

Hepatitis B: The Immaculate Infection

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The incidence of acute hepatitis B virus (HBV) saw a decline throughout the 1980s and early 1990s. This was the consequence of both a decrease in incidence among homosexual men (from 20% to 7%) due to safe sex education for HIV transmission, as well as a decrease in incidence among children and adolescents (from 8.5 cases to 2.1 cases per 100,000 people) due to the initiation of the HBV vaccination in 1991.¹ However, the incidence of HBV among adults has increased since 1999.² The most common risk factors for HBV infection include injection drug use, sex with multiple partners, and men having sex with men. Sexual transmission is the major mode of transmission in developed countries and accounts for more than 50% of acute HBV infection in the United States.³ Although the risk of chronic HBV infection after acute exposure is only 1–5% when infection occurs in adulthood, approximately 1.2 million individuals have chronic HBV in the United States and are sources of infection to others.⁴ The risk of HBV transmission from those chronically infected is thought to be highest among those who are hepatitis B e antigen (HBeAg)–positive and those with elevated HBV DNA levels.⁵ It is recommended that spouses and steady sex partners of those with chronic HBV be vaccinated and follow safe sex practices to prevent sexual transmission of the disease. Patients treated with interferon and/or antivirals with adequate response, as demonstrated by hepatitis B e antibody (HBeAb)–seroconversion and undetectable serum HBV DNA levels, are generally accepted to be no longer infective to others. The case we present challenges the accuracy of this principle.

Case Report

A 37-year-old man from Texas living in New York City was referred to our liver clinic for management of HBV. A

homosexual male in a monogamous relationship with his partner, he denied any history of occupational exposure or blood transfusion. He recalled a prior HBV vaccination in 2000.

The patient had initially presented to his primary care physician in Texas in November of 2005 for symptoms of jaundice, pruritus, fever, and joint pain. His limited physical examination was significant for scleral icterus, and his laboratory work-up at that time was significant for transaminitis (aspartate aminotransferase [AST] of 1,081 IU/L, alanine aminotransferase [ALT] of 1,831 IU/L), hyperbilirubinemia (total bilirubin of 8.6 mg/dL), as well as alkaline phosphatase of 283 IU/L, lactate dehydrogenase of 348 IU/L, and gamma glutamyl transferase of 375 IU/L. His hepatitis serologies tested hepatitis A antibody immunoglobulin (Ig)M–negative, hepatitis B surface antigen (HBsAg)–positive, hepatitis B core antibody (HBcAb)–positive, a hepatitis B surface antibody (HBsAb) level of less than 3.0 mIU/mL, HBeAg–positive, and hepatitis C virus antibody–negative, all of which are consistent with acute hepatitis B infection. His HIV test was negative. Repeat laboratory examinations 1 week and 1 month later demonstrated worsening transaminitis (AST of 1,400 IU/L rising to 1,625 IU/L and ALT of 1,970 IU/L rising to 2,111 IU/L). HBeAb was found to be negative. The patient was treated with hydroxyzine (Vistaril, Pfizer) and cholestyramine (Questran, Bristol-Myers Squibb) for symptomatic relief.

At the beginning of May 2006, the patient presented with recurrent symptoms to The Mount Sinai Faculty Practice Associates, where his partner was being followed and treated for chronic HBV with adefovir (Hepsera, Gilead) and lamivudine (Epivir, GlaxoSmithKline). During the initial evaluation, the patient recalled a discrete incident of condom breakage during anal receptive intercourse with his partner in August 2005. He otherwise reported adherence to safe sex practices with his partner and denied having sex outside of the relationship. Although the time course from condom breakage to initial presentation of symptoms was consistent with

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the incubation time of acute HBV, his partner had a documented undetectable serum viral load at that time (6/05: HBV DNA <100 IU/mL, HBeAg nonreactive, HBeAb reactive; 11/05: HBV DNA <100 IU/mL, HBeAg nonreactive, HBeAb nonreactive).

On physical examination, our patient was anicteric and revealed borderline hepatomegaly. His hepatitis serologies were unchanged, and his HBV DNA level measured 58,900,000 IU/mL. Laboratory findings were otherwise significant for AST of 1,010 IU/L, ALT of 2,423 IU/L and bilirubin within normal limits. By his second visit on May 4, 2006, his aminotransferases had started to trend down and his HBV genotype was found to be type A without resistance to polymerase inhibitors. Precore and basic core promoter mutations were not found. Genotyping of the patient's partner was attempted at this time but could not be performed, as his serum viral load remained undetectable. The patient was started on 1 mg of entecavir (Baraclude, Bristol-Myers Squibb) once daily at this point.

As of follow-up on June 26, 2006, the patient's AST and ALT measured 38 IU/L and 48 IU/L, respectively, and his HBV DNA level measured 283 IU/mL. The patient remains on treatment with entecavir.

Discussion

If HBV transmission to the patient occurred as described above, via exposure to the semen of a chronic HBV carrier with an undetectable serum viral load, it raises the question: do infective quantities of HBV DNA remain present in semen after HBV DNA serum levels become undetectable as a result of antiviral treatment?

Facets of this issue were previously addressed in the mid-1980s, independently, by Jenison and associates,⁶ Fagan and colleagues,⁷ and Davison and coworkers.⁸ Jenison and associates performed a quantitative analysis of HBV DNA levels in the saliva and semen of 15 chronically infected homosexual men via molecular hybridization techniques and southern blot analysis. HBV DNA levels were detected in the saliva of 8 patients (10^5 – 10^7 virions/mL) and in the semen of 3 patients (10^6 – 10^7 virions/mL). He concluded that “the presence of relatively high concentrations (10^5 – 10^7 virions/mL) of HBV particles in the saliva and semen of some carriers supports the hypothesis that these secretions play an important role in the nonparenteral transmission of HBV.”⁶ The threshold for detection of HBV DNA levels in this study was 1.0 pg/mL or 8×10^5 virions/mL. All 3 patients with detectable HBV DNA levels in their semen were HBsAg-positive, anti-HBs-negative, anti-HBc-positive, and HBeAg-positive, and had more than 2,500 pg/mL of HBV DNA detected in their serum.

The study did not, however, include a subset of patients whose serum HBV DNA levels were undetectable yet were semen HBV DNA-positive.⁶

In 1986, Fagan and colleagues concluded that “despite histologic remission and loss of HBV DNA from serum, the potential for transmission of HBV and reactivation of disease remain.”⁷ They cited the case of a 29-year-old homosexual man with chronic persistent hepatitis, seropositive for HBsAg, HBeAg, DNA polymerase activity, and HBV DNA for 18 months, who was enrolled in a clinical trial of treatment with a 9-week course of lymphoblastoid interferon. Approximately 7 weeks after initiating therapy, the patient developed the signs and symptoms of acute hepatitis. He subsequently cleared the HBeAg and achieved an undetectable serum HBV viral load. Six weeks later, he developed anti-HBe. His liver biopsy specimen at that time was histologically normal. However, 4 months after HBV DNA was no longer detectable in his serum, the patient's semen, saliva, and urine were examined and found to be positive for free and replicative HBV DNA. Fagan and coworkers speculated that mononuclear cells, present in both saliva and semen, were a likely source of the HBV DNA levels detected in these fluids. They also proposed that the “presence of free and replicating HBV DNA in tissues in the absence of detectable serum HBV DNA suggests that intracellular virus is protected in some way from immune surveillance.”⁷ This study utilized simple spot hybridization for quantitative analysis of HBV DNA levels, with a lower limit of detection of approximately 1 pg.⁷

In 1987, Davison and colleagues similarly concluded that sexual transmission of HBV could still occur without viral replication markers in serum. This was based on a prospective study examining the urine, saliva, and semen of 18 chronic HBsAg carriers for HBV DNA using molecular hybridization. In their study, free HBV DNA was identified in the semen of 5 patients whose sera were negative for DNA polymerase, including 2 patients whose sera were also HBV DNA-negative and 2 patients who had developed HBeAb. The presence of DNA polymerase or HBV DNA in serum was considered to be even more sensitive than the presence of HBeAg as an indication of active viral replication.⁸ The investigators subsequently concluded that “all HBsAg carriers must be assumed capable of transmitting HBV during sexual contact,” irrespective of serum HBV levels.⁸ They found that leukocytes isolated from peripheral blood contained HBV DNA. As leukocytes normally constitute saliva, semen, and urine, the authors speculated that leukocytes may be the major source of the HBV DNA identified in these fluids. The leukocytosis associated with other sexually transmitted diseases could, therefore, increase the risk of HBV infection via seminal fluid.⁸

It is worth noting that the patient reported previous vaccination against hepatitis B, as it provides an opportunity to review HBV vaccination. Hepatitis B vaccine is composed of recombinant HBsAg. The currently accepted dosing schedule, as recommended by the Centers for Disease Control (CDC), involves 3 doses at 0, 1–2, and 4–6 months. Efficacy is reported to be more than 90% in healthy adults and more than 95% in infants, children, and adolescents.⁹ The CDC does not recommend postvaccination testing for adequate antibody response after routine vaccination unless the person is immunocompromised, born to an HBsAg-positive mother, a healthcare worker, or a sex partner of a person with chronic HBV infection. Postvaccination testing, if indicated, should be completed 1–2 months after the third vaccine dose, and adequate response is 10 or more mIU/mL.¹ Booster dosing is not recommended as a part of HBV vaccination, as current data show that vaccine-induced HBsAb levels may decline over time; however, immune memory remains intact indefinitely following immunization and, therefore, people with declining antibody levels are still protected against clinical illness and chronic disease.¹ Our patient did not have documented antibody response to the HBV vaccine; however, it is unclear whether or not he actually completed the recommended dosing. In retrospect, he should have had postvaccination testing performed, as he is the sex partner of a person with chronic HBV infection.

Conclusion

These studies indicate that infective quantities of HBV DNA remain present in semen after HBV DNA levels become undetectable in serum. However, it is possible that those patients with undetectable serum HBV DNA levels cited may represent false-negatives. The threshold of laboratory testing available during this time of study

was as high as 1 pg of HBV DNA, which is equivalent to 800,000 IU of HBV DNA. That leaves a large range of HBV DNA that could have been missed because of the insensitivity of the testing performed at that time. As noted by Wei-Ping and coworkers, the southern blot techniques utilized by the above investigators were tedious, time-consuming, and possibly of limited accuracy.¹⁰ In comparison, conventional polymerase chain reaction (PCR) and real-time PCR are available for detection of HBV DNA levels with threshold values of 200 and 10 copies of HBV DNA, respectively. We propose that it would be prudent to reinvestigate whether HBV DNA remains present in semen after HBV DNA levels become undetectable in serum with real-time PCR.

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Review

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The case report by Huysman and colleagues raises a number of provocative questions and conclusions regarding hepatitis B.¹ First, can hepatitis B virus (HBV) be transmitted via bodily fluids such as semen and saliva from a hepatitis B surface antigen (HBsAg) carrier whose serum HBV DNA levels are repeatedly verified as negative? Second, what is the likelihood of acquiring lamivudine (Epivir, GlaxoSmithKline)-resistant HBV among treatment-naive patients? Finally, Huysman and coworkers demonstrate that, in the treatment of hepatitis B, prevention is superior to therapy.

Transmission of Hepatitis B Virus in the Absence of Detectable Hepatitis B Virus DNA

If undetectable levels of HBV DNA in the serum can be associated with transmission of the infection through bodily fluids, especially during sexual contact, significant public health implications may result. In order to establish the validity of this possible route of HBV transmission, one must have concrete scientific evidence based on well-designed clinical studies. As Huysman and colleagues cited in their discussion, most of the original studies addressing this issue were published in the 1980s.²⁻⁴ The limitations of these studies include lack of accuracy and of standardization of HBV DNA quantification. Over the past decades, significant advances have been made in the diagnosis and treatment of hepatitis B. We, therefore, must critically examine prior studies and determine whether their results still hold true with the availability of more sensitive HBV DNA quantification methods.

Fagan and coworkers reported in 1986 that a patient with hepatitis B achieved undetectable HBV DNA lev-

els in serum but had HBV DNA levels that could still be detected in other bodily fluids such as urine, semen, and saliva.² However, it is important to emphasize that the assays utilized for detecting HBV DNA levels in serum and other bodily fluids were heterogeneous. HBV DNA levels in sera, for instance, were measured by spot hybridization assay, whereas southern blots were applied to detect HBV DNA in urine, saliva, and semen. As the threshold for HBV DNA detection in these tests was different, definitive conclusions about the transmission of HBV via bodily fluids in the absence of HBV DNA in serum cannot be drawn from this study. The results of the study conducted by Jenison and associates actually indirectly refuted the hypothesis that HBV can be transmitted in the setting of undetectable HBV DNA in serum.³ All 3 subjects with detectable HBV DNA levels in semen had at least 100-fold higher levels of HBV DNA in their corresponding serum samples.

In the case report by Huysman and colleagues, the partner of their patient could theoretically transmit HBV during unprotected sex, but the causal relationship could not be established, as the partner's semen sample was not tested for HBV DNA. Furthermore, sequencing results were unavailable to determine whether both the patient and his partner had the same strain of HBV. Carefully conducted studies utilizing sensitive HBV DNA assays on different bodily fluids are essential to draw a definitive conclusion to this question.

There are many commercially available assays to quantify HBV DNA. Until recently, HBV DNA titers had been reported in different units of measurement such as copies/mL, genome equivalents/mL, or mega-equivalents/mL. Since the World Health Organization developed an international standard for HBV DNA nucleic acid amplification techniques, serum HBV DNA titers can and should be uniformly expressed in IU/mL to ensure comparability among different assays.⁵ The ideal HBV DNA quantification assay should be sensitive and reproducible and have broad dynamic range of at least 5 logs. Real-time polymerase chain reaction (PCR) quantification assays possess these properties and, therefore, are recommended for HBV DNA baseline determination and monitoring during therapy.⁶⁻⁸

The Prevalence of Lamivudine-Resistant Hepatitis B Virus Among Treatment-Naive Patients

It is interesting that the authors chose to treat the patient with 1 mg of entecavir (Baraclude, Bristol-Myers Squibb) versus the recommended 0.5 mg dose for treatment-naive HBV patients. Entecavir 1 mg daily is the US Food and Drug Administration–approved dose for patients with

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Table 1. Comparison of Different Genotypic Assays for Detection of Drug Resistance

Method	Detection Threshold	Information details	Commercially Available	Complexity of interpretation
Direct sequencing	15–50%	High	Yes	High
RFLP	5–10%	Low	No	Intermediate
RT-PCR	5–10%	Low	No	Intermediate
LiPA	5%	Low	Yes	Low
Flourescence	Not determined	Intermediate	No	Intermediate
MALDI-TOF	<5%	Intermediate	No	High

Adapted from Sablon and Shapiro.³³

RFLP=restriction fragment length polymorphism; RT-PCR=real-time polymerase chain reaction; LiPA=line probe assay; MALDI-TOF=matrix assisted laser desorption/ionization time-of-flight assay.

known lamivudine-resistance or history of being refractory to lamivudine therapy.^{9–10} Lamivudine is a nucleoside analog that directly inhibits HBV DNA polymerase.¹¹ Lamivudine resistance has been attributed mainly to a substitution of valine or isoleucine for methionine in the tyrosine-methionine-aspartate-aspartate (YMDD) motif in the catalytic site of the HBV polymerase gene rtM204V/I.^{12–13} In some patients, this is accompanied by a second mutation substituting methionine for leucine in an upstream region (rtL180M).^{12–13} Entecavir, a cyclopentyl guanosine analogue, is a potent inhibitor of HBV DNA polymerase, inhibiting both the priming and elongation steps of viral DNA replication.¹⁴ Cross-resistance between entecavir and lamivudine have been described in in-vitro and clinical observations.^{15–16} Entecavir resistance occurs in the setting of preexisting lamivudine-resistance plus additional substitutions at reverse transcriptase positions T184, S202, and/or M250.¹⁷ Thus, entecavir is less potent and is associated with a significantly higher rate of resistance when used in patients with lamivudine-resistance compared with treatment-naïve subjects, despite a higher dose of medication.^{16,18,19} The reported entecavir resistance rate is less than 1% in 4 years for treatment-naïve patients compared with 39% among those with a history of being refractory or resistant to lamivudine.²⁰

It is well documented that long-term lamivudine monotherapy leads to the emergence of lamivudine-resistant virus in patients with chronic hepatitis B. Approximately 25% of patients developed resistance within 1 year of treatment, and more than 40% after 2 years, which increases to 53% and 67% after 3 and 4 years, respectively.^{21–24} More recently, naturally occurring rtM204V/I and rtL180M mutant viruses were reported in HBV carriers who have never received lamivudine.^{25–31} Feeney and colleagues examined 108 samples from treatment-naïve

HBV patients from diverse ethnic backgrounds and HBV genotypes.²⁵ Among them, 34% were born in Ireland and the remainder were from Africa (22%), Asia (17.4%), Eastern Europe (10.1%) and other regions (16.5%). Genotypes were available in 98 samples. Lamivudine-resistant mutations were present in 16 (14.8%) of the patients tested. However, it is unclear whether these patients actually developed de novo lamivudine-resistant mutations or were infected with lamivudine-resistant viruses that persisted. The actual transmission rate of the lamivudine-resistant viruses is unknown. Thibault and associates documented a case of primary infection with lamivudine-resistant HBV via direct sequencing that was acquired presumably via homosexual activity. The patient was receiving a lamivudine-containing highly active antiretroviral treatment regimen when he developed acute hepatitis B. His HBsAg was negative 3 months prior to the onset of acute hepatitis, providing clues that lamivudine-resistant HBV can be transmitted from one individual to another. It would be more conclusive and convincing, however, if the source patient had been identified and tested.³²

In the case study by Huysman and coworkers, the patient's genotype profile did not reveal polymerase inhibitor-induced resistant mutants. However, the sensitivity of the different genotypic assays for the detection of drug resistance varies significantly in their ability to identify minor strains of viruses (Table 1).³³ Direct sequencing, for example, has relatively low sensitivity and can only detect mutant viruses if they exceed 15–50% of the total viral population. The advantage of direct sequencing is its ability to identify new mutations. Line probe and matrix assisted laser desorption/ionization time-of-flight assays, in contrast, can identify very low levels (5%) of the mutant viruses. It is, therefore, important to know which

resistance assay was applied. This is of clinical relevance to the case study patient of Huysman and associates, as it would influence the treatment choice if subpopulations of polymerase inhibitor-induced resistant mutants coexisted with the wild-type virus. Standardization of the genotypic resistance assays is necessary to determine the incidence and prevalence of the transmission of these nucleos(t)ide-induced resistant HBV mutations.

Prevention is Superior to Therapy

In this clinical case study, the patient recalled being vaccinated against hepatitis B in 2000. It is not known if he received all 3 of the recommended doses of the vaccine nor whether he had postvaccination testing. In November 2005, it was noted that he had an anti-HBs titer less than 3 mIU/mL, indicating either failed immunity or lack of immunity.

Among healthy adults receiving immunization with HBV vaccine at baseline, 1 month, and 6 months, approximately 90% develop protective antibodies. Response rates range from 20% to 30% after the first injection, approximately 75–80% after the second injection, and approximately 90–95% following the third injection.³⁴⁻³⁵ Response rates to the HBV vaccine tend to be lower in populations with chronic conditions such as hepatitis C, alcoholic liver disease, renal failure on dialysis, HIV, and liver transplantations.³⁶⁻⁴⁰ Other factors that are associated with reduced response include male gender, smoking cigarettes, obesity, and older age (>40 years).⁴¹⁻⁴³ In order to identify true nonresponders, the Center for Disease Control (CDC) recommends evaluating the anti-HBs titer at 1–6 months following the last dose of the vaccine when utilizing the recommended vaccination schedule. Individuals who achieved a titer greater than 10 mIU/mL are considered to be immune.⁴⁴ From the very first publication of the Advisory Committee on Immunization Practices in 1982, men having sex with men were targeted as adults at high risk for infection and vaccination was recommended. Despite subsequent guidelines to reinforce the importance of vaccination in this high-risk group, the incidence of new HBV cases increased from 7% to 18% among this population from 1990 to 2004.⁴⁵

The CDC does not recommend postimmunization testing for the general population because of the high response rate.⁴⁶ However, the CDC does recommend postvaccination testing for high-risk groups such as healthcare workers, public safety workers, chronic hemodialysis patients, HIV-infected persons, and sex or needle-sharing partners of HBsAg-positive persons. The patient in Huysman and colleagues' case study had a sex partner with chronic hepatitis B. Accordingly, he should have had postvaccine testing after receiving a full course of

HBV vaccine. Testing would have identified failed immunity or lack of immunity in the patient's case and would have allowed the opportunity for further intervention to prevent the acquisition of hepatitis B.

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