

Occult Hepatitis B Virus Infection in Immunocompromised Patients

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Occult hepatitis B infection is characterized by hepatitis B virus (HBV) DNA in the serum in the absence of hepatitis B surface antigen (HBsAg). We assessed occult HBV infection prevalence in two groups of immunocompromised patients (maintenance hemodialysis patients and HIV-positive patients) presenting HBsAg-negative and anti-HBc positive serological patterns, co-infected or not by HCV. Thirty-four hemodialysis anti-HIV negative patients, 159 HIV-positive patients and 150 blood donors who were anti-HBc positive (control group) were selected. HBV-DNA was detected by nested-PCR. Occult hepatitis B infection was not observed in the hemodialysis patients group but was found in 5% of the HIV-patients and in 4% of the blood donors. Immunosuppression in HIV positive patients was not a determining factor for occult HBV infection. In addition, no significant relationship between HBV-DNA and HCV co-infection in the HIV-positive patient group was found. A lack of significant associations was also observed between positivity for HBV-DNA and CD4 count, viral load and previous lamivudine treatment in these HIV-positive patients.

Key-Words: Occult HBV infection, HBV-DNA, anti-HBc alone, hemodialysis, HIV, immunosuppressed patients.

Hepatitis B virus (HBV) infection is a major global health problem. It is estimated that about 350,000,000 individuals are infected by HBV; infection can induce a wide spectrum of clinical forms, ranging from a healthy carrier state to cirrhosis and hepatocellular carcinoma [1]. HBV infection is usually diagnosed when circulating HBsAg is detected. However, the availability of highly sensitive molecular biology techniques has also allowed the identification of HBV infection in HBsAg-negative individuals, with or without circulating antibodies to HBsAg (anti-HBs) and/or hepatitis B core antigen (anti-HBc) [2-5].

The failure to detect HBsAg, despite the persistence of the viral DNA, is due in most cases to the strong suppression of viral replication and gene expression that characterizes this “occult” HBV infection [6-9]; although the mechanisms responsible for suppression of HBV are not well understood.

Occult HBV infection has been studied in selected groups or individuals in which its prevalence is believed to be higher, such as intravenous drug abusers, patients on maintenance hemodialysis, organ transplant patients, patients with chronic HBV infection [10], and patients co-infected with human immunodeficiency virus (HIV) or hepatitis C virus (HCV) [11].

However the magnitude and pattern of occult HBV infection among immunocompromised patients, such as hemodialysis and HIV patients, is not well established yet. In maintenance hemodialysis patients, the risk of acquiring HBV and HCV infection is high, due to the dialysis process [12,13]; consequently, the probability of patients becoming chronic carriers is higher than for the general population. In HIV-positive patients, co-infection with HBV and HCV is common due to the common route of transmission of these diseases. In patients coinfecting with HBV and HIV, it has been suggested that HIV interferes with the natural history of HBV infection by enhancing HBV replication [14] and that immunosuppression associated with HIV infection allows reinfection or reactivation of a past HBV infection [15,16].

We examined occult HBV infection prevalence in two groups of immunocompromised patients (maintenance hemodialysis patients and HIV-positive patients), who present HBsAg-negative and anti-HBc positive serological patterns, co-infected or not by HCV. Possible correlations were investigated between the prevalence of HBV DNA, viral loads, CD4 levels and previous lamivudine administration in the HIV group.

Material and Methods

The study was performed between 2002 and 2005 in the Laboratory of the Hepatitis Study Group of the Infectious Diseases Department, School of Medical Sciences, State University of Campinas (UNICAMP), after approval by the Institutional Ethics Committee. All adult hemodialysis patients originated from two dialysis centers in São João da Boa Vista, São Paulo State, Brazil. HIV-positive patients were selected during their clinical follow-up at the Clinical Hospital of the University of Campinas; only those presenting a serological pattern of HBsAg negative and anti-HBc positive, co-infected or not by HCV, were included.

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Table 1. Prevalence of occult HBV infection (HBV DNA positivity) in the groups studied.

Groups	No	HBV DNA positive (%)	HBV DNA negative (%)	p value
GI – Hemodialysis Patients	34	- -	34 (100)	
Anti-HBc+/Anti-HBs-/Anti-HCV-	8	- -	8 (100)	NS
Anti-HBc+/Anti-HBs+/-/Anti-HCV-	19	- -	19 (100)	NS
Anti-HBc+/Anti-HBs-/Anti-HCV+	1	- -	1 (100)	NS
Anti-HBc+/Anti-HBs+/-/Anti-HCV+	6	- -	6 (100)	NS
GII – HIV-positive patients	159	8 (5.0)	151 (95.0)	
Anti-HBc+/Anti-HBs-/Anti-HCV-	15	1 (6.7)	14 (93.3)	NS
Anti-HBc+/Anti-HBs+/-/Anti-HCV-	56	4 (7.2)	52 (92.8)	NS
Anti-HBc+/Anti-HBs-/Anti-HCV+	30	1 (3.3)	29 (96.7)	NS
Anti-HBc+/Anti-HBs+/-/Anti-HCV+	58	2 (3.5)	56 (96.5)	NS
GIII – Blood donors	150	6 (4.0)	144 (96.0)	
Anti-HBc+/Anti-HBs-/Anti-HCV-	100	6 (6.0)	94 (94.0)	NS
Anti-HBc+/Anti-HBs+/-/Anti-HCV-	50	- -	50 (100)	NS

A total of 343 samples were analyzed. The samples were distributed into three groups:

Group I

Thirty-four adult hemodialysis patients who were HBsAg negative, anti-HBc positive and anti-HIV negative (18 [53%] males and 16 [47%] females; mean age 53.7 ± 10.10 years; range 29-76 years) were included. Seven (20.6%) of them were also co-infected with HCV (Table 1).

Group II

One hundred and fifty nine HIV-positive patients co-infected by HBV (HBsAg negative and anti-HBc positive) were selected (129 [81%] male and 30 [19%] female; mean age 39.9 ± 8.31 years; range 22-67 years). Eighty-eight (55.3%) of them were also co-infected by HCV (Table 1).

Group III

One hundred fifty volunteer blood donors with HBsAg negative and anti-HBc positive serological patterns (102 [68%] males and 42 [32%] females; mean age 41.8 ± 10.78 years; range 18-64 years) were studied. This group was being analyzed for another study and was included in our study as a control group. All of these individuals were negative for syphilis, anti-HCV, anti-HIV and anti-HTLV I/II (Table 1).

Serology

Testing for HBsAg was performed with a third-generation monoclonal enzyme immunoassay (Abbott/Murex, Dartford, England). Anti-HBc was screened using a competitive enzyme immunoassay (Abbott/Murex, Dartford, England). Anti-HBs was tested using an enzyme immunoassay (ETI-AB AUK-3, Diasorin, Italy). Antibodies to HCV were detected with a fourth-generation enzyme immunoassay (Abbott/Murex Dartford, England). For detection of antibodies to HIV 1/2, two different assays were utilized, a third-generation assay (Abbott/Murex Dartford, England) and an antigen/antibody combo assay

(Abbott/Murex, Dartford, England), confirmed with a Western Blot. All samples were tested according to the manufacturer's instructions. The samples were considered positive when absorbance was greater than the cutoff and presented the same result in duplicate.

The samples that tested HBV-DNA positive were retested for HBsAg, in a fully automated quantitative chemiluminescent microparticle immunoassay (CMIA) Architect HBsAg (Abbott Laboratories, Sligo, Ireland) that is able to detect the most frequent mutants of the HBV S region, in order to confirm the status of occult HBV infection. This Architect HBsAg Qt assay was used according to the manufacturer's protocol. The resulting chemiluminescent reaction was measured in relative light units (RLUs). A direct relationship exists between the amount of HBsAg in the sample and the number of RLUs. Architect HBsAg Qt is capable of measuring a wide range of from 0.05 to 250 IU/mL.

HBV-DNA

Serum samples were stored at -20° C and thawed immediately before use. For detection of HBV DNA, nested *in house* PCR was used as described by Kaneko et al. [17]. The 5'-3' nucleotide (nt) sequences of the primers were as follows: outer primers HBV1763 (GCT TTG GGG CAT GGA CAT GA CCC GTA TAA)' and HBV2032R (CTG ACT ACT AAT TCC CTG GAT GCT GGG TCT-3'); inner primers HBV 1778E (GAC GAA TTC CATT GA CCC GTATAAAGAATT)' and HBV 2017RB (ATG GGA TCC CTG GAT GCT GGG TCT TCC AAA-3'). After both reactions, a 270 nucleotide fragment was obtained and detected by agarose gel electrophoresis. To prevent carryover contamination during PCR, each step of the procedure was performed in a separate room with dedicated equipment and directional flow from the beginning of the procedure to the end. Negative controls consisting of water were also included in each extraction run, and an extra negative control consisting of water was included as a last sample in each PCR run. Serial dilutions were made in an HBsAg low-titer performance

Table 2. Epidemiological, clinical and laboratorial findings in HIV-positive patients and blood donors.

Clinical features	No	HBV-DNA positive (%)	HBV DNA negative (%)
HIV-positive patients	159	8 (5.0)	151 (95.0)
Source of infection			
Intravenous drug abusers	46	3 (6.5)	43 (93.5)
Sexual	51	4 (7.8)	47 (92.2)
Sexual/Parenteral	17	1 (5.8)	16 (94.2)
Unknown	45	-	44 (100)
CD4+ cell count (cells/mL)			
<200	37	1 (2.7)	36 (97.3)
200-350	32	1 (3.1)	31 (96.9)
>350	90	6 (6.7)	84 (93.3)
Plasma HIV-RNA load (copies/mL)			
<10 ⁴	113	5 (4.4)	98 (94.6)
10 ⁴ to 10 ⁵	25	1 (4.0)	24 (96.0)
>10 ⁵	21	2 (9.5)	19 (90.5)
HAART treatment			
Yes	130	7 (5.4)	123 (94.6)
No	29	1 (3.5)	28 (96.5)
Lamivudine			
Yes	80	4 (5.0)	76 (95.0)
No	50	4 (8.0)	46 (92.0)
Blood donors	150	6 (4.0)	94 (94.0)
Risk Factors			
Sexual	29	2 (6.8)	27 (93.2)
Parenteral	35	1 (2.9)	34 (97.1)
Parenteral/Sexual	17	- (100)	17 (100)
Unknown	69	4 (5.8)	65 (94.2)

Table 3. Clinical aspects, serological data, plasma HIV-RNA load, CD4+ count and lamivudine as part of HAART therapy in HIV patients with occult HBV infection.

Patients	Date of diagnosis	Sex	Age in years	Risk factor	HIV status	HIV viral	CD4+ load	Lam	Anti-HBs (cells/mL)	Anti-HCV
1	1994	M	44	Bisexual	AIDS B3	2,500	521	no	no	no
2	1993	M	36	IDA	AIDS	750	418	no	yes	yes
3	1995	M	38	IDA	AIDS	170,000	280	yes	yes	yes
4	1995	M	33	IDA	AIDS C3	22,000	154	no	no	yes
5	1992	M	62	Homo	AIDS C3	<LD	461	yes	yes	no
6	1997	F	34	IDA	AIDS	110,000	551	yes	yes	no
7	1997	F	47	HIV partner	AIDS	<LD	409	yes	yes	no
8	1998	M	43	IDA/Homo	AIDS A2	<LD	501	no	yes	no

IDA=intravenous drug abusers; IDA/Homo=intravenous drug user and homosexual; Homo=homosexual; Lam=lamivudine; LD=limit of detection.

panel (PHA 105, Boston Biomedica Inc, Boston, Mass.), which made it possible to establish a limit of detection by nested PCR of 10² copies/mL.

Statistical Analysis

Statistical comparison of distribution was carried out using either the chi-square or the Fisher exact tests, as applicable. For comparison of means and variances, other tests were used (Student t, Kruskal-Wallis, and Mann-Whitney tests). The level of significance was set at p<0.05.

Results

Among 34 maintenance hemodialysis patients (Group I), 27 (79.4%) were anti-HBc positive and anti-HCV negative; among these, eight (29.6%) were anti-HBc alone and seven (20.6%) were anti-HBc plus anti-HCV positive. HBV-DNA was not detected in any serum from the patients in this group (Table 1).

Among the 159 HIV-positive patients (Group II), 71 (44.6%) were anti-HBc positive and anti-HCV negative, 15 (21%) of them were anti-HBc alone, while the other 88 (55%) patients in this group were co-infected with HCV; anti-HBc alone was

found in 30 (34%) of patients of this group. HBV-DNA was detected in eight (5%) of the patients in this group. Among the HIV-positive patients that were HCV-negative, HBV DNA was found in 5/71 (7.0%); one (6.7%) presented anti-HBc alone and four (7.2%) were anti-HBc and anti-HBs positive. HBV DNA was also observed in 3/88 (3.4%) HIV-positive patients co-infected with HCV, one (3.4%) was anti-HBc alone and two (3.5%) had anti-HBc and anti-HBs positive markers. No significant difference in the presence of HBV-DNA was observed in HIV-positive patients co-infected or not with HCV; similarly, no significant difference was observed in the presence or absence of anti-HBs (Table 1).

Among the 150 blood donors (Group III), HBV DNA was detected in six (4%). No HBV-DNA was found in blood donors that were anti-HBs positive only, among those that presented anti-HBc alone. The prevalence of HBV DNA in Groups II and III was similar (5 and 4% respectively); in Group II, HBV-DNA was also found in HIV-positive patients that were anti-HBs positive (Table 1).

Among the 159 patients infected with HIV-1, 51 (32%) had as a risk factor for acquired HIV and HBV unsafe sexual practices, 46 (29%) were intravenous drug abusers, 17 (10.7%) presented two risk factors (sexual and parenteral), and in 45 (28.3%) it was not possible to determine the risk factor. Among 150 blood donors, 35 (23.4%) related parenteral exposure, 29 (19.3%) reported unsafe sexual practices, 17 (11.3%) had both parenteral and sexual exposure, and in 69 (46%) no known risk factors were reported. The finding of HBV DNA was not significantly associated with risk factors in the two groups ($p > 0.05$, Table 2).

Among the other HIV-positive patients, 90 (57%) presented a TCD4+ count of more than 350 cells/mL, 113 (71%) presented a viral load of less than 10,000 copies/mL, 130 (82%) were under antiretroviral treatment, and 80 (61%) of the patients received lamivudine as part of a HAART scheme. HBV-DNA was detected in six (6.7%) of the HIV-positive patients, with CD4+ counts above 350 cells/mL, five (4.4%) had a viral load < 10,000, in four (5%) HIV-positive patients who were being treated with lamivudine, and in four (8%) of the patients who had not used lamivudine. The frequency of occult HBV infection was not significantly different, with variations in TCD4+ count, HIV viral load or lamivudine treatment ($p > 0.05$, Table 2).

Table 3 shows the clinical and serological features, as well as the TCD4+ counts and HIV viral load of the eight patients who tested positive for HBV-DNA. All patients who were HBV-DNA positive were classified as having AIDS. Six (75%) of them were anti-HBs positive; only one was anti-HBc alone. These patients were in follow-up for an average of 10 years since the initial anti-HIV serology-confirmed diagnosis. Among these patients, six (75%) had TCD4+ counts above 350 cells/mL and three (37.5%) presented a viral load below the detection limit.

Discussion

The relationship between occult HBV infection and liver disease is still unclear. Occult HBV infection has received

much attention recently, since it has been detected in patients with hepatocarcinoma [18], in blood donors and transfused patients [19,20], and in patients infected with hepatitis C virus [21-23]. In chronic HCV patients on hemodialysis, the prevalence of occult HBV infection is not yet well known [22,24]. Some studies have demonstrated that HIV infection modifies the natural history of HBV and HCV infections [25], increasing the progression to chronic disease. Among immunocompromised patients, HBV-DNA can be detected in 10% of HBsAg negative/anti-HBc positive patients. Reactivation of HBV is rare [10]; reactivation of HBV has recently been described in an HIV-positive patient who was anti-HBc alone [26].

The main characteristic of occult HBV infection is HBV DNA at low levels in serum and hepatic tissue [27]. Due to the high sensitivity (100 copies/mL) of the *in house* PCR used in our study, we were able to identify HBV DNA in the serum of HBV infected patients even when they had few copies. Commercial disposable tests only detect more than 1,000 copies/mL.

The data recorded from hemodialysed patients in our study were similar to those encountered in another study made in Italy [12], in which no occult infection was found in 213 patients from five different centers.

Clearance of HBV-DNA is expected to occur following loss of HBsAg and seroconversion to anti-HBs; anti-HBs is considered the classical marker of past-resolved infection. In some cases, however, HBV DNA has been detected in anti-HBs-positive sera [5] and even in individuals without HBV serological markers [28]. Persistence of HBV DNA after seroconversion to anti-HBs has been documented more frequently in patients with chronic liver disease than in asymptomatic carriers. HBV DNA was found in 10% of patients with anti-HBc alone and in 20% of patients with anti-HBs and anti-HBc. These results are in agreement with previous findings, which demonstrated that HBV DNA detection is elevated in HBsAg-negative, HIV-infected patients [29].

A number of explanations for the persistence of HBV DNA in HBsAg-negative samples have been proposed, including HBV DNA in low copy numbers [27], genetic variations of the S gene [30,31], and immune complexes in which HBsAg is hidden [32].

A key question to understanding the role of occult HBV infection is whether small amounts of HBV will lead to progressive liver disease. Systemic detection of HBV DNA levels in HIV-positive individuals, with and without HBsAg, as well as further study of the association between HBV DNA and markers of liver function, should help to answer this question.

Suppression of HBV DNA by lamivudine has been demonstrated in studies of patients chronically infected with HBV [33-35]. In cases of co-infected HBV-HIV patients who received lamivudine as part of their antiretroviral treatment, loss of HBV DNA after one year of treatment was observed in 96.3% of patients when assessed by molecular hybridization

and 88.5% of patients assessed by PCR [36]. However, trials on lamivudine treatment have been performed with HBsAg-positive patients alone, co-infected with HIV or not; the effects of lamivudine were analyzed at various intervals after the beginning of therapy [37,38].

The loss of HBV DNA due to lamivudine treatment is usually accompanied by significant histological and biochemical improvement [39]. The main limitation for the use of lamivudine is the selection of resistant mutations that affect the YMDD motif of HBV polymerase and that may arise during therapy. Such mutations have also been reported in studies of patients co-infected with HIV and HBV [37,40].

In our cross-sectional study, no significant differences in the prevalence of HBV DNA or of HBV viral load were observed between patients under lamivudine treatment and those not receiving lamivudine. Indeed, it is known that increased duration of lamivudine treatment is associated with an increasing number of resistance mutations. In a recent study conducted with HIV-HBV co-infected patients, it was observed that the rate of resistance mutations increased from 25% after one year of treatment to 52% after two years [38]. The efficacy of lamivudine treatment and the rate of resistance mutations in patients with occult HBV infection have not yet been investigated. To determine the effect of lamivudine treatment on HBsAg-negative patients, the rates and pattern of lamivudine-resistant mutations should be investigated in this particular group of HIV-HBV infected individuals.

In conclusion, the finding of HBV DNA in individuals without serological markers for HBV reinforces the importance of a revised definition of hepatitis B, which is currently based on HBsAg.

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