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Research article

High Prevalence of Hepatitis B Virus Markers in Romanian Adolescents With Human Immunodeficiency Virus Infection

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Abstract

Background.: We evaluated the frequency of hepatitis coinfection in Romanian adolescents who were diagnosed with human immunodeficiency virus (HIV) infection prior to 1995.

Methods.: One hundred sixty-one adolescents (1318 years of age) with symptomatic HIV infection, but without signs of hepatic dysfunction, and 356 age-matched, HIV-uninfected controls underwent laboratory testing for markers of parenterally acquired hepatitis virus infection.

Results.: Seventy-eight percent of HIV-infected adolescents had markers of past or present hepatitis B virus (HBV) infection, as compared with 32% of controls (P = .0001). The prevalence of HBV replicative markers was more than 5-fold higher in HIV-infected adolescents as compared with controls: 43.4% vs 7.9% (P = .0001), respectively, for hepatitis B surface antigen (HBsAg); and 11.2% vs 2.2% (P = .0001), respectively, for hepatitis B e antigen (HBeAg). The prevalence of HBsAg chronic carriers and the presence of HBV replicative markers was significantly higher in patients with immunologically defined AIDS (CD4+ cell counts < 200 cells/mcL): 59.6% vs 34.6% (P = .02) for HBsAg and 22.8% vs 5.7%, (P = .002) for HBV DNA. After 1 year of follow-up, the proportion of those who cleared the HBeAg was considerably lower in severely immunosuppressed coinfected patients: 4.7% vs 37.1% (P = .003). Four additional HIV-infected adolescents became HBsAg-positive over the term of follow-up (incidence rate, 24.9/1000 person-years), despite a record of immunization against hepatitis B.

Conclusion.: A substantial percentage of HIV-infected and HIV-uninfected Romanian adolescents have evidence of past or present HBV infection. In HIV-infected adolescents, the degree of immunosuppression is correlated with persistence of HBV replicative markers, even in the absence of clinical or biochemical signs of liver disease.

Introduction

Through the end of 2003, Romania had reported 9936 pediatric HIV/AIDS cases, representing an important part of all European pediatric HIV/AIDS cases. Most of these children became infected with HIV between 1988 and 1992 through the use of blood and blood products unscreened for HIV antibodies, and the use and reuse of disposable needles and syringes in hospitals and institutions.[1]

Because of the shared routes of transmission, Romanian children and adolescents with horizontally acquired HIV infection also may be at substantial risk for infection with hepatitis B virus (HBV) and hepatitis C virus (HCV). Although nosocomial transmission of these viruses in Romania has been almost completely eliminated, children and adolescents infected with HIV and/or HBV soon will be sexually active (if they are not already), and could pose substantial public health risks. In addition, coinfection with HIV and HBV has potential implications for the safety and effectiveness of antiretroviral therapy. Assessing the changing incidence and prevalence of viral hepatitis markers in a cohort of HIV-infected adolescents allows us to gauge the impact of HIV infection on the course of HBV infection. In this study we report the prevalence of HBV infection markers in a cohort of 161 HIV-infected adolescents with HIV infection acquired prior to 1995. Our objectives were to evaluate the influence of HIV disease status and level of immunosuppression on clearance of HBV infection, and to assess the impact of HIV/HBV coinfection on the occurrence of subclinical hepatic dysfunction.

Patients and Methods

Demographic, social, and clinical data were obtained from 161 HIV-infected adolescents, 1318 years of age, living in Constanta County, Romania. The cohort consisted of subjects diagnosed with HIV infection before 1995, who were followed up on a regular basis and who had no signs of acute or chronic hepatitis (absence of jaundice, fatigue, right upper quadrant pain, abdominal distension, nausea, poor appetite and malaise; and absence of abnormal biochemical data: alanine and aspartate aminotransferase less than twice the upper limit of normal, normal serum levels of gamma glutamyltransferase, alkaline phosphatase, albumin, and bilirubin). One hundred twenty-six patients (78.2%) were in US Centers for Disease Control and Prevention (CDC) clinical category B and only 35 were in category C; 79.5% were receiving antiretroviral therapy. A blood sample was obtained from each subject every 6 months during 2002 and 2003. A control group was established, consisting of 356 age-matched, healthy, HIV-uninfected adolescents, living in Constanta and 2 neighboring counties, from whom a blood sample was obtained during the same period as

part of a seroprevalence surveillance after a rubella catch-up vaccination program. Whole blood was collected by venipuncture into EDTA anticoagulant. Samples were centrifuged within 2 hours after collection, and plasma was transferred to cryovials, frozen at -80°C and stored at the Institute of Virology, Bucharest. Laboratory personnel were not aware of the clinical status of the adolescents from whom blood was obtained. The study was approved by review boards for human subject research in Romania and at Baylor College of Medicine, Houston, Texas. Informed consent was obtained from each subject's parent or legal guardian.

HBV Infection Markers

Hepatitis markers were tested by immunoenzymatic assays (Murex Biotech Limited; Kent, England) and nucleic acid amplification tests (Roche Molecular System, Inc; Branchburg, New Jersey). All sera were initially tested for total antibody against hepatitis B core antigen (HBcAg), for antibody to hepatitis B surface antigen (HBsAg), and for antibody against HCV. Sera positive for anti-HBc antibody were tested for HBsAg; sera positive for anti-HBs antibody were retested in a quantitative assay to determine the anti HBs titer (>10 mIU/mL is considered protective). Sera positive for HBsAg were tested for hepatitis B e antigen (Murex HBeAg/anti HBe) as a marker for active HBV replication. Detection of hepatitis B viral DNA was done using a commercial kit for quantitative in vitro nucleic acid amplification (*AMPLICOR HBV Monitor* test). The linear range for the *AMPLICOR HBV Monitor* test is between 1×10^3 and 4×10^7 copies/mL, with a lower limit of detection of 1000 HBV DNA copies/mL.

HIV Infection Markers

Virologic and immunologic HIV infection markers were determined, each by an independent investigator, only for samples collected from HIV-infected patients. CD4+ cell counts and viral loads were performed on blood samples anticoagulated in EDTA solution. CD4+ cell counts were made using the *COULTER Manual CD4+ Count Kit*. This is a light microscopy-based assay, which depends on the ability of monoclonal antibody-coated latex spheres to bind to the surface of cells expressing discrete antigen determinants. Plasma HIV-1 RNA quantification was performed with a commercial nucleic acid amplification test (*AMPLICOR HIV-1 MONITOR TEST version 1.5*). Quantitative measurement of plasma HIV RNA levels was expressed in the number of HIV RNA copies/mL (lower detection limit, 400 copies/mL; linear range, 400750,000 copies/mL).

Statistical Analysis

Differences in HBV marker prevalence rates were evaluated by Fisher's exact test analysis of contingency tables, using GraphPad QuickCalcs http://www.graphpad.com/

quickcalcs/index.cfm. Two-tailed *P* values were reported. *P* < .05 was considered statistically significant.

Results

Demographic Characteristics

Demographic data for HIV-infected adolescents and HIV-uninfected controls are shown in Table 1. The 2 groups did not differ significantly by age or sex ratio. Ninety-one percent of controls, but only 70.2% of HIV-infected adolescents, reside with their own families. In Romania, universal immunization against hepatitis B of all newborns was introduced in 1995 and extended to preschool children in 1999. The history of complete anti-hepatitis B vaccination was extracted from records of children in both groups and compared with the presence of protective titers of anti-HBs antibodies at baseline. Seven out of 35 (20%) HIV-infected adolescents vaccinated against hepatitis B demonstrated seroconversion to anti-HBs antibodies, compared with 40 out of 51 (78.4%) vaccinated adolescents in the control group. The successfully vaccinated adolescents were excluded from analysis in Table 2.

Prevalence of HBV Serologic Markers

The prevalence of HBV markers was significantly greater among HIV-infected adolescents than among HIV-uninfected controls (78.3% vs 31.7%, respectively [*P* < .0001]) (Table 2). Subjects with HBsAg and anti-HBc total antibodies were defined as being chronic HBsAg carriers. Forty-four percent of HIV-infected adolescents vs 7.9% of controls had chronic hepatitis B (*P* < .0001). Further evaluation of these individuals included HBeAg, anti-HBe, and HBV DNA to determine disease status. Eighteen (11.2%) HIV-infected adolescents and 8 (2.2%) controls were HBeAg-positive (*P* < .0001), suggesting that HIV-infected adolescents have decreased rates of clearance of HBsAg and HBeAg. All subjects without HBe antigen had undetectable levels of HBV DNA, while the HBeAg presence was accompanied by high viral load values (range, 1.54×10^7 copies/mL). Chronic HBsAg carriers were tested for hepatitis D virus (HDV) superinfection. Nine out of 70 (12.8%) HBsAg-positive HIV-infected adolescents, but

none of the controls, had anti-HDV antibodies, which confer a HDV seroprevalence rate of 5.6% in the HIV cohort.

The serologic pattern for viral clearance was defined as negative HBsAg but positive anti-HBs and anti-HBc antibodies. The difference between the proportions of HIV-infected subjects (14.3%) and controls (8.4%) who had this pattern of HBV clearance is not statistically significant. The burden of past or chronic HCV infection was low in both groups, possibly reflecting the absence of intravenous-drug use in both groups. The prevalence of anti-HCV antibody was 1.8% among HIV-infected adolescents and 0.8% among controls.

During the term of follow-up, 4 more HIV-infected adolescents (11% of the previously HBV-uninfected) became HBsAg-positive. Acute HBV infection was defined as presence of IgM anti-HBc antibody and the incidence was computed as 24.9 cases/1000 person-years. No new cases of HCV seroconversion were observed. There were 3 deaths among the HIV-infected adolescents, 2 of whom were coinfected with HBV.

The relation between HBV replicative markers and degree of immunosuppression

Most evidence supports the idea that HIV accelerates progression of HBV disease.[2,3] To understand the influence of advanced HIV disease on the evolution of HBV infection, we compared HIV-infected adolescents with severe immunosuppression (CD4+ cell count < 200 cells/mcL) vs those with moderate immunosuppression (CD4+ cell count > 200 cells/mcL). Subjects of all immunologic strata were similarly exposed to HBV, as shown by the prevalence of anti-HBc antibody, but fewer adolescents with severe immunosuppression cleared HBsAg and HBeAg. As shown in Table 3, 59.6% of the severely immunosuppressed patients (CD4+ cell counts < 200/mcL) were HBsAg-positive compared with 34.6% of those with CD4+ cell counts > 200/mcL (*P* = .02). The seroprevalence of HBeAg was more than double in AIDS patients: 17.5%

Table 1: Characteristics of Study Participants

Characteristic	Patients (n = 161)	Controls (n = 356)
Sex ratio M/F	89:72 (1.23)	194:162 (1.19)
Mean age [range] years	13.9 ± 1.2 [1318]	14.2 ± 0.8 [1318]
Living in family/institution	113/48 (2.35)	323/33 (9.8)
Vaccinated against hepatitis B	35 (21.7%)	51 (14.3%)
Efficient seroconversion after hepatitis B immunization (anti HBs+, anti HBc-)	7/35 (20%)	40/51 (78.4%)

Table 2: Prevalence of Viral Hepatitis Markers in HIV-Infected Adolescents and Controls

Viral Hepatitis Markers	HIV-Infected Adolescents (n = 161)	Controls (n = 356)	*P*
Anti-HBc	126 (78.3%)	113 (31.7%)	.0001
HBsAg	70 (43.4%)	28 (7.9%)	.0001
HBeAg	18 (11.2%)	8 (2.2%)	.0001
Anti-HBs+, anti HBc, +	23 (14.3%)	30 (8.4%)	.06
Anti-HDV	9 (5.6%)	0	NA
Anti-HCV	3 (1.8%)	3 (0.8%)	NS

vs 7.7%. Patients with severe immunosuppression were more likely to maintain active HBV replication: 22.8% vs 5.7% (*P* = .002) had high viremic samples (> 10^7 HBV DNA copies/mL). No correlation between the HIV viral load and the HBV DNA copies number was found.

The Specific Humoral Immune Response in HIV/HBV-Coinfected Patients

In the absence of HIV coinfection, it is likely that most HBV carriers who are HBeAg-positive will eventually seroconvert to anti-HBe antibodies, even if untreated.[4] Clearance rates of HBsAg and HBeAg defined as the proportion of those who seroconvert to anti-HBs and anti-HBe antibodies, respectively, from the total number of coinfected adolescents after 1 year of follow-up are indicated in Table 4. After 1 year of follow-up, 31.7% (95% confidence interval [CI] = 24.240.3) of all patients coinfected with HIV and HBV seroconverted to anti-HBe. However, in severely immunosuppressed coinfected patients, the proportion of those who cleared HBeAg and developed anti-HBe antibodies was substantially lower.

Only 4.7% of the anti-HBc-positive patients with < 100 CD4+ cells/mcL vs 37.1% of the remainder developed anti-HBe antibodies (*P* = .003). The development of anti-HBs antibody and the persistence of the protective titer (> 10 mIU/mL) was very low in patients with marked immunosuppression. The geometric mean of the anti-HBs antibodies titer was 21 mIU/mL in patients with < 100 CD4+ cells/mcL, compared with 374 mIU/mL in the rest of the coinfected patients. On 1-year follow-up, only 23 (74.2%; 95% CI = 56.586.5) out of the total 31 coinfected HIV/HBV-coinfected patients who cleared HBsAg maintained a protective anti-HBs antibodies titer of > 10 mIU/mL (data not shown).

Antiviral Efficacy of Combivir in HIV/HBV-Coinfected Patients

Seventeen HBsAg-positive patients received 150 mg of lamivudine + 300 mg zidovudine (*Combivir*; GlaxoSmith-Kline; Research Triangle Park, North Carolina) twice daily, as part of their antiretroviral regimen, consisting of 2 nucleoside reverse transcriptase inhibitors (NRTIs) and

Table 3: Influence of the Degree of Immunosuppression on HBV Serologic Markers

HBV Markers	HIV Patients With CD4+ Cell Counts > 200 cells/mcL (n = 104)	HIV Patients With CD4+ Cell Counts < 200 cells/mcL (n = 57)	*P**
Anti-HBc	77 (74.1%)	49 (85.9%)	.11
HBsAg	38 (34.6%)	32 (59.6%)	.02
HBeAg	8 (7.7%)	10 (17.5%)	.06
HBV DNA (>10^7 copies/mL)	6 (5.7%)	13 (22.8%)	.002
Anti-HBs	15 (14.4%)	8 (14%)	1
Without HBV markers	27 (25.9%)	8 (14.1%)	.11

* Differences, calculated by Fisher's exact test, were considered to be statistically significant at 2-tailed P values < .05
NS = non significant; NA = not applicable

Table 4: The Clearance Rates of HBsAg and HBeAg in Coinfected Adolescents After 1-Year Follow-up

CD4+ Cell Count (cells/mcL)	Total Number of HBV-Infected Patients (anti-HBc antibody-positive)	Anti-HBs Antibody-Positive	Anti HBe Antibody-Positive
< 100	21	2 (9.5%)	1 (4.7%)
< 200	28	7 (25%)	11(39.3%)
< 500	50	16 (32%)	17 (34%)
> 500	27	6 (22.2%)	11 (40.7%)
Total	**126**	**31 (24.6%)**	**40 (31.7%)**

1 protease inhibitor (PI). After 1 year of treatment, 4 patients (23.5%; 95% CI = 947.7) achieved undetectable levels of serum HBV DNA, while HBsAg loss was recorded in 7 patients (41.2%; 95% CI = 21.564) and sustained normalized alanine aminotransferase (ALT) was reported in 13 patients (76.5%; 95% CI = 52.290.9). HBeAg-positive patients did not lose this replicative marker, and seroconversion to anti-HBe antibodies was not demonstrated.

The influence of immune reconstitution on the clearance of HBsAg and HBeAg was evaluated in 57 patients who had evidence of increased level of CD4+ cells after 1 year of highly active antiretroviral therapy (HAART). A group of 5 out of 21 patients (23.8%; 95% CI = 10.245.4) with previous CD4+ cell count < 100 cell/mcL and another of 7 out of 28 (25%; 95% CI = 12.443.6) patients with CD4+ cell count < 200 cells/mcL improved their immunologic status, achieving levels of > 500 CD4+ cells/mcL. Four patients in the first group and 3 in the second cleared the HBsAg, but none the HBeAg.

Discussion
In our study, a substantial percentage of both HIV-infected and HIV-uninfected Romanian adolescents have serologic evidence of past or present HBV infection, despite the absence of clinical signs of hepatitis or of biochemical abnormalities. Previous studies had shown that Romania is a high endemic region for HBV infection (> 7% population prevalence for all hepatitis B markers),[5] mostly acquired in childhood by either maternal-fetal or parenteral routes. The frequency of asymptomatic hepatitis cases has important public health consequences concerning the transmission route and the effective prophylactic measures. Surveillance data collected by the Romanian Ministry of Health during 19971998 indicated that acute HBV infection was associated with receiving injections among children younger than 5 years.[6] In Romania, injection was and is frequently used to administer medications.[7] Shortage of infection-control supplies, including puncture-proof sharps containers,

disinfecting solutions, and single-use gloves, has been identified in Romania as one possible explanation for HBV transmission in hospitals and orphanages.[8] Also, in outpatient clinics, it has been suggested that sterile equipment might have become contaminated with blood before use (eg, blood specimens were collected in open wide-mouthed vials that were handled and placed on tables where injections were prepared, needles were placed in multidose vials to serve as access ports, and finger lacerations were left uncovered before preparing or administering injections). The high endemicity level of HBV infection in Romania can be attributed to all these practices.

Significant overlap exists for risk factors for acquisition of HIV, HBV, and HCV. The epidemiology of pediatric and adolescent HIV infection in Romania is unique in that almost all cases are attributable to horizontal, nosocomial transmission of the virus, in early childhood. In our study, the rate of HBV infection was significantly greater among HIV-infected adolescents than among HIV-uninfected controls: 78.3% vs 31.7% (*P* < .0001). In addition, the coinfection with HDV in chronic HBsAg carriers was present in 5.6% of HIV-infected adolescents but in none of the HIV-uninfected controls. The HIV transmission efficiency through unsafe medical injections in Romanian orphanages was estimated at 2% to 7%; the transmission efficiency for HBV is probably about 10-fold greater.[9] The magnitude of HBV coinfection contrasts with the amplitude of intervention measures: reduction of risks factors for acquisition of blood-borne pathogens, hepatitis B vaccination, and antiretroviral therapy. The low rate of HCV infection in both groups could reflect the fact that none of the study subjects reported intravenous or intranasal drug use. However, this also implies that others risk factors for HBV acquisition may be taken into consideration. For severely immunosuppressed HIV-infected patients, close contact with HBsAg carriers might multiply the risk.[10] In our study group, many HIV-infected adolescents lived for at least some period in small group

homes (812 children) where they may have come in close contact with HBV carriers, even if their present caretakers are efficiently vaccinated against hepatitis B. Severely immunosuppressed patients may act as "supershedders" and maintain a high rate of HBV infection in the community. This is supported by the fact that HIV/HBV-coinfected patients manifest decreased responses to the HBV vaccine. Their seroconversion rates to the recombinant 3-dose HBV vaccine were 20%, compared with 78.4% in the control group (Table 1); a waning over time of protective antibody titers in HIV-infected subjects has also been reported.[11] The low efficiency of immunization in HIV/HBV-coinfected adolescents suggests that other HBV prevention strategies also must be considered, including behavioral and barrier precautions, and avoidance of sharing of personal-care items (eg, razors). It also prompts us to suggest inclusion of lamivudine in the treatment of pregnant HIV adolescents for prevention of vertical transmission of both viruses. Lamivudine can suppress HBV plasma viral load, making vertical transmission unlikely, without any effects on reproduction, fertility, or risk for birth defects.

The results of our study suggest that in HIV-infected adolescents, degree of immunosuppression is correlated with persistence of HBV replicative markers. Natural seroconversion from HBeAg to anti-HBe is associated with sustained remission of hepatitis in about two thirds of patients infected with HBV alone. The HIV/HBV-coinfected adolescents we studied showed decreased clearance rates for HBsAg and HBeAg that were related to degree of immunosuppression. This fact amplifies concerns about the possible long-term consequences of chronic HBV infection and liver disease for HIV-infected children, as well as the need for effective therapeutic strategies in the management of patients coinfected with HIV and HBV.

Reactivation to HBeAg can occur at any time. Recent follow-up data indicate that a 4% spontaneous reactivation rate occurs over 118 years.[12] HIV infection sometimes is associated with reactivation of HBV, accelerated loss of anti-HBs, higher levels of HBV DNA, and lower ALT levels, because of a depressed immune response.[13] When HBeAg seroconversion has been prompted by antiviral therapy, the short-term stability (6 months) of this seroconversion may be reduced.[14] Following treatment with interferon-alpha, reactivation rates between 10% and 24% are described over a similar period (> 6 months) of follow-up.[15] The data supporting the stability of lamivudine-induced seroconversion are even more varied. A reactivation rate as high as 50% has been reported in HIV/HBV-coinfected patients after withdrawal of lamivudine[16]; this may portend decreased effectiveness of anti-HBV therapy in HIV/HBV-coinfected individuals. Those who are inactive carriers (HBeAg-negative with < 10^5 cop-

ies/mL of HBV DNA and normal ALTs) do not need treatment, but should have periodic monitoring of ALT, aspartate aminotransferase (AST), and HBV DNA. Liver enzyme elevations due to HAART-related hepatotoxicity as well as to coinfection with HBV or HCV have been frequently reported in HIV patients.[17] However, in our study, only 19% (95% CI = 1326.8) of samples from coinfected patients had aminotransferases level beyond the upper normal limit of 30 IU/mL, none with associated clinical signs. The highest values were always found in patients with more than 10^7 HBV DNA copies/mL. On the contrary, the majority of those who cleared HBeAg had undetectable viremia and normal ALT levels.

Although we did not observe it in the present study, immune reconstitution sometimes can precipitate the evolution of HBV infection, increasing the risk for progression to cirrhosis. Thus, a short-term goal of antiviral therapy in the HIV/HBV-coinfected patient is to prevent this progression to cirrhosis. Only long-term follow-up studies will indicate whether antiretroviral therapy will have the same beneficial effect on HBV transmission as it has on HIV transmission.

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Authors and Disclosures
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