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Incidence of transaminitis among HIV-infected patients with occult hepatitis B☆

Vincent Lo Re III[a,b,c,*], Benjamin Wertheimer[d], A. Russell Localio[b,c], Jay R. Kostman[a], Janel Dockter[e], Jeffrey M. Linnen[e], Cristina Giachetti[e], Zachariah Dorey-Stein[a], Ian Frank[a], Brian L. Strom[b,c], Robert Gross[a,b,c]

[a] *Division of Infectious Diseases, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, USA*
[b] *Department of Biostatistics and Epidemiology, Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA*
[c] *Center for Education and Research on Therapeutics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA*
[d] *Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA, USA*
[e] *Gen-Probe Incorporated, San Diego, CA, USA*

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Abstract

Background: The clinical significance of occult hepatitis B virus (HBV) infection, defined as the presence of HBV DNA in individuals with HBV core antibodies (anti-HBc) in the absence of HBV surface antigen (HBsAg), is unclear in HIV-infected patients. This information is needed to determine the importance of detecting and treating occult HBV in this population.
Objective: To determine if HIV-infected patients with occult HBV infection have an increased incidence of transaminitis.
Study design: We performed a cohort study among randomly selected HBsAg−/anti-HBc+ HIV-infected patients in the Penn CFAR Database and Specimen Repository. HBV DNA was qualitatively detected using a transcription-mediated amplification assay. Hepatic transaminase levels, the main study outcome, were collected at 6-month intervals from the time of occult HBV determination.
Results: Among 97 randomly selected subjects without baseline transaminitis, 13 (13%) had occult HBV. These subjects more frequently had detectable HIV RNA. The 2-year incidence of transaminitis among HIV-infected subjects with occult HBV (50 events/100 person-years) was not significantly different from those without occult HBV (38 events/100 person-years; adjusted incidence rate ratio = 1.36 [95% CI, 0.72–2.59]).
Conclusions: Occult HBV did not increase the incidence of hepatic transaminitis over 2 years. Future studies should determine whether occult HBV is associated with other clinically important outcomes, particularly hepatocellular carcinoma.

Keywords: Occult hepatitis B virus; HIV/hepatitis B virus coinfection; HIV; Transaminitis

1. Background

Occult hepatitis B virus (HBV) infection is defined as the presence of HBV DNA in the serum and/or liver tissue of individuals with HBV core antibodies (anti-HBc) in the absence of HBV surface antigen (HBsAg) [Conjeevaram and Lok, 2001; Hu, 2002; Torbenson and Thomas, 2002]. The reported prevalence of occult HBV among HIV-infected patients has ranged from 0% to 89.5% [Hofer et al., 1998; Nunez et al., 2002; Shire et al., 2004]. The mechanisms responsible for occult HBV remain unclear, but prior investigators have proposed that mutations in the S region of the HBV genome prevented production of HBsAg; host immune dysfunction allowed low-level HBV DNA levels; or that chronic hepatitis C virus (HCV) in HIV/HCV-coinfected individuals inhibited HBV replication [Brechot et al., 2001].

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* Corresponding author at: Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, 711 Blockley Hall, 423 Guardian Drive, Philadelphia, PA 19104-6021, USA.
Tel.: +1 215 573 5964; fax: +1 215 349 5111.
E-mail address: vincent.lore@uphs.upenn.edu (V. Lo Re III).

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A major question about occult HBV infection is whether the low levels of HBV DNA typically associated with occult HBV infection induce necroinflammation and progressive liver damage [Raimondo et al., 2005]. The clinical impact of occult HBV has been examined primarily among HIV-uninfected patients with chronic HCV infection. Among these patients, occult HBV infection promotes hepatic inflammation [Cacciola et al., 1999; Kannangai et al., 2007], accelerates hepatic fibrosis [Raimondo, 2001], and increases the risk of hepatocellular carcinoma [Pollicino et al., 2004]. However, the clinical significance of occult HBV infection in HIV-infected patients remains unclear. We previously reported no association between occult HBV infection and transaminitis in a cross-sectional study of HIV-infected patients [Lo Re et al., 2007], but no longitudinal study has yet examined this question. These data are needed to determine whether testing and treatment of occult HBV infection is warranted for HIV-infected patients. To address this question, we determined whether HIV-infected patients with occult HBV infection had a greater incidence of transaminitis over time than those without occult HBV infection.

2. Patients and methods

2.1. Subjects

We performed a cohort study among subjects enrolled in the Penn Center for AIDS Research (CFAR) Adult/Adolescent Database and Specimen Repository, which was initiated in November 1999 to track demographic, clinical, and laboratory data from HIV-infected patients cared for in Penn affiliated hospitals. Subjects in the CFAR Database have laboratory-confirmed HIV infection, provide informed consent, and complete a standardized questionnaire that collects demographic, medical, psychosocial, and HIV data at enrollment. A serum sample is obtained from each enrolled subject and stored at −70 °C in the CFAR Specimen Repository. Subjects complete a follow-up questionnaire and have a serum sample obtained every 6 months. The study was approved by the Institutional Review Board of the University of Pennsylvania.

All HBsAg−/anti-HBc+ subjects enrolled between November 1, 1999 and December 31, 2002 were eligible for inclusion. Subjects with transaminitis at baseline were excluded. A random sample of 100 subjects was targeted to provide 90% power to detect an incidence rate ratio (IRR) of transaminitis of 2.0 between subjects with and without occult HBV infection. We over-sampled to account for subjects who might not have serum samples available in the CFAR Specimen Repository and who had baseline transaminitis.

2.2. HBV DNA detection

Exposure status was defined by the presence of HBV DNA, which was qualitatively evaluated from each subject's most recent serum sample (through December 31, 2002) using a transcription-mediated amplification (TMA) nucleic acid test for HBV (modified Procleix® Ultrio® Assay; Gen-Probe, Incorporated) that has a lower limit of detection of 15 HBV genome copies/mL [Linnen and Phelps, 2005; McCormick et al., 2006].

2.3. Serologies

To avoid misclassification of hepatitis serostatus, we repeated HBsAg (Elecsys 2010; Roche Diagnostics, Indianapolis, IN), anti-HBc (HBV Core Antibody Assay; Diagnostic Products Corporation, Los Angeles, CA), and hepatitis C virus (HCV) antibody (anti-HCV; Abbott HCV EIA 2.0 or 3.0 enzyme immunoassay; Abbott Laboratories, Abbott Park, IL) tests on the same serum sample in which HBV DNA testing was performed. HBsAg and anti-HBc were performed on the serum of all subjects, while anti-HCV testing was repeated only for HIV-infected subjects who were previously recorded as anti-HCV−. All subjects with HCV coinfection had detectable HCV RNA.

2.4. Study outcomes

The main study outcome was transaminitis, defined as either alanine aminotransferase (ALT) >40 U/L or aspartate aminotransferase (AST) >30 U/L. This definition was chosen because these ALT and AST cut-offs have been recommended as upper limits of normal [Katkov et al., 1991; Kratz and Lewandrowski, 1998]. Although transaminase levels cannot predict definitively the degree of hepatic fibrosis, they are followed routinely to evaluate the clinical status of acute and chronic liver diseases, as well as to determine the hepatotoxicity of various medications. As a secondary outcome, we examined severe transaminitis, defined as an ALT and/or AST level >3 times the upper limit of normal.

2.5. Data collection

Demographic and clinical data, including age, sex, race/ethnicity, duration of HIV diagnosis, possible mode of HIV acquisition, self-reported alcohol use within the past 30 days, use of highly active antiretroviral therapy (HAART, defined as use of three antiretroviral agents), CD4 T lymphocyte count, HIV viral load (determined by Versant HIV-1 RNA 3.0 Assay; Bayer Diagnostics; lower limit of detection: 75 copies/mL), ALT, AST, and platelet counts were collected from the CFAR Database. Transaminase levels and platelet counts were collected at 6-month intervals from the time of occult HBV infection determination, which represented the baseline observation for each subject.

2.6. Statistical analysis

Differences between subjects by occult HBV infection status were assessed using Fisher's exact tests for

categorical data and Wilcoxon rank–sum tests for continuous data. Poisson regression was used to determine unadjusted and adjusted incidence rate ratios (IRRs) of transaminitis with 95% confidence intervals (CIs) [Rosner, 2000]. Potential confounders evaluated included age, sex, race, HIV risk factors, baseline CD4 count, baseline HIV viral load, HAART use, and chronic HCV infection. All data were analyzed using Stata version 8.2 (Stata Corp., College Station, TX). Statistical significance was declared with two-sided p-values < 0.05.

3. Results

Of 1193 HIV-infected patients enrolled in the CFAR Database, we identified 699 (59%) HBsAg−/anti-HBc+ subjects, 398 (33%) HBsAg−/anti-HBc− subjects, 84 (7%) HBsAg+/anti-HBc+ subjects, and 12 (1%) with unknown HBsAg and anti-HBc status. A total of 222 HBsAg−/anti-HBc+ subjects were randomly selected. Thirty-three did not have a serum sample and were excluded. Upon repeat HBsAg and anti-HBc testing, 3 which were HBsAg+ and 7 which were anti-HBc− were also excluded. All anti-HCV− subjects remained HCV-uninfected on repeat testing. Among the 179 remaining subjects, 82 had baseline transaminitis and were excluded. Subjects excluded due to baseline transaminitis less commonly had occult HBV (4/82 [5%] versus 13/97 [13%]; $p = 0.07$), more frequently had chronic HCV (62/82 [76%] versus 37/97 [38%]; $p < 0.001$); had lower median HIV RNA levels (75 copies/mL [interquartile range (IQR), 50–5908] versus 484 copies/mL [75–11,810]; $p = 0.05$); and similar median CD4 cell counts (369 cells/mm^3 [IQR, 219–550] versus 355 cells/mm^3 [IQR, 198–549]; $p > 0.5$) compared to those without baseline transaminitis. The final HBsAg−/anti-HBc+ sample included 97 subjects.

Thirteen (13%) subjects were identified with occult HBV, and they more frequently had detectable HIV RNA (Table 1). There were no statistical differences between the groups in age, sex, race, alcohol use, HCV coinfection, mode of HIV transmission, duration of HIV infection, CD4 cell count, or use of antiretroviral drugs that are active against HBV. The median duration of follow-up from HBV DNA testing was 25.2 months (IQR, 17.1–30.4 months). Subjects with occult HBV had shorter follow-up than those without occult HBV (median 17.2 versus 25.4 months), but this difference was not statistically significant ($p = 0.3$). The median number of 6-month follow-ups for transaminase measurements was also similar between those with occult HBV infection (3 [IQR, 2–4]) and without occult HBV infection (4 [IQR, 3–5]) ($p > 0.5$).

During longitudinal observation, transaminitis occurred at least once in 11 (85%; 95% CI, 55–98%) occult HBV-infected subjects compared to 66 subjects without occult HBV infection (79%; 95% CI, 68–87%; $p > 0.5$). Differences by occult HBV infection status in the frequency of abnormal ALT (7% versus 13%; $p = 0.2$) or AST (50% versus 41%; $p = 0.3$) levels were small. The incidence rates of transaminitis among subjects with (50 events/100 person-years) and without occult HBV infection (38 events/100 person-years) were similar (IRR = 1.32 [95% CI, 0.63–2.53]; $p > 0.5$). There was little change in the IRR after adjusting for alcohol, HAART use, and chronic HCV (adjusted IRR = 1.36; 95% CI, 0.72–2.59). Differences were also slight for the incidence rates of transaminitis between subjects with and without occult HBV infection among HIV/HCV-co-infected subjects (71 events/100 person-years versus 45 events/100 person-years; IRR = 1.57 [95% CI, 0.40–4.44]; $p = 0.4$) and HIV-monoinfected subjects (43 events/100 person-years versus 33 events/100 person-years; IRR = 1.30 [95% CI, 0.49–2.98]; $p > 0.5$). Severe transaminitis occurred at least once during follow-up in 5 subjects, but none had occult HBV infection.

4. Discussion

The clinical impact of occult HBV infection has been examined primarily among HIV-uninfected patients with chronic HCV infection. Among these patients, occult HBV infection promotes transaminitis [Cacciola et al., 1999] and increases the risk of advanced fibrosis and cirrhosis [Cacciola et al., 1999; De Maria et al., 2000; Raimondo, 2001]. Occult HBV infection has also been found to increase the risk of hepatocellular carcinoma in these individuals, possibly by integrating with the host genome and/or synthesis of pro-oncogenic proteins by free intra-hepatic HBV genomes [Brechot et al., 1998; 2001; Hu, 2002; Kubo et al., 2001; Paterlini et al., 1993; Pollicino et al., 2004; Shibata et al., 1999]. In contrast, the clinical significance of occult HBV infection in HIV-infected patients is unclear.

We found that occult HBV infection did not increase the incidence of hepatic transaminitis in HIV-infected patients over a 2-year period, after adjusting for chronic HCV, HAART, and alcohol use. The likely low levels of HBV DNA in occult HBV might not be sufficient to induce clinically significant inflammation. However, longitudinal studies with longer periods of observation that examine other hepatic outcomes, such as hepatic fibrosis by liver biopsy and hepatocellular carcinoma, could indicate the clinical impact of occult HBV in HIV.

Our results differ somewhat from prior longitudinal studies examining the clinical significance of occult HBV infection in HIV-infected patients. Hofer et al. (1998) reported that HCV-uninfected subjects with occult HBV in the Swiss HIV Cohort had an increased frequency of abnormal ALT values compared to those without occult HBV infection (30.1% versus 0%; $p = 0.084$) over a median 31 months of follow-up. The frequency of abnormal ALT values was also higher among HCV-infected occult HBV-infected subjects than HCV-uninfected (54.4% versus 30.1%;

Table 1
Baseline characteristics of subjects, by occult hepatitis B status ($N = 97$)

Characteristic	HBV DNA Negative (N=84)	HBV DNA Positive (N=13)	P-Value
Median age (yrs, IQR)	45 (39-51)	51 (42-54)	0.08
Male sex (%, no.)	85% (71)	85% (11)	>0.5
Race (%, no.)			0.3
African-American	67% (56)	85% (11)	
Caucasian	33% (28)	15% (2)	
HIV risk factor (%, no.)			0.2
Unprotected sex	70% (59)	85% (11)	
Injection drug use history	17% (14)	8% (1)	
Median duration HIV diagnosis (yrs, IQR)	10 (5-13)	10 (6-13)	0.4
Self-reported alcohol use within last 30 days (%, no.)	42% (35)	62% (8)	0.2
No. with HBV surface antibody (%, no.)	63% (53)	46% (6)	0.4
Chronic hepatitis C virus infection (%, no.)	38% (32)	38% (5)	>0.5
Median CD4 cell count (cells/mm^3, IQR)	361 (218-554)	273 (123-378)	0.06
CD4 cell count <200 cells/mm^3 (%, no.)	24% (20)	38% (5)	0.3
Median HIV RNA level (copies/mL, IQR)	187 (75-6,867)	31,663 (1,118-31,663)	0.04
HIV RNA >75 copies/mL (%, no.)	43% (36)	77% (10)	0.03
On HAART[‡] (%, no.)	71% (60)	54% (7)	0.2
Receipt of anti-HBV antiretroviral (%, no.)	61% (51)	31% (4)	0.06
Anti-HBV antiretroviral usage (%, no.)			
Lamivudine	55% (46)	31% (4)	0.1
Tenofovir	11% (9)	0% (0)	>0.5
Emtricitabine	1% (1)	0% (0)	>0.5
No. of anti-HBV antiretroviral agents (%, no.)			0.1
0	39% (33)	69% (9)	
1	55% (46)	31% (4)	
2	6% (5)	0% (0)	

HIV: human immunodeficiency virus; HBV: hepatitis B virus; HAART: highly active antiretroviral therapy; IQR: interquartile range.
[a]HAART defined as use of three antiretroviral agents.

$p < 0.005$). Filippini et al. (2006) found that hepatic flares (defined as an increase in ALT >3 times prior value) occurred more commonly among HIV patients with occult HBV infection than in those without occult HBV infection (64.7% versus 24.6%, $p < 0.005$) over a median 18 months follow-up after the initiation of antiretroviral therapy. Our results might differ because we controlled for relevant confounders (i.e., alcohol use, HAART, and chronic HCV). Differences in the method used to identify occult HBV, the study outcome and its frequency of ascertainment, and the study population might also account for the disparate results between studies.

Our study had several limitations. We used a qualitative assay to detect occult HBV. Second, while the observed difference in the incidence rate of transaminitis was not statistically significant, our study's power was limited by the small number of subjects with occult HBV infection. Third, subjects receiving anti-HBV antiretrovirals might be more likely to be misclassified as occult HBV-negative. However, the qualitative assay used to examine occult HBV infection in this study had a lower limit of detection of 15 HBV genome copies/mL, making such misclassification less likely. Finally, we determined the presence of occult HBV infection from a single serum sample, and HBV DNA levels may fluctuate over time.

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