

## Hepatitis B Virus DNA in Sera of Blood Donors and of Patients Infected with Hepatitis C Virus and Human Immunodeficiency Virus

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Received 10 January 2003/Returned for modification 9 April 2003/Accepted 8 May 2003

**With the use of PCR, we searched for hepatitis B virus (HBV) DNA in serum samples from 415 HBsAg-negative, anti-HBc-positive patients: 150 were blood donors, 106 had only hepatitis C virus (HCV) infection, and 159 had human immunodeficiency virus (HIV) infection (of which 88 were HCV positive and 71 were HCV negative). HBV DNA was detected in 4% of blood donors, 3.4% of HIV- and HCV-positive patients, and 24% of HCV-positive patients.**

Until recently, it has been considered that clearance of hepatitis B virus surface antigen (HBsAg) from the serum normally indicates resolution of biochemical and histological hepatitis in patients with chronic hepatitis B virus (HBV) infection (15). Ten to twenty percent of all individuals with HBV have antibodies to the HBV core antigen (anti-HBc) as the only marker of this infection (7). Since the development of PCR, it has become possible to detect HBV DNA in the sera of about 10% of patients that are HBsAg negative and anti-HBc positive (7). The HBV infections in patients who lack detectable HBsAg are called occult infections (3), and the serum HBV DNA levels are usually less than  $10^4$  copies/ml (9). Low levels of HBV DNA have also been identified in the sera of patients with chronic hepatitis C virus (HCV) infection with undetectable serum HBsAg levels (2, 3, 6, 9, 16) and in the sera of patients coinfecting with human immunodeficiency virus (HIV) (7, 8).

Recently we analyzed the prevalence of occult HBV infection in the sera of 415 individuals from the city of Campinas in southeastern Brazil. All the patients were negative for HBsAg and positive for anti-HBc (Hepanostika anti-HBc Uniform; Organon Teknika, Boxtel, The Netherlands) and were tested for anti-HBs (AUSAB, MEIA; Abbott Laboratories, North Chicago, Ill.), anti-HCV (anti-HCV assay version 3.0; Abbott Murex, Dartford, United Kingdom), and anti-HIV (anti-HIV 1.2.O assay; Abbott Murex). In group 1 (G1), there were 150 blood donors negative for anti-HCV and anti-HIV. In group 2 (G2), there were 106 patients with chronic HCV infection (anti-HCV positive and HCV RNA positive). All these patients were anti-HIV negative. Group 3 (G3) was constituted

of 159 patients with HIV infection (anti-HIV positive and HIV RNA positive). Among them, 88 were HCV positive and 71 were HCV negative. A total of 81 of 159 patients were taking lamivudine at the time the samples used to detect HBV DNA were obtained.

The nested-PCR method for detection of HBV DNA was performed essentially as described by Kaneko et al. (13). Serial dilutions were made in an HBsAg low-titer performance panel (PHA 105; Boston Biomedica, Inc., Boston, Mass.), which made it possible to establish a limit of detection by PCR of  $10^2$  copies/ml. Samples considered positive by “in-house” PCR were subjected to a commercial test for HBV DNA (HBV Monitor; Roche) with a lower detection limit of 1,000 copies/ml. Fisher’s exact test and the chi-square test, when applicable, were used for comparing proportions (1). All the *P* values were two-tailed, and a *P* value of  $<0.05$  was considered significant.

HBV DNA was detected in 8.2% of HIV-negative patients (Table 1) and in 5% of HIV-positive patients (Table 2) (*P* = 0.2179). Among HCV-positive, HIV-negative patients, 14.2% were HBV DNA positive, which was significantly more than the percentage (4%) of HBV DNA-positive blood donors (*P* = 0.0036). In G2, 24% of anti-HBc-positive, anti-HBs-negative patients were HBV DNA positive, while among anti-HBs-positive patients, 5.4% were HBV DNA positive (*P* = 0.0102). In G3, among HCV-negative patients, 7% were HBV DNA positive, and among HCV-positive patients, 3.4% were HBV DNA positive (*P* > 0.99). All samples positive for HBV DNA by in-house PCR were negative for HBV DNA when assayed with an automatic commercial test. Among the 81 HIV-positive patients who were taking lamivudine, four (4.9%) were HBV DNA positive, compared to four (5.1%) HBV DNA-positive patients among the 78 that were not receiving lamivudine (*P* > 0.7580).

Among the HIV-negative patients and blood donors, we found that sera from 8.2% were HBV DNA positive; that percentage is higher than, but not significantly different from,

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TABLE 1. HBV DNA in sera of HBsAg-negative, anti-HBc-positive, HCV-negative blood donors and HBsAg-negative, anti-HBc-positive, HCV-positive patients uninfected with HIV

| Patient status                                     | No. (%) positive for HBV DNA | No. (%) negative for HBV DNA | TOTAL (%) |
|--|------------------------------|------------------------------|-----------|
| HCV negative, anti-HBc positive, anti-HBs negative | 6 (6)                        | 94 (94)                      | 100 (100) |
| HCV negative, anti-HBc positive, anti-HBs positive |                              | 50 (100)                     | 50 (100)  |
| Total (G1) <sup>a</sup>                            | 6 (4)                        | 144 (96)                     | 150 (100) |
| HCV positive, anti-HBc positive, anti-HBs negative | 12 (24)                      | 38 (76)                      | 50 (100)  |
| HCV positive, anti-HBc positive, anti-HBs positive | 3 (5.4)                      | 53 (94.6)                    | 56 (100)  |
| Total (G2) <sup>b</sup>                            | 15 (14.2)                    | 91 (85.8)                    | 106 (100) |
| Total <sup>c</sup>                                 | 21 (8.2)                     | 235 (91.8)                   | 256 (100) |

<sup>a</sup> P = 0.1790 for comparison of patients in G1.  
<sup>b</sup> P = 0.0102 for comparison of patients in G2.  
<sup>c</sup> P = 0.0036 for comparison of patients in G1 and G2.

that of HIV-positive patients noted to be HBV DNA positive (5%). A factor that could be associated with a relatively low positivity for HBV DNA among our HIV-positive patients is therapy with lamivudine, which by acting indirectly on HBV could decrease the frequency of occult infection. However, there was no significant difference in the frequencies of positivity for HBV DNA among patients who were taking lamivudine and patients who were not using this drug.

Recently, with serum samples of 240 HIV-infected patients enrolled in a nationally distributed study cohort, the prevalence of both active HBV infection and occult HBV infection was investigated. HBV infection with active viremia is more prevalent in HIV patients than in the general population, and the prevalence of anti-HBc alone is lower (15.8%) than the one seen in European cohorts. Only 2.6% of the U.S. subjects with anti-HBc alone were HBV DNA positive (K. E. Sherman, N. Shire, S. Rouster, and N. Rajcic, Program Abstr. 10th Conf. Retrovir. Opportunistic Infect., abstr. 820, 2003). This percentage of subjects with occult HBV infection is lower than that observed among our HIV-positive patients. It is possible that the immunodeficiency observed in HIV-positive patients may accelerate the HBV replication, increasing the viremia and facilitating the detection of HBsAg by serological tests.

The percentage of blood donors positive for HBV DNA

(4%) was similar to the percentages of HBV DNA-positive Swiss and German blood donors (3.9 to 7.4%) observed in previous studies (7, 11, 14). This detection rate, however, rises to more than 30% when HBV DNA is researched in diagnostic settings (7, 12), as observed with the detection of HBV occult infection in 14.2% of our HCV-positive patients. The prevalence of anti-HBc in patients with chronic HCV infection ranges from 50 to 55%, and using PCR amplification to detect the HBV DNA is possible in about 46% of patients with anti-HBc alone (9). Studies on HBV and HCV coinfection revealed a mutual viral interference between HBV and HCV (9), with some reports suggesting that HCV possesses the strongest suppressing effect (4, 5, 6, 10).

The main characteristic of occult infection is the low level of HBV DNA detected in the blood and in the liver tissue (2, 9, 10). This characteristic was also observed in our patients with or without infection by HIV. We utilized a highly sensitive in-house PCR test with a lower detection limit of 100 copies/ml. The positive samples were tested afterwards with a commercial test that had a lower detection limit of 1,000 copies/ml, and all were negative. Thus, our HBV DNA-positive patients had more than 100 and less than 1,000 copies/ml, which confirms the low levels of HBV DNA generally present in the sera of patients with occult HBV infection.

TABLE 2. HBV DNA in sera of HBsAg-negative, anti-HBc-positive patients infected with HIV

| Patients status                                    | No. (%) positive for HBV DNA | No. (%) negative for HBV DNA | TOTAL (%) |
|--|------------------------------|------------------------------|-----------|
| HCV negative, anti-HBc positive, anti-HBs negative | 1 (6.7)                      | 14 (93.3)                    | 15 (100)  |
| HCV negative, anti-HBc positive, anti-HBs positive | 4 (7.1)                      | 52 (92.9)                    | 56 (100)  |
| Subtotal <sup>a</sup>                              | 5 (7)                        | 66 (93)                      | 71 (100)  |
| HCV negative, anti-HBc positive, anti-HBs negative | 1 (3.3)                      | 29 (96.7)                    | 30 (100)  |
| HCV negative, anti-HBc positive, anti-HBs positive | 2 (3.5)                      | 56 (96.5)                    | 58 (100)  |
| Subtotal <sup>b</sup>                              | 3 (3.4)                      | 85 (96.6)                    | 88 (100)  |
| G3 total <sup>c</sup>                              | 8 (5)                        | 151 (95)                     | 159 (100) |

<sup>a</sup> P > 0.99 for comparison of HCV-negative patients.  
<sup>b</sup> P > 0.99 for comparison of HCV-positive patients.  
<sup>c</sup> P = 0.4683 for comparison of HCV-negative and HCV-positive patients.

The rate of HBV occult infection was higher in HCV-infected patients who were HIV negative. In HIV-positive patients, the rate of HBV occult infection was lower than that observed in HIV-negative patients and apparently was not affected by lamivudine therapy. Viral coinfections, with their complex and unknown interactions, need to be fully investigated in order to be completely understood.

## REFERENCES

1. Armitage, P., and G. Berry. 1994. Statistical methods in medical research, 3rd ed. Blackwell Scientific, London, England.
2. Bréchet, C., V. Thiers, D. Kremsdorf, B. Nalpas, S. Pol, and P. Paterlini-Bréchet. 2001. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult." *Hepatology* **34**:194–203.
3. Cacciola, I., T. Pollicino, G. Squadrito, G. Cerenzia, M. E. Orlando, and G. Raimondo. 1999. Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. *N. Engl. J. Med.* **341**:22–26.
4. Chemin, I., F. Zoulim, P. Merle, A. Arkhis, M. Chevallier, A. Kay, L. Cova, P. Chevallier, B. Mandrand, and C. Trépo. 2001. High incidence of hepatitis B infections among chronic hepatitis cases of unknown aetiology. *J. Hepatol.* **34**:447–454.
5. Crespo, J., J. L. Lozano, B. Carte, B. Heras, F. Cruz, and F. P. Romero. 1997. Viral replication in patients with concomitant hepatitis B and C virus infections. *Eur. J. Clin. Microbiol. Infect. Dis.* **16**:445–451.
6. Fukuda, R., N. Ishimura, S. Hamamoto, M. Moritani, Y. Uchida, S. Ishihara, S. Akagi, M. Watanabe, and Y. Kinoshita. 2001. Co-infection by serologically-silent hepatitis B virus may contribute to poor interferon response in patients with chronic hepatitis C by down-regulation of type-I interferon receptor gene expression in the liver. *J. Med. Virol.* **63**:220–227.
7. Grob, P., W. Jilg, H. Bornhak, G. Gerken, G. Gerlich, S. Günther, G. Hess, H. Hüdig, A. Kitchen, H. Margolis, G. Michel, C. Trepo, H. Will, A. Zanetti, and I. Mushahwar. 2000. Serological pattern "anti-HBc alone": report on a workshop. *J. Med. Virol.* **62**:450–455.
8. Hoffer, M., H. I. Joller-Jemelka, P. J. Grob, R. Lüthy, M. Opravil, and Swiss HIV Cohort Study. 1998. Frequent chronic hepatitis B virus infection in HIV-infected patients positive for antibody to hepatitis B core antigen only. *Eur. J. Clin. Microbiol. Infect. Dis.* **17**:6–13.
9. Hu, K. Q. 2002. Occult hepatitis B virus infection and its clinical implications. *J. Viral Hepat.* **9**:243–257.
10. Jeantet, D., I. Chemin, B. Mandrand, F. Zoulim, C. Trepo, and A. Kay. 2002. Characterization of two hepatitis B virus populations isolated from a hepatitis B surface antigen-negative patient. *Hepatology* **35**:1215–1224.
11. Jilg, W., B. Hottenträger, K. Weinberger, K. Schlottmann, E. Frick, A. Holstege, J. Schölmerich, and K. D. Palitzsch. 2001. Prevalence of markers for hepatitis B in the adult German population. *J. Med. Virol.* **63**:96–102.
12. Jilg, W., E. Sieger, R. Zchoval, and H. Schätzle. 1995. Individuals with antibodies against hepatitis B core antigen as the only serological marker for hepatitis B infection: high percentage of carriers of hepatitis B and C virus. *J. Hepatol.* **23**:14–20.
13. Kaneko, S., S. M. Feinstone, and R. H. Miller. 1989. Rapid and sensitive method for the detection of serum hepatitis B virus DNA using the polymerase chain reaction technique. *J. Clin. Microbiol.* **27**:1930–1933.
14. Kherfouche, C. 1999. Bedeutung des isolierten hepatitis-serologischen Befundes "anti-HBc-positiv" bei Blutspendern. M.S. thesis. University of Zürich, Zürich, Switzerland.
15. Perrillo, R. P., and E. M. Brunt. 1991. Hepatic histologic and immunohistochemical changes in chronic hepatitis B after prolonged clearance of hepatitis B e antigen and hepatitis B surface antigen. *Ann. Intern. Med.* **115**:113–115.
16. Uchida, T., Y. Kaneita, K. Gotoh, H. Kanagawa, H. Kouyama, T. Kawanishi, and S. Mima. 1997. Hepatitis C virus is frequently coinfecting with serum marker-negative hepatitis B virus: probable replication promotion of the former by the latter as demonstrated by in vitro cotransfection. *J. Med. Virol.* **52**:399–405.