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Limited infection with occult hepatitis B virus in drug users in the United States

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Abstract

Occult HBV infection (O-HBV) is defined as low level HBV replication in the absence of detectable circulating HBV surface antigen. O-HBV has been implicated in HBV reactivation, advanced liver fibrosis and cirrhosis, reduced interferon response rates, elevated liver enzyme levels, and the development of hepatocellular carcinoma. However, prevalence of O-HBV has not been clearly established in certain at-risk populations, such as injection drug users. Therefore, the current pilot study examined the prevalence of O-HBV in a prospective cohort designed to assess the role of injection and non-injection drug use (IDU) on HIV-associated co-morbidities. Utilizing two distinct real-time PCR assays, HBV DNA was not detected in 99 participants examined. This finding is in contrast to other data from US IDU cohorts and suggests that the prevalence of O-HBV infection is very specific to the cohort studied, is sensitive to other confounding variables such as HCV and/or HIV serostatus, and should not be generalized across risk groups or distinct cohorts.

Keywords

HIV co-infection; Injection drug use; Occult HBV; Surface antigen negative

Introduction

Globally, 350 million people are chronically infected with hepatitis B virus (HBV) - the world's leading cause of cirrhosis and hepatocellular carcinoma (HCC) (20, 28). Chronic HBV infection is characterized by the presence of hepatitis B surface antigen (HBsAg) in the serum. In contrast, occult HBV infection (O-HBV) is defined as low level HBV replication in the absence of detectable circulating HBsAg. Previous studies have demonstrated that O-HBV infection can be transmitted through blood donation, liver transplantation, and perinatal exposure and establish chronic HBV infection in the recipient (3, 10, 13, 23). O-HBV is common in household contacts of persons with chronic HBV infection (11). O-HBV has also been implicated in HBV reactivation, advanced liver fibrosis and cirrhosis, reduced interferon response rates, and elevated liver enzyme levels, as well as the development of HCC (1, 9, 16). Thus, O-HBV infection represents a significant diagnostic challenge with important clinical ramifications.

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disclosures

The authors report no conflicts of interest.

In recent years, the proportion of injection drug users with acute HBV infection has increased (27). A previous study found that 70% of injection drug users (IDUs) were infected with HBV within a year of initiating injection drug use (7). Similarly, others have reported a high prevalence of chronic HBV in IDUs (12, 15). Markers of HBV infection are also elevated among African-American and Hispanic IDUs compared to Caucasian IDUs (reviewed in (4)). The prevalence of O-HBV infection is frequently elevated in persons with HCV (reviewed in (25)). While some studies have reported high levels of O-HBV infection in persons with HIV infection compared to uninfected controls, similar findings were not confirmed in other studies (reviewed in (17)). Data regarding the prevalence of O-HBV infection in the US and its association with recreational drug use and race/ethnicity are currently limited. Thus, the purpose of this pilot study was to examine the prevalence of O-HBV in a cohort of Hispanics infected with or at risk for HBV from illicit drug use.

Methods

Study subjects and laboratory data

Data were derived from the BIENESTAR study, a community-based prospective cohort of adult Hispanics living in the greater Boston area designed to examine the role of injection and non-injection drug use on HIV-associated co-morbidities as described elsewhere (5). Participants eligible to join the BIENESTAR cohort were Hispanic adults (≥ 18 years of age) who spoke Spanish fluently and were HIV seropositive or at risk of HIV through current illicit drug use, including heroin, cocaine, amphetamines, or methadone taken by any route. Between September 1999 and March 2009, 512 participants were recruited, including a control group of 93 Hispanics who did not use illicit drugs. Information regarding demographics, HIV disease progression, and current/past use of antiretroviral therapies (ART) was collected at each visit. At the first study visit, participants were asked to provide a detailed history of their past alcohol and drug use that was updated at subsequent visits. To examine the prevalence of O-HBV, a subgroup of 99 participants from the original cohort was selected based on the following criteria: 1) baseline serology was HBsAg negative, 2) known HCV status (positive or negative), 3) not a control participant, and 4) sufficient serum from the baseline visit available for additional analyses.

With the exception of hepatitis testing, all assays were conducted in the clinical laboratories of the Tufts Medical Center. Self-reported HIV status was confirmed by enzyme immunoassay (Genetic Systems™ HIV1 /HIV2 Plus O EIA, Biorad Laboratories, Redmond, WA). HIV RNA levels were measured by reverse transcriptase polymerase chain reaction (RT-PCR) using a Roche Amplicor Monitor system (Roche Molecular Systems, Somerville, NJ) with a lower detection limit of 400 copies/mL. CD4 cell counts were determined by FACS analysis. Among participants who were not healthy controls, 411 were tested for hepatitis C virus (HCV) RNA at baseline using the AMPLICOR Hepatitis C Virus test, Version 2.0 (Roche Molecular Systems Inc., Branchburg, NJ, USA). HBsAg was tested at baseline in 283 participants using the HBsAg Enzyme Immunoassay 3.0 (Biorad Laboratories, Redmond, WA, USA). The Institutional Review Board at Tufts Medical Center approved the study, and all participants provided informed consent.

Testing for O-HBV infection

HBV DNA was isolated from 200uL of patient serum using the QIAamp UltraSens virus kit. 5uL of each DNA sample was tested for HBV DNA utilizing a real-time PCR assay that simultaneously co-amplified two separate regions of the HBV genome, including a 432 base pair fragment of the surface gene (nucleotides 261-692) and a 119 base pair fragment of the core gene (nucleotides 2269-2387) as reported previously (22) (method #1). The primer sets were designed to amplify highly conserved regions among published HBV genomes

representing genotypes A-F as reported by Chen *et al* (2). The lower limit of detection was determined to be 100 IU/mL. Any replicate with a detectable Ct value and its corresponding duplicate were subjected to gel electrophoresis to determine the PCR product size. A randomly chosen subset of 17 samples with non-specific PCR products was evaluated using a second real-time PCR method. 2uL from each DNA sample was used to amplify the same HBV fragments utilizing the same primer sets in duplicate with the SsoFast EvaGreen Supermix (Bio-Rad). The thermal profile consisted of 98°C for 2 min, followed by 40 cycles of 98°C for 3 seconds, 56°C for 3 seconds, and 72°C for 3 seconds. A melting curve from 65°C to 95°C at 0.5°C increments for 3 seconds/step, was used for all replicates and run on a Bio-Rad CFX96 real-time system (method #2). As with method #1, all replicates were subjected to gel electrophoresis to determine the PCR product size.

Statistical analyses

Statistical analyses were done using SAS version 9.1 (SAS Inc., Cary NC). In Table 1, participant characteristics were reported as the mean and standard deviation (SD) for normally distributed continuous data or the median (25th, 75th percentile) for non-normally distributed continuous data. The differences in baseline characteristics among the groups were tested for significance with analysis of variance or Student's t-test for normally distributed variables and the Wilcoxon rank sum test for skewed variables, using either the raw or log-transformed data, when appropriate. Chisquare or Fisher's exact test was used to test between group differences for categorical variables.

Results

The 99 selected participants included 43 HIV-positive drug users, 28 HIV-positive non-drug users, and 28 HIV-negative drug users. Overall, 64% were male, and 54% were HCV RNA positive. The median age, gender distribution, and prevalence of HCV RNA differed significantly among the three groups (Table 1). ART utilization was 72% and 79% in the HIV-positive IDU and HIV-positive non-IDU groups, respectively. Lamivudine – which is active against both HIV and HBV – was used by 26% and 21% of participants in these groups. Among the 99 participants, none had detectable HBV DNA in the serum at the baseline visit, while all positive control reactions were clearly positive. A random subset of 17 serum samples was retested via an alternative real-time PCR strategy for HBV DNA. Again, no HBV DNA was detected, despite all positive control reactions being positive.

Discussion

A high prevalence (45%) of O-HBV infection has been reported in the ALIVE study, a US-based cohort of HCV-positive IDUs who are predominantly (95%) African-American (24). While no other studies have specifically focused on the prevalence of O-HBV in IDUs in the US to date, several US studies have included IDUs as a risk group. Lo Re *et al.* reported a 9.5% prevalence of O-HBV in a US population that included 24% IDUs (14). Others have reported prevalence rates of 0% – 2% in cohorts that included IDUs (18, 21, 22, 26). In the current study, we observed no O-HBV infections despite the use of two previously validated real-time PCR assays for the detection of low levels of HBV DNA from diverse HBV genotypes. It is unclear why the prevalence of O-HBV was elevated in the ALIVE cohort (24) compared to the BIENESTAR cohort, although differences in racial distribution (95% black in ALIVE versus 100% Hispanic for BIENESTAR), HCV serostatus (100% versus 54%), HIV serostatus (30% versus 72%), and HBV vaccination rates (not evaluated in ALIVE) in the two cohorts could be contributing factors. Other studies of O-HBV included only a modest percentage of IDUs and/or a low percentage of Hispanics (14, 21, 24). Additionally, studies that preselected individuals based on Core antibody (HBcAb) only status may limit the comparability to our study that examined HBsAg negative individuals

regardless of HBcAb status. Additionally, the sensitivity of the HBV DNA assay utilized should be considered, although assays for HBV DNA have similar limits of detection (15-200 copies/mL).

Social networking among IDUs may play an important role for HBV transmission as previously described for HIV (6, 19). Interestingly, a study in Hungary identified two clusters of IDUs – a marginalized group and a socially integrated group (8). While these groups shared several common characteristics, there were also differences in their drug use practices, social and injecting risk networks, sex partner selection, and travel patterns. Preliminary analyses have also explored social networks in relation to HBV infection, although no specific data on O-HBV are available currently (29). Overall, these data strongly suggest that the prevalence of O-HBV infection is very specific to the cohort studied and cannot be generalized across risk groups or distinct cohorts. Given the modest number of samples included in this pilot study, larger studies in cohorts with several distinct risk factors – HIV, HCV, and/or IDU – are warranted, as are longitudinal investigations to more accurately establish the impact of O-HBV on liver disease.

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Table 1

Characteristics of study participants at baseline

	HIV-positive drug user (n = 43)	HIV-positive non-drug user (n = 28)	HIV-negative drug user (n = 28)
Age (years)	41 (7)	40 (10)	34 (8) *
Male %	35 (81)	8 (28)	20 (71) *
HCV RNA positive	29 (67)	4 (14)	20 (71) *
BMI (kg/m ²)	25.5 (23.1, 29.0)	25.8 (21.4, 29.5)	25.4 (23.6, 30.5)
CD4 cell count	310 (178, 485)	331 (210, 508)	1046 (848, 1140)
HIV RNA (log ₁₀ copies/mL)	3.4 (1.1)	2.9 (0.94)	NA
Detectable HIV RNA	19/42 (45)	17/27 (63)	NA
ART use	31 (72)	22 (79)	NA
Lamivudine use	11 (26)	6 (21)	NA

Data are number (%)

mean (SD)

or median (25th, 75th percentile)

HIV, human immunodeficiency virus

HCV, hepatitis C virus

BMI, body mass index

ART, antiretroviral therapy

NA, not applicable

*
p <0.01