Occult Hepatitis B Virus Infection: A Hidden Menace?

SEE ARTICLE ON PAGE 194

Recovery from an acute hepatitis B virus (HBV) infection is associated with loss of HBV DNA from serum, hepatitis B e antigen seroconversion, hepatitis B surface antigen (HBsAg) seroconversion, and normalization of serum aminotransferases. These changes generally imply clearance of virus, but clinical observations have shown that reactivation of HBV infection can occur either spontaneously or after immunosuppression.[1-3] Recent studies showed that immune response to HBV remains vigorous long after an acute infection. In addition, HBV DNA can be detected by polymerase chain reaction (PCR) assays in serum, liver, and peripheral blood mononuclear cells more than a decade after an apparent recovery from HBV infection.[4-6] These findings suggest that recovery from acute hepatitis B may not result in complete virus elimination, but rather the immune system keeps the virus at very low levels. There is, however, no clear evidence that patients who have persistently low levels of HBV after recovery from acute hepatitis B develop progressive liver disease.

The availability of PCR assays for HBV DNA allows the detection of 10^2 copies/mL compared with 10^6 copies/mL using hybridization assays. Using PCR assays, HBV DNA has been detected in some subjects who are HBsAg negative including those with no serologic markers of HBV infection. In this issue of the HEPATOLOGY, Bréchot et al. review the prevalence, virologic basis, and clinical significance of occult HBV infection.[7] For the clinician, several issues regarding occult HBV infection are pertinent: Does it exist? How common is it? What is the risk of transmission? What is the risk of progressive liver disease? How can it be diagnosed? Is antiviral therapy indicated? Before these issues can be addressed, it must be recognized that there is currently no standardized definition or diagnostic criteria of occult HBV infection. A simple definition would be the detection of HBV DNA in HBsAg-negative subjects. However, more specific information must be provided, as occult HBV infection is a heterogeneous clinical entity.

Bréchot et al. provide very strong evidence that occult HBV infection exists and that most cases are related to very low levels of HBV rather than to HBV mutants that do not express or produce aberrant surface proteins and therefore are undetected by standard testing.[7] Because HBV-DNA detection is the key to diagnosis of occult HBV infection, the type of assay used and its sensitivity must be specified. The sensitivity of PCR assays for HBV DNA in studies on occult HBV infection varies from 10^1 to 10^3 copies/mL.[8] However, most PCR assays including commercially available assays are not standardized.[9] Other factors that may affect the detection rates of HBV DNA include the volume of sample used and the material tested. Thus, the limit of detection can be increased by using a larger volume of serum as in the case of the hybrid capture assay. Most studies on occult HBV infection have reported higher rates of HBV-DNA detection in liver or peripheral blood mononuclear cells compared with serum or plasma. In addition, snap-frozen liver tissue has a higher rate of HBV-DNA detection than paraffin-embedded liver tissue. More importantly, specificity and reproducibility of assay results must be ensured. This requires meticulous steps to prevent contamination of samples, inclusion of negative controls, and performance of assays in duplicate using two independent sets of HBV primers.

Occult HBV infection should also be reported in the context of other HBV serology markers. Broadly, individuals should be classified as being seropositive or seronegative. "Seropositive" subjects are positive for antibodies to hepatitis B core antigen (anti-HBc) and can be further divided into 2 subgroups: with and without anti-HBs. "Seronegative" subjects are negative for both anti-HBc and anti-HBs. As indicated in the review by Bréchot et al.,[7] the HBV-DNA detection rate is highest in subjects who are anti-HBc positive/anti-HBs negative; some of these individuals probably have low-level HBV infection with subdetectable HBsAg. The HBV-DNA detection rate is intermediate in subjects who are positive for both anti-HBc and anti-HBs. These individuals may have recovered from previous infection but may have persistent low levels of HBV. The HBV-DNA detection rate is lowest in seronegative subjects. These individuals have recovered from previous infection but lost all serologic markers of HBV infection. Rarely, they may be infected with HBV mutants that do not express HBV serologic markers.

Geographic differences in the prevalence of occult HBV infection are most likely related to the endemicity of HBV infection. Thus, occult HBV infection is most commonly reported in high endemic areas where 70% to 90% of the population have been exposed to HBV and infrequently reported in low endemic areas where 5% to 20% of the population had prior infection with HBV.[10-12] The prevalence of occult HBV infection also depends on the population studied, being more common in patients with chronic liver disease and less common among healthy blood or organ donors. Patients with fulminant hepatitis B may be misdiagnosed as occult HBV infection because of rapid virus clearance with undetectable HBsAg at the time of presentation.

Transmission of HBV infection has been documented from HBsAg-negative, anti-HBc–positive blood and organ donors. The risk is variable (0.4%-90%)[13-17]; it is highest when livers

Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; PCR, polymerase chain reaction; anti-HBc, antibody to hepatitis B core antigen; HCC, hepatocellular carcinoma.

From the Division of Gastroenterology, University of Michigan Health System, Ann Arbor, MI.

Received March 14, 2001; accepted March 30, 2001.

Address reprint requests to: Anna Lok, M.D., Division of Gastroenterology, University of Michigan Health System, 3912 Taubman Center, Box 0362, Ann Arbor, MI 48109-0362. E-mail: aslok@umich.edu; fax: 734-936-7392.

0270-9139/01/3401-0028$35.00/0
doi:10.1053/jhep.2001.25225

from anti-HBc–positive donors are transplanted to seronegative recipients. The possibility of HBV transmission from HBsAg-negative donors raises several questions: Should blood/organ donors be screened for anti-HBc? Should blood/organs from HBsAg-negative, anti-HBc–positive donors be used? Would PCR testing for HBV DNA differentiate infectious from noninfectious donors? In the United States, blood and organ donors are routinely screened for anti-HBc. In general, blood and organs from HBsAg-negative, anti-HBc–positive donors are not used. The shortage of donor organs has led to a widespread practice of transplanting organs from anti-HBc–positive donors to HBsAg-positive recipients. The transplantation of such organs (especially livers) to HBsAg-negative recipients has been reported to result in *de novo* HBV infection.[14-17] Additional testing for HBV DNA by PCR in the setting of solid organ transplantation is logistically difficult and several studies found that PCR testing of donor blood is not sensitive enough to identify donors who are infectious.[17,18] Thus, use of organs from anti-HBc–positive donors in HBsAg-negative recipients in the United States should be restricted to dire emergencies only. However, anti-HBc screening and exclusion of anti-HBc–positive donors is impractical in countries where HBV infection is prevalent and greater than 20% of the population are anti-HBc positive. Whether anti-HBc screening and direction of organs from anti-HBc–positive donors to seropositive recipients should be implemented deserves careful study. The risk of transmission of HBV infection from seronegative individuals has not been well studied. Although there have been case reports of such transmission, the overall risk is likely to be negligible and does not warrant routine screening of seronegative blood/organ donors for HBV DNA.

Occult HBV infection is usually associated with very low levels of HBV DNA. A key question is whether the presence of small amounts of HBV will lead to progressive liver disease. Available data suggest that the likelihood is extremely low although definitive data are lacking. Among patients with persistent low levels of HBV DNA after apparent recovery from acute hepatitis B, there is no evidence that they have increased risks of cirrhosis or hepatocellular carcinoma (HCC). Cirrhosis and HCC have been reported in patients with seropositive occult HBV infection, but it is not clear if the cirrhosis or HCC is a result of persistent low levels of HBV or other causes of liver disease. In patients with other etiologies of chronic liver disease such as alcoholism and hepatitis C virus infection, HBV may be a bystander or a cofactor in the pathogenesis of liver disease. Other patients may have chronic HBV infection for decades leading to liver damage but HBsAg is no longer detectable when cirrhosis or HCC is diagnosed.[19-21] The role of occult HBV infection in the etiology of liver disease in seronegative individuals is less clear. Data are scanty and based on case reports or case series. Thorough evaluation for other causes of liver disease and duplicate testing for HBV DNA under stringent conditions must be performed. At the recent National Institutes of Health Conference on Hepatitis B, an arbitrary HBV DNA level of 10^5 copies/mL was chosen as a diagnostic criterion for chronic hepatitis B and an indication for antiviral therapy.[22] However, more studies using standardized assays are needed to determine if a threshold HBV-DNA level exists for pathogenicity of liver disease and to define this level.

When should occult HBV infection be considered? In patients with cryptogenic acute or chronic liver disease, it is not unreasonable to test for HBV DNA particularly in anti-HBc–positive individuals but routine testing of seronegative individuals is not necessary. HBV-DNA testing is also not recommended in HBsAg-negative patients with other identifiable etiologies of liver disease. An exception may be in patients who are hepatitis C antibody positive but hepatitis C virus RNA negative on repeat testing. HBV-DNA testing should be performed on anti-HBc–positive donors regardless of aminotransferase levels if their blood or organs may be used. However, the logistics of obtaining reliable results in a timely manner are difficult and a negative test result has a very low accuracy in predicting infectivity. Thus, blood or organs from anti-HBc–positive donors should in general be used in HBsAg-positive or seropositive recipients only. Based on the limited data available, universal screening for HBV DNA among seronegative donors is not warranted.

There are no data on the use of antiviral treatment in patients with occult HBV infection. Intuitively, the benefit is anticipated to be low as the vast majority of patients with occult HBV infection have very low levels ($\sim 10^3$ copies/mL) of HBV DNA, which may not be sufficient to cause progressive liver disease. However, the threshold level of HBV DNA that causes liver disease has not been defined and may be different in different clinical situations. Thus, patients with active liver disease and no other identified etiology should have HBV DNA levels monitored. Antiviral treatment may be considered in the context of clinical trials in patients with HBV DNA levels that are persistently or intermittently above 10^3 copies/mL.

In summary, we agree with Bréchot et al. that occult HBV infection exists and, in most instances, is associated with very low levels of HBV rather than HBV mutants. We propose that occult HBV infection be defined as the detection of HBV DNA by PCR or other amplification assays in HBsAg-negative individuals. The prevalence of occult HBV infection is unclear but appears to be more common in endemic areas and among seropositive individuals (anti-HBc positive with or without anti-HBs). There is, as yet, no proof that occult HBV infection exists in individuals with anti-HBs only. Standardized definition and diagnostic criteria of occult HBV infection are needed for future research to determine the prevalence and clinical significance of occult HBV infection and the role of antiviral therapy. Reports of occult HBV infection should specify the type and sensitivity of HBV-DNA assay, the material used for detection of HBV DNA, the HBV serology profile, and the presence or absence of liver disease as determined biochemically and/or histologically.

HARI S. CONJEEVARAM, M.D.
ANNA S. LOK, M.D.
Division of Gastroenterology
University of Michigan Health System
Ann Arbor, MI

REFERENCES

1. Perrillo RP. Acute flares in chronic hepatitis B: the natural and unnatural history of an immunologically mediated liver disease. Gastroenterology 2001;120:1009-1022.
2. Hoofnagle JH, Dusheiko GM, Schafer DF, Jones EA, Micetich KC, Young RC, Costa J. Reactivation of chronic hepatitis B virus infection by cancer chemotherapy. Ann Intern Med 1982;96:447-449.
3. Lok ASF, Liang RHS, Chiu EKW, Wong KL, Chan TK, Todd D. Reactivation of hepatitis B virus replication in patients receiving cytotoxic

therapy. Report of a prospective study. Gastroenterology 1991;100:182-188.
4. Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. Nat Med 1996;2:1104-1108.
5. Fong TL, Di Bisceglie AM, Gerber MA, Waggoner JG, Hoofnagle JH. Persistence of hepatitis B virus DNA in the liver after loss of HBsAg in chronic hepatitis B. HEPATOLOGY 1993;18:1313-1318.
6. Blackberg J, Kidd-Ljunggren K. Occult hepatitis B virus after acute self-limited infection persisting for 30 years without sequence variation. J Hepatol 2000;33:992-997.
7. Bréchot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Brechot P. Persistent hepatitis B virus: clinically significant or purely occult? HEPATOLOGY 2001;34:194-203.
8. Pawlotsky JM, Bastie A, Hezode C, Lonjon I, Darthuy F, Remire J, Dhumeauz D. Routine detection and quantification of hepatitis B virus DNA in clinical laboratories: performance of three commercial assays. J Virol Methods 2000;85:11-21.
9. Heermann KH, Gerlich WH, Chudy M, Schaefer S, Thomssen R, and the Eurohep Pathobiology Group. Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. J Clin Microbiol 1999;37:68-73.
10. Liang TJ, Baruch Y, Ben-Porath E, Enat R, Bassan L, Brown NV, Rimon N, et al. Hepatitis B virus infection in patients with idiopathic liver disease. HEPATOLOGY 1991;13:1044-1051.
11. Scully LJ, Sung H, Pennie R, Gill P. Detection of hepatitis B virus DNA in the serum of Canadian hepatitis B surface antigen negative, anti-HBc positive individuals, using the polymerase chain reaction. J Med Virol 1994;44:293-297.
12. Zhang YY, Hansson BG, Kuo LS, Widell A, Nordenfelt E. Hepatitis B virus DNA in serum and liver is commonly found in Chinese patients with chronic liver disease despite the presence of antibodies to HBsAg. HEPATOLOGY 1993;17:538-544.
13. Hoofnagle JH, Seeff LB, Bales ZB, Zimmerman HJ. Type B hepatitis after transfusion with blood containing antibody to hepatitis B core antigen. N Engl J Med 1978;298:1379-1383.
14. Wachs ME, Amend WJ, Ascher NL, Bretan PN, Emond J, Lake JR, Melzer JS, et al. The risk of transmission of hepatitis B from HBsAg(−), HBcAb(+), HBIgM(−) organ donors. Transplantation 1995;59:230-234.
15. Dickson RC, Everhart JE, Lake JR, Wei Y, Seaberg EC, Wiesner RH, Zetterman RK, et al. Transmission of hepatitis B by transplantation of livers from donors positive for antibody to hepatitis B core antigen. The National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database. Gastroenterology 1997;113:1668-1674.
16. Uemoto S, Sugiyama K, Marusawa H, Inomata Y, Asonuma K, Egawa H, Kiuchi T, et al. Transmission of hepatitis B virus from hepatitis B core antibody-positive donors in living related liver transplants. Transplantation 1998:65:494-499.
17. Prieto M, Gomez MD, Berenguer M, Cordoba J, Rayon JM, Pastor M, Garcia-Herola A, et al. De novo hepatitis B after liver transplantation from hepatitis B core antibody positive donors in an area with high prevalence of anti-HBc positivity in the donor population. Liver Transpl 2001;7: 51-58.
18. Chazouilleres O, Mamish D, Kim M, Carey K, Ferrell L, Roberts JP, Ascher NL, et al. "Occult" hepatitis B virus as source of infection in liver transplant recipients. Lancet 1994;343:142-146.
19. Chung HT, Lai CL, Lok AS. Pathogenic role of hepatitis B virus in hepatitis B surface antigen-negative decompensated cirrhosis. HEPATOLOGY 1995;22:25-29.
20. Yotsuyanagi H, Shintani Y, Moriya K, Fujie H, Tsutsumi T, Kato T, Nishioka K, et al. Virologic analysis of non-B, non-C hepatocellular carcinoma in Japan: frequent involvement of hepatitis B virus. J Infect Dis 2000;181:1920-1928.
21. Shiota G, Oyama K, Udagawa A, Tanaka K, Nomi T, Kitamura A, Tsutsumi A, et al. Occult hepatitis B virus infection in HBs antigen-negative hepatocellular carcinoma in a Japanese population: involvement of HBx and p53. J Med Virol 2000;62:151-158.
22. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000, summary of a workshop. Gastroenterology 2001 (in press).