



## CASE REPORT

## Reactivation of Chronic HBV Infection Leading to HCC following Treatment of HCV with an Interferon-Based Regimen

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### Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most significant hepatocarcinogens. Increased risk for the development of Hepatocellular carcinoma (HCC) was observed in patients with dual infection compared to those with either HBV or HCV infection alone [1-3]. In the case of co-infection, the inhibitory effect of HCV against HBV replication has been observed in clinical studies [4-6] and in virological investigation as well [7,8]. Removal of the suppressive effect of HCV in dual infection may activate the then suppressed HBV which could lead to progression of liver disease and ultimately to HCC associated with HBV.

Here we report a unique case of HCC in a patient who has had chronic HBsAg and anti-HBc total positive and HBeAg negative hepatitis B for years without detectable HBV DNA, whose course was complicated by super infection with HCV and development of HCC following successful treatment of HCV with interferon and ribavirin.

### Case Presentation

A 62-year-old Asian man was found to be HBsAg (+) in 1998 at age 45 with undetectable Hepatitis B virus (HBV) DNA (< 0.0004 pg/ml). No family history of HBV was available: His father passed away before he was born, and his mother was remarried when he was one-year-old and left him to the care of his uncle.

A detailed summary of the patient's labs and imaging can be found in Table 1. In October 1999, he was involved in a car accident and incurred multiple severe injuries requiring two times surgery and two units of blood.

During his first visit to our institution in 10/2000, he was HBsAg (+), anti-HBc total (+) and HBeAg (-) indicating that he has been infected for years [9,10]. HBV DNA was undetectable. Initially ALT was elevated but became normal one month later. Without antiviral treatment he was closely monitored by his physician.

Nearly nine years later in 7/2009, he was referred back by his family physician because of an ALT of 163 IU/L. Again, He was HBsAg (+), anti-HBc (+), HBeAg (-), HBV DNA undetectable (< 100 IU/ml). Abdominal MRI showed moderate to severe hepatic steatosis with no evidence of cirrhosis or HCC. With undetectable HBV DNA (< 29 IU/ml) and abnormal ALT, investigation for HCV revealed him to be anti-HCV (+) with HCV RNA  $1.75 \times 10^4$  IU/ml.

He was started on peginterferon (IFN) alfa-2a 180 µg/week and ribavirin 400 mg twice daily in September 2010 and completed 6 months of treatment in March 2011. After one month on treatment, HCV RNA became undetectable (< 43 IU/ml) and remained negative throughout the rest 5-month treatment period. However, ALT fluctuated between 99-150 IU/L and nor-

**Table 1:** Summary of patient labs and imaging.

Date	Lab
1998	HBsAg (+), HBV DNA < 0.0004 pg/mL
10/1999	Pt sustained severe car accident with multiple severe injuries, requiring surgeries and blood transfusion
10/2000	HBV DNA < 0.01 pg/mL ALT 145 IU/L could have been due to HCV but anti-HCV was not tested
11/2000	HBsAg (+), anti-HBs (-), anti-HBc (+), HBeAg (-)/anti-HBe (-) ALT 35 IU/L AFP 4 ng/mL Abdominal ultrasound: Liver with normal and homogeneous parenchyma No return for 9 years
7/2009	ALT 163 U/L
Sent by FMD for abnormal ALT	HBsAg (+), anti-HBs (-), HBeAg (-)/anti-HBe (-) HBV DNA undetectable (< 100 IU/ml) AFP 7.2 ng/ml Abdominal MRI: Moderate to severe hepatic steatosis with no evidence of cirrhosis or HCC
9/2009	HBV DNA < 29 IU/mL
3/2010	ALT 71 IU/L Since ALT continued elevated with negative HBV DNA, HCV was tested: Anti-HCV (+) with HCV RNA $1.75 \times 10^4$ IU/ml, HCV genotype 2 HBV DNA < 29 IU/mL, Platelets $160 \times 10^3/\mu\text{L}$ PegIFN + Ribavirin treatment for 6 months (9/2010-3/2011)
7/2011	HCV RNA (< 43 IU/ml) and HBV DNA (< 169 IU/ml)
10/2011	Abdominal MRI: $1.6 \times 1.6$ cm lesion in segment 8 that had T2 hyperintensity, arterial enhancement and washout consistent with HCC, minimally cirrhotic appearing liver AFP 2.8 ng/ml ALT 40 IU/L Platelets $200 \times 10^3/\mu\text{L}$ HBV DNA: $8.9 \times 10^4$ copies/ml (from a special lab)

malized only near the end of treatment in March 2011. HBV DNA had remained undetectable. In July 2011, 4 months after completion of peginterferon with ribavirin treatment, both HCV RNA and HBV DNA remained undetectable.

In October 2011, 7 months after completion of peginterferon therapy, abdominal MRI showed a  $1.6 \times 1.6$  cm lesion in segment 8 that had T2 hyperintensity, arterial enhancement and washout consistent with HCC. The liver showed mild atrophy of the medial segment of the left hepatic lobe suggestive of mild cirrhosis. He underwent Transarterial Chemoembolization (TACE) in November 2011, followed by laparoscopic Radiofrequency tumor ablation (RFA) in April 2012.

Based on the unexpected development of HCC in the setting of multiple non-detectable HBV DNA levels, additional virologic investigation was undertaken. There was a small amount of leftover serum from October 2011 (the day of HCC diagnosis) that was sent to a special lab for detection of HBV DNA. This new laboratory-developed assay [11,12] showed an HBV DNA level of  $8.9 \times 10^4$  copies/ml. Incidentally, the amount of leftover serum was insufficient for the clinical lab to measure HBV DNA.

With the detectable HBV DNA, he was started on tenofovir 300 mg daily with lamivudine 150 mg daily for prevention of HCC recurrence [13].

During the past 5 years following tumor ablation and anti-HBV treatment, he has remained with no recurrence of HCC based on labs or imaging. The patient remains on his anti-HBV regimen. He has maintained undetectable HCV RNA (< 15 IU/mL) and HBV DNA (< 20 IU/mL). The most recent AFP was 3.3 ng/ml, ALT 29 IU/L, and Platelets  $240 \times 10^3/\mu\text{L}$ .

## Discussion

This case represents a unique case of HCC in an HBsAg (+) patient with undetectable HBV DNA (by commercial assay) for over 10 years who was superinfected with HCV later. It is likely that HCV infection took place in 1999 when he sustained severe injury, requiring multiple surgery and blood transfusion. He has never used IV drugs. For his active HCV infection, he was treated with IFN and ribavirin with success and has remained HCV free for years. Following the treatment of HCV, he later developed HCC and found to have detectable HBV DNA using a special serum analysis.

On average, HBeAg loss occurs in the second to fourth decade of life, peaking at mid-thirties in the HBV endemic region where he had lived until 35-years-old [9,10]. Therefore, it is likely that this patient has been infected with HBV for more than 30 years before he was tested to be HBsAg (+) at age 42.

Elevated ALT was shown on three occasions, 145 IU/L in October 2000 which was one year after car injury and multiple surgery with blood transfusion. His HBV DNA was < 0.01 pg/ml.

In July 2009, he returned 9 years later with an ALT of 163 IU/L. HBV DNA was undetectable, In March 2010, ALT was 71 IU/L. He was found to have HCV infection. Therefore, his abnormal ALTs were considered to have been from HCV infection. In addition, he did never drink and had normal BMI that excluded other cause of abnormal liver enzymes.

Studies have shown a 5 to 100-fold increase in the risk of developing HCC in patients with chronic HBV infection [14,15]. The World Health Organization estimates that more than 500 million people worldwide are chronically coinfecting with HBV and HCV. Study results vary on the development of HCC in patients with HBV/HCV coinfection. Three meta-analyses from 1998, 2005 and 2011 demonstrate an additive effect on the risk of HCC [16].

Increased use of oral antiviral agents for hepatitis C has allowed for a better understanding of the interaction between the two viruses. *In vitro* studies dating back to the 1990s demonstrated that the HCV "core" protein strongly inhibits HBV replication [16,17]. This causes a 2-4-fold reduction of HBV mRNA and HBV antigen expression in the presence of HCV structure genes, as well as a 20-fold suppression of HBV particle secretion. More recent studies show that the two viruses can replicate in the same hepatocyte without interference, but one often exerts a more dominant effect (which virus has a large degree of variation and can be influenced by ethnic factors) [16]. It has been proposed that replication can coexist, but serum HBsAg titers and HBV DNA levels are markedly reduced in patients with coinfection [17] making it more difficult to identify the true burden of a suppressed virus.

In recent years, with more frequent use of oral direct acting antivirals (DAAs) for HCV treatment, multiple cases have emerged with reactivation of HBV in coinfecting patients who had a rapid reduction in HCV viral load, particularly in those who are additionally immunocompromised [18,19]. The burden of this reactivation led the FDA and AASLD/IDSA to recommend screening all HCV infected patients that are planned to initiate DAAs for current or prior HBV infection before initiating treatment with a DAA. In those with a positive screen, (positive HBsAg, anti-HBc or anti-HBs) HBV DNA measurement should also be pursued before HCV treatment

[19]. This phenomenon is becoming more common; a recent meta-analysis including data through September 2016 cited 7 studies with evidence of DAA induced reactivation of HBV [20]. The aforementioned meta-analysis conducted by Chen, et al. compared HBV reactivation in patients treated with DAAs to those treated with interferon-based therapy, as the patient in this case, and it found that rates of reactivation are similar despite choice of treatment, however, time to overt HBV infection after achieving sustained virologic response was shorter in those who received DAAs [20]. Additionally, it was found that there was more clinically significant hepatitis in those treated with DAAs compared to IFN-based therapy. Other reviews stated similar results in regard to the decreased risk of significant HBV reactivation with IFN therapy [21].

We believe our patient has had chronic HBV infection over 40 years that eventually led to HCC. He had HCV superinfection during the massive injuries related to car accident and subsequent surgery. HCC appeared 12 years (at maximum) after HCV infection. Therefore, it is unlikely for HCV responsible for the development of HCC. Furthermore, anti-HBV treatment after HCC ablation has kept him without recurrence demonstrating that the HBV was causally associated for his HCC [13,22].

This patient had an abnormal constellation of hepatitis laboratory values by routine serologic testing, which decreased the initial index of suspicion for active infection. However, after sending his samples for specialized testing, HBV DNA burden was found to be in the thousands. Upon reactivation, the HBV most likely placed the patient at an increased risk of developing HCC which he eventually did within one year of HCV viral load becoming undetectable.

Variability of laboratory values in co-infected patients treated with DAAs or IFN therapy makes it difficult to definitively rule out active HBV infection. Lack of early identification of HBV can lead to cirrhosis and eventual HCC as demonstrated in this case, potentially increasing morbidity. Recently, efforts have been made to develop a more sensitive HBV DNA assay [11,12]. Given the inconsistency of standard HBV serology in co-infected patients and increased risk of reactivation after treatment, there may be utility in sending serum from treated HCV patients for more sensitive testing to aid in early recognition of an active infection. In those patients with abnormal HBV serology by standard laboratory assays, such as positive HBsAg without anti-HBs or findings consistent with occult HBV, advanced testing should be considered.

This patient, following Transarterial Chemoembolization (TACE) and laparoscopic radiofrequency tumor ablation received concomitant antiviral therapy with nucleotide analogues (NAs). He has had no recurrence of HCC for the last 5 years. Improved survival of patients with HCC who received antiviral therapy following tu-

mor ablation has been observed in the past by multiple institutions in Asian countries and in our institution [13]. These reports were recently summarized by Yuan, et al. in his meta-analysis [22]. The best results were seen in our institution where a statistically significant difference in survival of 16 versus 80 months, respectively, was documented for patients who did not or did receive antiviral therapy [13]. Whether the risk of relapse is more prevalent in previously co-infected patients who were treated for HCV is not clearly documented, but is an area for further study, and would allow more selectivity in deciding which patients will benefit from anti-HBV treatment.

## Conclusion

HBV and HCV clearly increase the risk of HCC, however recent paradigms in treatment have made monitoring for HBV reactivation and the associated risk of HCC a critical issue. As in the case above, patients with HBV and HCV co-infection who are subsequently treated for HCV are at increased risk for HBV reactivation and potential HCC development. These patients should be monitored closely for HBV reactivation, hopefully including special laboratory assays that can detect HBV DNA with higher levels of specificity.

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