbenson M, Thomas DL. Occult hepatitis B. Lancet Infect Dis 2002;2:479-86.
20. Lindh M, Hannoun C, Malmstrom S, Lindberg J, Norkrans G. Lamivudine resistance of hepatitis B virus masked by coemergence of mutations in probe region of the cobas amplicor assay. J Clin Microbiol 2006;44:2587-9.
21. Saitta C, Tripodi G, Barbera A, Bertuccio A, Smedile A, et al. Hepatitis B virus (HBV) DNA integration in patients with occult HBV infection and hepatocellular carcinoma. Liver Int 2015;35:2311-7.
22. Wong DK, Cheng SCY, Mak LL, To EW, Lo RC, et al. Among patients with undetectable hepatitis B surface antigen and hepatocellular carcinoma, a high proportion has integration of HBV DNA into hepatocyte DNA and no cirrhosis. Clin Gastroenterol Hepatol 2020;18:449-56.
23. Tan AT, Yang N, Lee Krishnamoorthy T, Oei V, Chua A, et al. Use of expression profiles of HBV-DNA integrated into genomes of hepatocellular carcinoma cells to select t cells for immunotherapy. Gastroenterology 2019;156:1862-76.e9.
24. Yip TC, Chan HL, Wong VW, Tse YK, Lam KL, et al. Impact of age and gender on risk of hepatocellular carcinoma after hepatitis B surface antigen seroclearance. J Hepatol 2017;67:902-8.
25. Ahn SH, Park YN, Park JY, Chang HY, Lee JM, et al. Long-term clinical and histological outcomes in patients with spontaneous hepatitis B surface antigen seroclearance. J Hepatol 2005;42:188-94.
26. Yuen MF, Wong DK, Fung J, Ip P, But D, et al. Hbsag seroclearance in chronic hepatitis B in asian patients: Replicative level and risk of hepatocellular carcinoma. Gastroenterology 2008;135:1192-9.
27. Koga H, Kai K, Aishima S, Kawaguchi A, Yamaji K, et al. Occult hepatitis B virus infection and surgical outcomes in non-B, non-C patients with curative resection for hepatocellular carcinoma. World J Hepatol 2017;9:1286-95.
28. Hann HW, Bergin D, Coben R, DiMarino AJ. Prevention of new hepatocellular carcinoma with concomitant antiviral therapy in chronic hepatitis B patients whose initial tumor was successfully ablated. Int J Cancer 2011;128:739-42.
29. Hann HW, Coben R, Brown D, Needleman L, Rosato E, et al. A long-term study of the effects of antiviral therapy on survival of patients with HBV-associated hepatocellular carcinoma (HCC) following local tumor ablation. Cancer Med 2014;3:390-6.
30. Yuan P, Chen P, Qian Y. Evaluation of antiviral therapy performed after curative therapy in patients with HBV-related hepatocellular carcinoma: an updated meta-analysis. Can J Gastroenterol Hepatol 2016;2016:5234969.