Occult Hepatitis B in Cuban HIV Patients

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ABSTRACT

INTRODUCTION Co-infections between hepatitis B and HIV viruses are frequent due to their similar epidemiological characteristics. Worldwide, hepatitis B infection is one of the main causes of hepatocellular carcinoma and cirrhosis. In Cuba as elsewhere, prevalences of hepatitis B and hepatitis C viral infections are higher in persons with HIV. These hepatitis viruses act as opportunistic infections in persons with HIV. In other contexts, persons with HIV have been found to be at higher risk for occult hepatitis B, defined as the presence in serum or plasma of hepatitis B virus DNA and antibodies to its core antigen, in the absence of hepatitis B surface antigen.

OBJECTIVES Describe occult hepatitis B prevalence in Cuban HIV-positive patients and explore possible associations with their clinical characteristics.

METHODS A total of 325 serum samples from patients positive for HIV and negative for hepatitis B surface antigen were studied, divided into two groups, Group 1, negative for hepatitis C virus; and Group 2, positive for hepatitis C virus. Exposure to hepatitis B was determined by testing for hepatitis B core antigen; samples positive for hepatitis B core antigen were then examined for presence of antibodies to hepatitis B surface antigen. Both determinations were done by ultramicroELISA. In samples positive for hepatitis B core antigen with levels of antibodies to hepatitis B surface antigen of <50 IU/L, real-time polymerase chain reaction was used to detect hepatitis B DNA and its presence examined in relation to several clinical variables. All data were obtained from patients' clinical records.

RESULTS In the hepatitis-C–negative group, 27.9% (68/243) of serum samples tested were positive for hepatitis B core antigen. In the hepatitis-C–positive group, 37.8% (31/82) were positive for hepatitis B core antigen. Total hepatitis B virus exposure prevalence was 30.4% (99/325); 54.5% (54/99) showing low immunity (hepatitis B virus surface antigen <50 IU/L) and 24% of these (13/54), occult hepatitis. There was no statistically significant association between hepatitis B virus DNA and any of the clinical variables studied.

CONCLUSIONS Low-immunity HIV-positive persons in our study were exposed to hepatitis B virus. Diagnosis of occult hepatitis B infection is frequent in these patients. This study suggests that diagnostic protocols for persons with HIV and without hepatitis B surface antigen should include testing for hepatitis B core antigen, with positive results followed by molecular techniques to detect occult hepatitis B. This study makes a useful contribution to prevention and control of hepatitis B in Cuba.

KEYWORDS Hepatitis B, hepatitis B antigens, hepatitis C, AIDS, AIDS-related opportunistic infections, Cuba

INTRODUCTION

Co-infections with hepatitis B (HBV) and HIV viruses are a public health challenge, due to their increasing incidence, population impact and similar epidemiological characteristics.[1] HIV affects the prognosis and clinical course of HBV and thus prospects for its prevention and control. HBV is considered an opportunistic infection in HIV patients, when diminished immunity accelerates the progress of hepatitis B virus (HBV) infection to chronic hepatitis.[2–4] HBV prevalence is higher in HIV-infected individuals than in the general population; in some regions of the world, one of every three HIV-infected individuals has HBV or hepatitis C (HCV) markers, or both.[5]

Traditionally, HBV is diagnosed by serology techniques to detect antigens or antibodies. Since the 1990s, in Cuba and elsewhere, molecular techniques have enabled detection of HBV DNA.[6,7] HBV surface antigen (HBsAg) in serum or plasma is the marker commonly used to diagnose HBV infection, but there are persons in whom HBV DNA is detected in the absence of HBsAg.[8,9] Such occult HB infection has been found in patients:[9]

- with HBV risk factors;
- with hepatic carcinoma;
- who are chronic HBV carriers;
- with HBV–HCV co-infection;
- who are immunosuppressed;
- with inexplicable increases in hepatic enzymes (cryptogenic cirrhosis);
- who are co-infected with HIV and HCV.

Occult HBV is frequent in persons with HIV, its detection depending on the molecular techniques used and the endemic pattern of both viruses in the populations studied.[1] It is also reported to be very frequent in persons with HIV−HCV co-infection. Immunosuppressed HIV-positive persons may not respond to recombinant HBV vaccine or have low antibody response to HBsAg.[1,2] Hence, in HIV patients who test HBsAg negative, HBV DNA should be determined before starting high activity antiretroviral therapy (HAART) so that anti-HBV antiretrovirals can be included if necessary.

At the end of 2009, there were 12,217 HIV-positive persons in Cuba,[10] but occult HBV studies among these individuals have not been carried out. Research on such patients with HBsAg negative sera would have important implications for appropriate clinical management and improved prognosis.

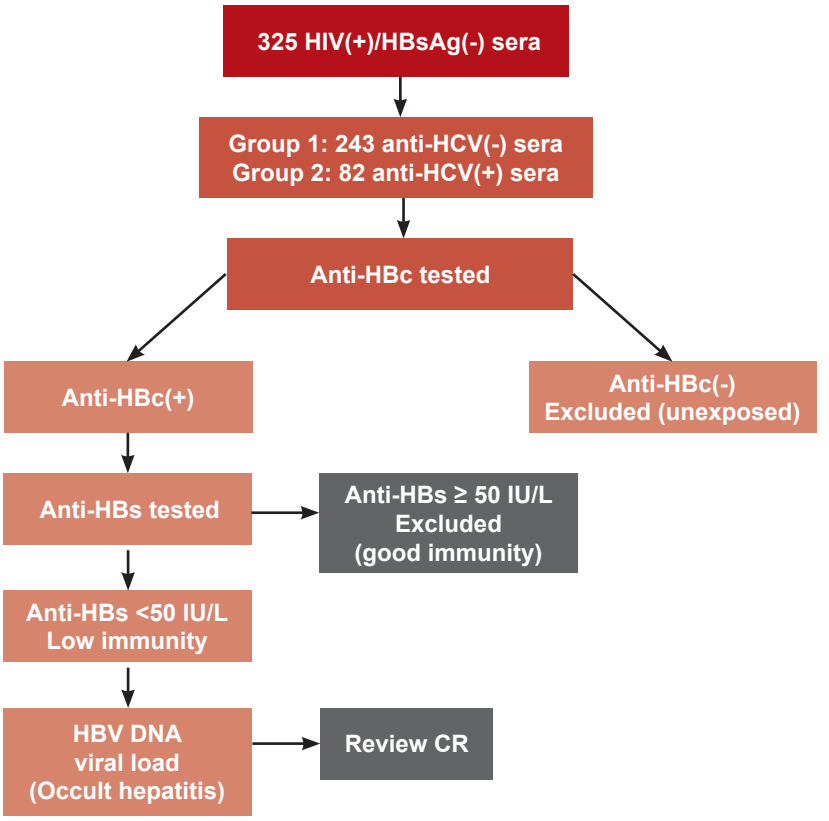
The objectives of this study were to describe occult HBV prevalence in HIV-positive persons in Cuba and to explore possible associations between occult HBV infection and patients' clinical characteristics.

METHODS

A cross-sectional study was carried out using 325 HBsAg-negative serum samples from among the 1770 HIV-positive adults treated at the Pedro Kourí Tropical Medicine Institute (IPK, its Spanish acronym) during 2008 and 2009. At IPK, HBsAg and HCV antibody (anti-HCV) tests are included in annual follow up of HIV-positive persons because of the frequent association between HIV and these two hepatitis viruses. The HBsAg-negative

samples were divided into two groups: anti-HCV–positive and anti-HCV–negative. HBV exposure was determined by presence of antibodies to HBV core antigen (anti-HBc); anti-HBc–negative sera were excluded from the study. Immunity was assessed by detection of antibodies to HBV surface antigen (anti-HBs). Anti-HBc–positive sera with anti-HBs levels of <50 IU/L were examined for viral load by HBV DNA detection. The diagnostic algorithm is displayed in Figure 1.

Figure 1: Diagnostic algorithm



CR: clinical record

A form was prepared to record variables from clinical records: time elapsed from HIV diagnosis, AIDS stage, alanine aminotransferase (ALT), aspartate aminotransferase (AST), erythrosedimentation rate and CD4 cell count. Not all data were available for all cases, so totals are not uniform across variables.

Serological techniques UltramicroELISA (enzyme-linked immunosorbent assay) was used to test all serum samples for anti-HBc, to determine HBV exposure. Samples with fluorescent values equal to or below the threshold value (0.2 x median of the negative controls) were considered positive. All samples positive for anti-HBc were tested for anti-HBs, also with ultramicroELISA. Anti-HBs levels of ≥10 IU/L were considered protective; levels <10 IU/L were considered non-protective or negative.

Traditionally, the degree of immune response is classified thus:[11]

- Non-protective: anti-HBs <10 IU/L
- Hypo response: anti-HBs 10 IU–99.9 IU/L
- Normal response: anti-HBs 100 IU–999.9 IU/L
- Hyper response: anti-HBs ≥1000 IU/L

In this study, low immunity was defined by anti-HBs levels of <50 IU/L, and a normal or good response by levels of ≥50 IU/L, as recommended by several authors.[1,9]

Diagnostic kits (UMELISA Anti-HBsAg, UMELISA Anti-HBcAg, UMELISA HBsAg) used were produced by the Immunoassay Center, Havana, Cuba. For both techniques the MW2001 washer (TecnoSuma, Cuba) and the PR-521 reader, (TecnoSuma, Cuba) were used. Test validity and results interpretation were performed automatically by the manufacturer's program (software package for strip readers, version 8.0).

Molecular techniques Real-time polymerase chain reaction (RT-PCR) was used to detect and quantify HBV DNA in samples that had exhibited exposure to HBV and low immunity to it (anti-HBc−positive and anti-HBs <50 IU/L). The technique was standardized following the protocol described by Chen et al.[12] with slight modifications, using a probe kit amplifying a 120 bp region of the HBV genome core. An in-house serum standard was used to construct the calibration curve, enabling DNA quantification.

DNA extraction from serum samples using proteinase K Eighty µL of serum were used, adding 20 µLlysis buffer [20 mM Tris-HCl (pH = 8.0), 150 mM NaCl, 10 mM EDTA and 2% SDS] and 1 µL proteinase K (20 mg/mL) were added. After mixing, they were incubated at 56 °C for 1 hour and then at 100 °C for 10 min, to inactivate proteinase K. The mixture was then centrifuged at 8000 rpm (Eppendorf 5417R centrifuge, Germany) for 3 minutes and the clear aqueous supernatant transferred to a new tube.

Amplification, detection and quantification The commercial kit LightCycler TaqMan Master Mix (Roche, Germany) was used, consisting of a ready-to-use reaction mixture including: FastStar Taq DNA polymerase, reaction buffer, $MgCl_2$, dideoxynucleotide mixture and nuclease-free water. Primers and probe were added to the mixture at 0.7 µM and 0.15 µM concentrations, respectively. Finally, 5 µL of control and sample DNA were added for a final volume of 20 µL. Amplification was carried out in 20 µL capillaries (LightCycler Capillaries, Roche, Germany) in a LightCycler 1.5 (Roche, Germany) with the following program:

- one 10-minute cycle at 95 °C (allows activation of Taq polymerase and denaturing of nucleic acids);
- 45 cycles of: denaturation at 95 °C for 15 sec, hybridization at 58 °C for one minute, and an extension at 72 °C for one second.

Analysis of results was performed automatically by the program at the end of the sample run. Results were considered positive from one copy forward, and nondetectable when there was no genome amplification.

Statistical analysis A database was prepared and processed using SPSS 11.5 and EpiInfo version 6.0. Absolute and relative frequencies, anti-HBc prevalence and HBV DNA presence were calculated. HBV DNA positivity was determined; univariate analysis performed, and prevalence ratio (PR) calculated as a measure of association, with 95% significance intervals. PR values

greater than 1.5 were considered clinically significant. Socio-demographic and clinical variables were evaluated for samples that tested positive for anti-HBc and had anti-HBs levels of <50 IU/L (exposed to HBV with low immunity).

Ethical aspects The IPK Ethics Committee approved this study, including prior written consent by patients seen in clinical services, to allow performance of tests for additional viral markers to detect occult HBV infection. Results were stored in a password-protected database accessible only to researchers from the laboratory. Data obtained were used only in this study and for patient well-being.

RESULTS

Exposure to HBV and presence of immunity (anti-HBs) In Group 1, 28% (68/243) of samples were anti-HBc positive, compared to 37.8% (31/82) in Group 2. Thus, global exposure prevalence as measured by anti-HBc was 30.5% (99/325) (Table 1).

Table 1: Prevalence of HBV exposure (anti-HBc) in serum samples of HIV-positive/HBsAg-negative patients (n=325)

Serum Group	Anti-HBc (+)		Anti--HBc (-)		Total	
	n	%	n	%	n	%
Group 1 anti-HCV(-)	68	28.0	175	72.0	243	74.8
Group 2 anti-HCV(+)	31	37.8	51	62.2	82	25.2
Total	99	30.5	226	69.5	325	100.0

Source: National Reference Laboratory for Viral Hepatitis, IPK

HBV immunity levels were determined in positive anti-HBc serum samples (Table 2). Of 99 samples analyzed, 38 (38.4%) did not show protective titers of anti-HBs (<10 IU/L); 16 (16.1%) had low immunity (10–49.9 IU/L) and the remaining 45 (45.5%) had levels of ≥50 IU/L. The overall prevalence of HBV seroprotection (low or complete) was 61.6%.

Protective anti-HBs titers were more frequent in sera in the anti-HCV–negative group: 63.2% (43/68); the frequency of titers ≥50 IU/L was also higher in anti-HCV–negative group (50% vs. 35.5%) (Table 2).

Table 2: HBV exposure (anti-HBc+) by anti-HBs titers and presence of anti-HCV in study samples

Anti-HBs (IU/L) titer	Anti-HBc(+)/ anti-HCV(-) (n=68)	%	Anti-HBc(+)/ anti-HCV(+) (n=31)	%	Total Anti-HBs (n=99)	%
<10	25	36.8	13	41.9	38	38.4
10–49.9	9	13.2	7	22.6	16	16.1
≥50	34	50.0	11	35.5	45	45.5

Source: National Reference Laboratory for Viral Hepatitis, IPK

HBV DNA detection and quantification When the 54 anti–HBc-positive sera with anti-HBs levels of <50 IU/L were tested for HBV DNA, HBV genetic material was identified in 24.1% (13/54) (Table 3). The mean number of HBV copies detected by RT-PCR was 10.5/5 µL DNA, ranging between 1 and 47 copies/5 µL.

HBV DNA was found in 28.9% (11/38) of sera exposed to HBV (anti-HBc–positive) without protective titers (anti-HBs levels of <10 IU/L). In exposed individuals with low immunity (anti-HBs levels of 10–49.9 IU/L), 12.5% (2/16) of serum samples were viremic (Table 4).

In anti-HCV–positive individuals, HBV genetic material was detected in 9.5% (2/21), in the absence of protective titers (anti-HBs <10 IU/L). In contrast, HBV DNA was identified in 33.3% (11/33) of anti-HCV–negative serum samples, predominantly in those with anti-HBs levels of <10 IU/L (Table 4).

Relation between presence of occult HBV and clinical variables There was no statistically-significant association between occult HBV infection and any of the clinical variables studied, although prevalence ratio point estimates exceeded 1.0 for some variables.

Table 3: HBV DNA detection by anti-HCV presence and anti-HBs titers (n=54)

Serum Group	HBV/DNA(+)		HBV/DNA(ND)	
	n	%	n	%
Anti-HBs <10 IU/L / anti-HCV(+)	2	15.3	11	84.6
Anti-HBs <10 IU/L / anti-HCV(-)	9	37.5	15	62.5
Anti-HBs 10–49.9 IU/L / anti-HCV(+)	0	0.0	8	100.0
Anti-HBs 10–49.9 IU/L / anti-HCV(-)	2	22.2	7	77.8
Total	13	24.1	41	75.9

ND: non-detectable
Source: National Reference Laboratory for Viral Hepatitis, IPK

Table 4: HBV DNA positivity by study variables in HBV-exposed HIV-positive sera with low immunity

Variable	HBV/DNA(+) / %	Total	PR (CI 95%)
AST (IU/L)			
≤40	9 (25.7)	35	
41–75	4 (33.3)	12	1.05 (0.37–2.94)
≥76	0 (0.0)	5	
ALT (IU/L)			
<40	10 (31.2)	32	
41–75	0 (0.0)	9	0.36 (0.09–6.02)
>76	2 (22.2)	9	
Anti-HCV			
Yes	2 (9.5)	21	0.31 (0.08–1.26)
No	11 (33.3)	33	
Erythrosedimentation			
<20	7 (21.8)	32	1.44 (0.57–3.66)
≥20	6 (31.5)	19	
Anti-HBs (IU/L)			
<10	11 (28.9)	38	2.32 (0.58–9.29)
10– 49.9	2 (12.5)	16	
CD4 (%)			
≤15	5 (21.7)	23	
16–24	3 (16.6)	18	0.51 (0.20–1.28)
≥25	5 (38.4)	13	
Time elapsed from HIV diagnosis (years)			
≤5	7 (24.1)	29	
6–10	4 (33.3)	12	
11–15	2 (28.5)	7	1.06 (0.26–4.42)
≥16	0 (0.0)	4	
AIDS			
Yes	6 (25.0)	24	1.07 (0.41–2.77)
No	7 (23.3)	30	
Therapy (lamivudine)			
Yes	5 (18.5)	27	1.60 (0.60–4.27)
No	8 (29.6)	27	

PR: Prevalence ratio
CI: Confidence interval
Source: National Reference Laboratory for Viral Hepatitis, IPK

DISCUSSION

HBV exposure prevalence observed was lower than the 45.5% anti-HBc prevalence reported by Rodríguez et al. in an earlier study of Cuban HIV-positive patients,[13] but this prevalence was in all patients, irrespective of HBsAg status. The lower prevalence observed in our study is not surprising; Cuba's strategy of immunizing at-risk groups and the population aged <27 years has reduced morbidity from HBV.[14] A Cuban study of hemodialysis patients at risk for HBV exposure found a higher anti-HBc prevalence than we detected (50.3% vs. 30.4%).[15] Research by Ballester et al. to determine anti-HBc prevalence in at-risk groups in Cuba found prevalences of 52.5% in persons on hemodialysis, 56.8% in hemophiliacs, 34.7% in persons with malignant hemopathies, and 40.5% in persons with sickle cell disease.[16]

In HBsAg-negative persons with HIV in Brazil, anti-HBc positivity was higher than in this study (56% vs. 30.4%).[17] In Senegal, which has a high prevalence of HBV, the presence of anti-HBc in the general population was similar to that in persons with HIV (66.6%),[18] and notably higher than that obtained in this study. At the same time, relatively low prevalence in studies similar to ours were reported in Argentina (12.1%), South Africa (10.6%) and Spain (6.6%).[19–21]

Nebbia et al. had similar results to those reported in this study (39%) in HBsAg-positive persons with HIV in the United Kingdom.[22] Similarly, 35.5% anti-HBc prevalence was found in a comparable population studied in Italy.[23] Sucupira et al. reported 30.0% anti-HBc positivity in HIV patients in Brazil, similar to our findings.[24]

Licourt et al., found 5.6% anti-HBc reactivity in a preliminary analysis carried out in blood banks in low-HBV prevalence areas of Cuba,[25] in contrast with the 30.4% reported in this study of persons with HIV, supporting the notion that this group is at risk for HBV exposure.

Laguno et al. stratified anti-HBc by anti-HCV levels, and detected higher values than ours for HIV co-infected patients (60% vs. 37.8%).[26] Results similar to ours were reported by Arababadi et al. in a comparable population in Iran (33%).[27]

Our observation of higher anti-HBc levels in anti-HCV–positive individuals than in anti-HCV–negative persons has also been reported in blood donors.[28] This may be explained by the viruses' shared transmission routes, independent of population group.

In Spain, a study similar to ours found a stronger association between the HBV exposure marker (anti-HBc) and anti-HCV (80.1%) than we did.[29] It is well known that the main transmission route for HBV and HCV in hemodialysis patients is parenteral. In the case of HIV, the parenteral route is pertinent mainly in persons who use intravenous drugs, a population with a high prevalence of HCV infection and in which often the only HBV marker found is anti-HBc.[30]

In Cuba, HIV transmission is predominantly sexual, which is relatively inefficient for transmitting HCV.[31] Our results reinforce the observation that frequency of HBV and HCV exposure markers in persons with HIV is likely related to their shared transmission routes.

Hemodialysis patients are another immunosuppressed population with increased risk of exposure to HBV; in hemodialysis patients with HBV exposure markers in Cuba, 91.2% have protective levels of anti-HBs. This higher level of seroprotection could be explained by Cuba's HBV immunization schedule for persons on hemodialysis: given their immunosuppression, they receive annual boosters to prevent anti-HBs titers declining to non-protective levels.

Several researchers have reported lower rates of dual anti-HBc and anti-HBs reactivity than ours in HIV patients, ranging from 11.9% to 34.1%.[33–35] This could be related to the Cuban HBV vaccine (Heberbiovac) schedule, which calls for additional vaccinations if HIV-positive status is detected.[13]

Levels of anti-HBs ≥10 IU/L detection were found slightly more frequently in anti-HCV–negative than in anti-HCV–positive patients (63.2% vs. 58%). In a US study of persons with HIV, some of whom were co-infected with HCV, French et al. detected low anti-HBs titers, which could suggest influence of viral co-infection on protective levels or duration of anti-HBs.[36]

Declining anti-HBs titers have already been reported in persons with HIV after a standard vaccination schedule; which is why boosters are recommended,[37,38] suggesting that the immunocompromised status of HIV-positive persons influences loss of HBV immunity; that is, anti-HBs titers decline to non-detectable levels, a considerably different picture than that of immunocompetent persons. So, for example, the prevalence of protective levels of anti-HBs in anti–HBc-positive blood donors was 85.7%.[39]

Our observed occult HBV prevalence of 24.1% is within the range of values reported in the international literature.[22] Lukhwareni et al. reported a lower value (7.4%) in HIV patients under highly active antiretroviral therapy.[1]

Occult HBV has frequently been reported in HIV patients without anti-HBs protective levels.[40] The presence of HBV DNA in HIV-positive individuals with low or absent immunity to HBV was also reported by Nebbia et al., who consider lack of HBV immunity in HBsAg-negative persons who are anti-HBc positive a marker of occult HBV.[22] Lower values than in this study were detected in South African HIV patients on retroviral therapy (5.4%).[1] Generally, viral loads detected in occult HBV are low (<10^3 copies/mL), which is consistent with the present study.[1,41,42]

Occult HBV infection was identified in 33.3% (11/33) of anti-HCV–negative patients in this study, with levels of anti-HBs of <10 IU/L predominating. Other authors report higher frequencies of occult HBV in HIV patients co-infected with HCV.[5,26]

We did not find an association between occult HBV and elevated ALT or AST. Similarly, Lo Re et al. carried out a study in HIV individuals with and without occult HBV and did not find significant differences in transaminase levels.[43]

Elevated erythrocyte sedimentation rates are frequent in HIV-positive individuals, since they may have different infectious and non-infectious conditions that can lead to erythrocyte sedimentation rates of up to three figures.[44] We encountered no difference in this respect between people with and without occult Hepatitis B infection.

Contrary to reports in international literature,[5] we found HBV DNA more frequently in anti-HCV–negative sera than in positive. This could be explained by HCV replication interfering with HBV replication, reducing HBV load to levels undetectable with the techniques used.[26]

This study suggests a possible inverse association between level of anti-HBs and occult hepatitis B, but because of the cross-sectional design, it is impossible to determine which came first, so we cannot infer a causal relationship. Other authors have found this association; some even hold that HBV molecular diagnosis should be considered when anti-HBs is absent in sera from persons positive for both HIV and anti-HBc, and even more so in those lacking all HBV markers.[1,29,45]

Like Fabris et al.,[5] we found no association between occult HBV infection and CD4 cell count, time elapsed from HIV diagnosis and AIDS stage. Similarly, a study in Mexico detected no correlation between occult HBV and AIDS status.[46]

Besides its cross-sectional design, a further limitation of our study is that we could not identify the presence of active HVC infection by PCR in persons positive for both HIV and anti-HCV, which would allow determination of its effect on HBV replication and occult HBV detection.

CONCLUSIONS

Occult HBV diagnosis is frequent in HIV patients with poor immune response.

We recommend that the diagnostic algorithm for persons with HIV who are HBsAg-negative include anti-HBc detection and, if positive, follow-up with molecular diagnosis to detect occult hepatitis B. Such a diagnostic strategy could help reduce sequelae and HBV transmission in this population, thus improving their quality of life. It is feasible in Cuba, which has a domestically-manufactured diagnostic kit, enabling anti-HBc and anti-HBs testing for those requiring it.

This is the first study of its type in Cuba. Its results contribute to understanding hepatitis B in persons with HIV, which should help improve treatment, prognosis, control and prevention of this disease, as well as contribute to the well-being of those affected.

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