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[1]. 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\(^1\). 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\(^3,4\). 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\(^5\).

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\(^6\). 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 imaging and 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. 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. 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%) and in cccDNA-negative patients (88%). 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.

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. 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.

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. 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.

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

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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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