# Impact of Hepatitis D Virus Infection on the Long-Term Outcomes of Patients with Hepatitis B Virus and HIV Coinfection in the Era of Highly Active Antiretroviral Therapy: A Matched Cohort Study

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***Background.*** Triple infection with human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis D virus (HDV) is rare. The influence of HDV infection on the responses to highly active antiretroviral therapy and hepatic complications in patients with HBV-HIV coinfection remains uncertain.

***Methods.*** Twenty-six HDV-infected case patients and 78 HDV-uninfected matched control subjects were identified between 1 January 1995 and 30 June 2003. Clinical and immunologic outcomes were noted, and HBV and HIV loads and genotypic resistance of HBV to lamivudine were determined.

***Results.*** Case patients had a higher rate of injection drug use (7.7% vs. 1.3%; $P = .05$) and lower serum levels of HBV DNA (median level, 4.04 vs. 5.75 $\log_{10}$ copies/mL; $P = .07$) than control subjects. During a median observation period of 54.7 months, HDV infection did not have an adverse impact on clinical, virological, or immunologic responses to highly active antiretroviral therapy. However, case patients had higher rates of hepatitis flares (57.7% vs. 23.1%; $P = .002$), hyperbilirubinemia (34.6% vs. 14.1%; $P = .04$), liver cirrhosis (26.9% vs. 5.1%; $P = .009$), hepatic decompensation (23.1% vs. 5.1%; $P = .007$), and death (adjusted hazard ratio, 5.41; 95% confidence interval, 1.39–23.85; $P = .02$), although these patients had a lower risk of genotypic resistance to lamivudine (0% vs. 57.1%; $P = .003$).

***Conclusions.*** HDV infection did not affect clinical, virological, or immunologic responses to highly active antiretroviral therapy in patients with HBV-HIV coinfection. HDV infection increased risk of hepatitis flares, liver cirrhosis, hepatic decompensation, and death in patients with HBV-HIV coinfection.

It is estimated that 6%–10% of HIV-infected patients have hepatitis B virus (HBV) coinfection in Western countries [1, 2]. Coinfection with HBV has been shown to increase the risk of acute hepatitis, hepatic decompensation, liver-related mortality, and virological failure in HIV-infected patients receiving HAART [1–3].

Hepatitis D virus (HDV) is a defective satellite virus that requires a helper function provided by HBV [4]. It has been estimated that ~5% of HBV carriers are also coinfected with HDV, resulting in ~15 million persons infected with HDV worldwide [5]. Most studies suggest that the majority of HDV infections are acquired through parenteral and sexual routes [6–8], which are also important routes for HIV transmission. In HIV-uninfected patients with chronic HBV infection, HDV coinfection may suppress HBV replication with subsequent clearance of hepatitis B surface antigen (HBsAg) [9–11] by exerting an inhibitory effect on the host DNA-dependent RNA polymerase that is involved in HBV transcription [12, 13].

Clinical studies regarding the impact of HDV infection on patients with HBV-HIV coinfection were limited and yielded inconsistent results before the introduction of HAART [14–17]. Some suggested that HIV

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coinfection might worsen chronic liver damage caused by HDV [6, 14], and patients with long-term HDV infection were more likely to develop cirrhosis than were patients with HBV infection alone [18], whereas others showed that the course of long-term HDV infection was not influenced by concomitant HIV infection [15–17]. Regarding the interaction with HBV, HDV coinfection was shown to significantly suppress HBV replication, which might ameliorate the damage incurred as a result of HBV infection [18]. However, HDV coinfection may lead to exacerbation and rapid progression of chronic liver disease, hepatic failure, and death in patients with HBV infection [6, 8]. These discrepancies may be related to patient selection and the shorter survival of patients before the introduction of HAART. The long-term impact of HDV infection on clinical outcomes and on the emergence of lamivudine-resistant HBV in HIV-infected patients with chronic HBV infection receiving prolonged lamivudine therapy is unknown. The improved survival rates among HIV-infected patients since the introduction of HAART in 1996 may allow complications and liver-related deaths involving chronic hepatotrophic virus infection to emerge [19]. Taking advantage of a higher prevalence of chronic HBV infection (15%–20%) in the general population and a higher prevalence of patients with HIV infection in Taiwan (21.7%) [3], we conducted a matched cohort study to investigate the impact of HDV infection on the immunologic, virological, and clinical responses to HAART of patients who had HBV-HIV coinfection.

## PATIENTS AND METHODS

***Setting.*** HIV-infected patients with test results positive for HBsAg for at least 6 months (i.e., with chronic HBV infection) who were seen at the National Taiwan University Hospital from 1 January 1995 to 30 June 2003 were enrolled. Although HAART has been provided without charge to all patients with HIV infection since April 1997, newer therapeutic agents for treating HBV infection, such as adefovir, entecavir, and tenofovir, were not available in Taiwan during this study period.

***Case-control matching.*** For patients with chronic HBV infection, serum samples were tested for antibody to HDV (anti-HDV); those patients with positive results were considered to be HDV-infected patients (case patients), and those patients with negative test results were considered to be control subjects. Each case patient was matched with 3 control subjects with respect to age ($\pm 2$ years), sex, baseline $CD4^+$ cell count, date of enrollment ($\pm 3$ months), serum albumin level ($\pm 0.5$ g/dL), and serum bilirubin level ($\pm 0.3$ mg/dL). When several potential control subjects were found, the control subject with the date of enrollment nearest to that of the case patient was selected. Patients with chronic alcoholism, test results positive for antibody to HCV or HCV viremia, total bilirubin levels >2.0 mg/dL, decompensated liver disease, and cirrhosis of the liver documented by abdominal sonography at enrollment were excluded. Six patients who had negative anti-HDV test results at enrollment but who experienced seroconversion and had positive test results at the last visit were also excluded. The study was approved by institutional review board of the hospital (NTUH-9261700889).

***Laboratory tests and radiographic investigations.*** Liver function tests were performed and serum aminotransferase and bilirubin levels, $CD4^+$ cell count, and plasma viral load of HIV (HIV-PVL) were determined every 3–4 months. HIV-PVL was quantified using the Cobas Amplicor HIV-1 Monitor test, version 1.5 (Roche Diagnostics), with a lower limit of detection of 400 copies/mL (2.60 $\log_{10}$ copies/mL), and $CD4^+$ cell count was determined using FACFlow (BD FACS Calibur; Becton Dickinson).

Patients underwent testing with an EIA for HBsAg, antibody to HBsAg, hepatitis B e antigen, antibody to hepatitis B e antigen, and anti-HDV at enrollment and either in December 2004 or at the last hospital visit. Antibody to HCV was assayed using a third-generation EIA (Ax Sym HCV III; Abbott Laboratories). HCV RNA level was determined using the Cobas Amplicor HCV Monitor assay, version 2.0 (Roche Diagnostics), for patients with a baseline $CD4^+$ cell count of <200 cells/$\mu$L.

HDV RNA was extracted from preserved serum samples from patients with test results positive for anti-HDV at enrollment using the QIAamp Viral RNA Mini Kit (Qiagen), and the purified RNA was subjected to nested RT-PCR. The primer sets for HDV are shown in the Appendix. The amplification condition was 30 cycles at 94°C for 30s, 55°C for 1 min, 72°C for 2 min, and a final extension at 72°C for 7 min. A 1-$\mu$L aliquot of the first-round PCR product was used for the second-round PCR, which was performed under the same conditions as the first round. The expected size for the PCR product was 419 base pairs, and the PCR results were visualized by gel electrophoresis.

HBV DNA was extracted from 200 $\mu$L of serum using the High Pure Viral Nucleic Acid Kit (Roche Molecular Biochemicals); real-time PCR was performed using a LightCycler hybridization probe assay system, as described elsewhere [20]. The primer sets for HBV are shown in the Appendix. It was estimated that the sensitivity corresponded to ~$10^3$ copies/mL. HBV genotypes were determined using PCR restriction fragment–length polymorphism of the surface gene of HBV, as described elsewhere [9], and 6 genotypes (A–F) could be identified. To analyze genotypic resistance to lamivudine, we amplified the polymerase gene containing the tyrosine-methionine-aspartate-aspartate (YMDD) motif of patients with detectable HBV DNA at the last hospital visit using a PCR assay. The presence of the YMDD variant (rt pol gene mutations rtM204V plus rtM204I) and/or rtL180M was confirmed by

directly sequencing the PCR product with an automatic ABI-DNA sequencer, model 377 A (Applied Biosystems).

Abdominal sonography and quantification of α-fetoprotein by chemiluminescent microparticle immunoassay (Architect AFP; Abbot Laboratories) were performed for patients with chronic HBV infection twice per year. In patients with abnormal liver function test results or abdominal symptoms localized at the right upper quadrant or the epigastrium, abdominal sonography was performed on an as-needed basis. CT of the abdomen was performed when a space-occupying lesion was detected by abdominal sonography. During the study period, 13 patients (4 case patients and 9 control subjects) underwent liver biopsy when hepatitis was diagnosed. The biopsy specimens were submitted for immunohistochemical staining of HBsAg and HBV core antigen in addition to routine staining and microbiological culturing.

***Assessment of virological and immunologic responses to antiretroviral therapy and HIV progression and definitions.*** Virological response to HAART was assessed by the proportion of patients achieving an undetectable HIV-PVL within 6 months of the end of study or patient death, whichever occurred first. Virological failure was defined as failure to achieve an undetectable HIV-PVL after ⩾4 months of HAART. Patients with missing HIV-PVL data for an interval of ⩾6 months were also counted as having experienced treatment failure (on the basis of the intention-to-treat principle). Immunologic response was assessed by the change in $CD4^+$ cell count from baseline to within 6 months of the end of the study or patient death and by the proportion of patients achieving an increase in $CD4^+$ cell count of either ⩾100 cells/μL or ⩾200 cells/μL during the follow-up period. HIV progression was defined as a relapse or the development of an AIDS-defining opportunistic illness [21] within 1 month after study entry. To better define the mortality rate and survival duration, we searched mortality report data from the vital statistics office of the Department of Health, Taiwan, to identify deaths among patients who might have been followed up at other designated hospitals.

Hepatitis flare was defined as 5-fold elevation in serum aspartate and alanine aminotransferase levels (upper limits of normal for aspartate and alanine aminotransferase levels, 31 U/L and 41 U/L, respectively), and hyperbilirubinemia was defined as a total serum bilirubin level ⩾2.0 mg/dL (upper limit of normal, 1.0 mg/dL) with >50% conjugated bilirubin without evidence of hemolysis. Hepatic decompensation was defined according to the Child-Pugh criteria [22] as presence of hepatic encephalopathy, coagulopathy, ascites, and prolonged hyperbilirubinemia for ⩾3 months, which was not attributable to concurrent AIDS-defining opportunistic illness and other medical causes. HAART was defined as the combination of at least 3 antiretroviral agents containing protease inhibitors or nonnucleoside reverse-transcriptase inhibitors. Cirrhosis of the liver was documented if cirrhotic changes were noted on histological examination of the liver or the presence of coarse echogenecity and irregular liver surface accompanied by splenomegaly was detected by sonography or CT.

***Statistical analysis.*** All statistical analyses were performed using SPSS software, version 12.0 (SPSS). Categorical variables were compared using $\chi^2$ or Fisher's exact test, and noncategorical variables were compared using the Wilcoxon rank-sum test. Logistic regression was used to assess the impact of HDV coinfection on the risk for acute hepatitis, progression of HIV disease, immunologic and virologic responses to HAART with adjustment for baseline HIV-PVL, risk behavior for HIV transmission, baseline opportunistic illness, use and duration of lamivudine and HAART, HBV genotypes, baseline HBV load, and genotypic resistance to lamivudine of HBV. ORs and 95% CIs were calculated for logistic regression analyses. The survival probabilities were estimated using the Kaplan-Meier method. The Cox proportional-hazard model was used to compare the difference in mortality rate between the 2 groups, with the same adjustments as above. Hazard ratios and 95% CIs were calculated for survival analyses. The survival duration of patients was estimated from the date of enrollment to death, last follow-up visit at our hospital (National Taiwan University Hospital, Taipei, Taiwan) or at another designated hospital in Taiwan, or the end of this observational study on 30 June 2005.

## RESULTS

***Patients.*** Over the 8-year study period, 36 (22.2%) of 162 HIV-infected patients with chronic HBV coinfection had test results positive for anti-HDV antibody. Two of the 36 patients with anti-HDV antibody and 4 of the 126 patients without anti-HDV antibody were excluded because of decompensated liver disease and cirrhosis at baseline. Of the remaining 34 patients with anti-HDV antibody, 3 with antibody to HCV at baseline and 5 with new HDV infection during follow-up were also excluded. Therefore, 26 patients with HDV, HBV, and HIV triple infection (case patients) and 78 matched control subjects with HBV and HIV dual infection were enrolled.

The patients' baseline demographic data and clinical characteristics are summarized in table 1. Case patients had a higher proportion of injection drug use than did control subjects (7.7% vs. 1.3%; $P = .05$). Almost all patients received lamivudine-containing antiretroviral therapy (100% and 98.7% for case patients and control subjects, respectively) during the observation period. There was no significant difference regarding duration of exposure to HAART and lamivudine-containing antiretroviral therapy (median duration of HAART, 51.5 vs. 50.3 months; $P = .81$; median duration of lamivudine therapy, 36.2 vs. 40.4 months; $P = .62$).

***HBV genotype, viral loads, evolution of serologic markers, and lamivudine resistance.*** Data on HBV genotypes, baseline

**Table 1. Demographic and clinical characteristics and antiretroviral treatment of patients with HIV, hepatitis B virus (HBV), and hepatitis D virus (HDV) coinfection and patients with HIV-HBV coinfection.**

| Characteristic | HIV-HBV-HDV coinfected ($n$ = 26) | HIV-HBV coinfected ($n$ = 78) | $P$ |
|---|---|---|---|
| Age, median years (range) | 35 (25–61) | 34 (25–62) | .93 |
| Male sex | 25 (96.2) | 75 (96.2) | 1.0 |
| Risk factor for HIV infection | | | |
| MSM | 14 (53.8) | 52 (66.7) | .13 |
| Heterosexual sex | 9 (34.6) | 24 (30.8) | .47 |
| IDU | 3 (7.7) | 1 (1.3) | .05 |
| Hemophilia | 0 (0) | 2 (2.6) | .56 |
| $CD4^+$ cell count at baseline | | | |
| Median cells/$\mu$L (range) | 100 (2–723) | 101 (0–739) | .89 |
| <100 cells/$\mu$L | 11 (42.3) | 33 (42.3) | 1.0 |
| 100–199 cells/$\mu$L | 6 (23.1) | 18 (23.1) | 1.0 |
| 200–349 cells/$\mu$L, (%) | 5 (19.2) | 15 (19.2) | 1.0 |
| $\geqslant$350 cells/$\mu$L | 4 (15.4) | 12 (15.4) | 1.0 |
| HIV-PVL at baseline[a] | | | |
| Median $\log_{10}$ copies/mL (range) | 4.99 (2.60–5.88) | 4.66 (2.60–5.88) | .83 |
| $\geqslant$5 $\log_{10}$ copies/mL | 9 (42.8) | 33 (47.1) | .71 |
| OI at baseline | 12 (46.2) | 32 (41.4) | .65 |
| ART containing lamivudine | 26 (100) | 77 (98.7) | .55 |
| Duration of lamivudine use, median months (25th–75th percentile) | 36.2 (20.0–57.2) | 40.4 (26.5–62.6) | .62 |
| Duration of HAART, median months (25th–75th percentile) | 51.1 (25.5–69.2) | 50.3 (29.7–68.8) | .81 |
| AST level at baseline, median IU/L (range) | 36 (17–158) | 35 (14–153) | .63 |
| ALT level at baseline, median IU/L (range) | 30 (11–148) | 27 (8–168) | .41 |
| Albumin level at baseline, median g/dL (range) | 3.4 (2.7–4.4) | 3.6 (2.5–4.6) | .65 |
| Total bilirubin at baseline, median mg/dL (range) | 0.7 (0.1–2.0) | 0.5 (0.2–2.0) | .72 |

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; IDU, injection drug use; MSM, men who have sex with men; OI, AIDS-defining opportunistic illness; PVL, plasma viral load.

[a] Baseline HIV-PVL data were available for 21 of the HIV-HBV-HDV–coinfected patients and 70 of HIV-HBV–coinfected patients.

HBV loads, and changes of HBV serologic markers during follow-up are shown in table 2. Case patients tended to have lower HBV loads ($P = .07$) and a higher rate of HBsAg clearance during follow-up ($P = .02$). Control subjects had a higher rate of genotypic resistance to lamivudine than did case patients at the end of study (57.1% vs. 0%; $P = .003$) (table 2). Three (75%) of 4 case patients and 5 (55.6%) of 9 control subjects had immunohistochemical staining of hepatocytes of the liver biopsy specimens with results positive for HBsAg and HBV core antigen, suggesting acute exacerbation of chronic hepatitis B.

***Hepatic outcomes and immunologic, virological, and clinical responses to HAART.*** During follow-up, case patients were more likely than control subjects to develop hepatitis flares, hyperbilirubinemia, liver cirrhosis, and hepatic decompensation (table 3 and figure 1). For example, 57.7% of case patients developed hepatitis flares, compared with 23.1% of control subjects, with an adjusted OR of 5.88 (95% CI, 1.96–17.54; $P = .002$). HDV infection has no statistically significant impact on responses to HAART in patients with HBV and HIV coinfection (table 3). The median increase of $CD4^+$ cell count from baseline was 201 cells/$\mu$L for case patients, compared with 237 cells/$\mu$L for control subjects ($P = .69$); 50% of case patients and 57.7% of control subjects had an increase in $CD4^+$ cell of $\geqslant$200 cells/$\mu$L ($P = .45$). At the end of the study, 65.4% of case patients and 88.5% of control subjects achieved an undetectable HIV-PVL ($P = .09$), and 23.1% and 15.4%, respectively, developed virological failure ($P = .17$). A similar proportion of the case patients (26.9%) and control subjects (12.8%) developed new AIDS-defining opportunistic illness during follow-up ($P = .38$) (table 3).

***Mortality.*** Ten patients died during follow-up (table 3 and figure 2). Compared with control subjects, the adjusted hazard ratio for death in case patients was 5.41 (95% CI, 1.39–23.85; $P = .02$). A total of 4 patients died of complications of AIDS-related opportunistic infections (2 patients), pseudomonal bacteremia (1 patient), and lymphoma (1 patient). Six patients died of end-stage liver disease, including 4 case patients and 2 control subjects. Compared with control subjects, the adjusted

**Table 2. Characteristics of hepatitis markers and hepatic outcomes of patients with HIV, hepatitis B virus (HBV), and hepatitis D virus (HDV) coinfection and patients with HIV-HBV coinfection.**

| Characteristic | HIV-HBV-HDV coinfected ($n$ = 26) | HIV-HBV coinfected ($n$ = 78) | $P$ |
|---|---|---|---|
| HBV genotype | | | |
| Genotype B[a] | 12 (92.3) | 50 (79.4) | .28 |
| Genotype C[a] | 1 (7.7) | 13 (20.6) | .28 |
| HBV load at baseline[b] | | | |
| Median $\log_{10}$ copies/mL (range) | 4.04 (2.76–9.80) | 5.75 (2.01–10.01) | .07 |
| $\geqslant$5 $\log_{10}$ copies/mL | 5 (38.5) | 31 (54.4) | .06 |
| Viral hepatitis markers | | | |
| HBeAg positive at baseline | 5 (19.2) | 25 (32.1) | .32 |
| Anti-HBe positive at end of study | 1 (3.8) | 5 (6.4) | .53 |
| HBsAg clearance at end of study | 7 (26.9) | 6 (7.7) | .02 |
| New HCV infection | 2 (7.7) | 3 (3.8) | .59 |
| Genotypic resistance to lamivudine[c] | | | |
| Any | 0 | 20 (57.1) | .003 |
| HBV load of 3–6 $\log_{10}$ copies/mL | 0 | 12 (34.3) | .04 |
| HBV load >6 $\log_{10}$ copies/mL | 0 | 8 (22.8) | .19 |

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. Anti-HBe, antibody to hepatitis B e antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.

[a] HBV genotype data were available for 13 of the HIV-HBV-HDV–coinfected patients and 63 of the HIV-HBV–coinfected patients during the study period.

[b] Baseline HBV loads were available for 13 of the HIV-HBV-HDV–coinfected patients and 57 of the HIV-HBV–coinfected patients.

[c] HBV DNA had been detected in 5 of the HIV-HBV-HDV–coinfected patients and 35 of the HIV-HBV–coinfected patients at the end of the study. The mutations conferring genotypic resistance to lamivudine for 20 HBV DNA from HIV-HBV-HDV–coinfected patients were rtM204V plus rtL180M (18 patients) and rtM204I (2 patients).

hazard ratio for hepatic death in case patients was 6.49 (95% CI, 1.16–6.85; $P = .03$). There was no significant difference in mortality between patients with anti-HDV who cleared HBsAg at the end of study, compared with those who did not (1 [14.3%] of 7 vs. 5 [26.3%] of 19; $P = .47$).

***Impact of HDV viremia at enrollment.*** HDV RNA was detectable in preserved serum samples from 7 (36.8%) of 19 case patients at enrollment. There were no significant differences in demographic data, risk factors for HIV infection, baseline $CD4^+$ cell count, plasma HIV and HBV loads, and changes of hepatitis B markers between the 7 patients with HDV viremia and their 21 matched control subjects (data not shown). After adjustment, there were no significant differences in $CD4^+$ cell count increase (median $CD4^+$ cell count increase, 189 cells/$\mu$L vs. 232 cells/$\mu$L; $P = .47$) or in the percentage of individuals with undetectable HIV-PVL after HAART (57.1% vs. 85.7%; $P = .32$) between patients with HDV viremia and their matched control subjects. However, patients with HDV viremia had higher rates of hepatitis flares (71.4% vs. 14.3%; $P = .01$), hepatic decompensation (42.9% vs. 9.5%; $P = .08$), liver cirrhosis (42.9% vs. 9.5%; $P = .08$), and death (42.9% vs. 4.8%; $P = .06$), but they had fewer occurrences of genotypic resistance to lamivudine (0% vs. 47.6%; $P = .03$).

## DISCUSSION

Our results demonstrate that HDV infection may increase risk for progression of chronic liver disease in patients with chronic HBV-HIV coinfection who may otherwise benefit from receipt of HAART that prolongs AIDS-free survival, although HDV coinfection does not have an adverse impact on clinical, virological, or immunologic responses to HAART. It is estimated that 1.9%–5% of HIV-infected patients are coinfected with both HBV and HDV; coinfection is especially common among patients who are injection drug users [23, 24]. In our cohort, which had a lower proportion of injection drug users, we showed a higher prevalence of HDV infection (22.2%) among patients with HBV-HIV coinfection than that in the general population of Taiwanese HBsAg carriers (2.7%–5%) [9, 10, 25]. The higher rate of HDV coinfection among our HIV-infected patients in Taiwan, where HBV infection is hyperendemic and sexual contact is the major risk factor for HIV transmission [26], may be related to multiple sexual exposures [7].

Although prolonged use of lamivudine with resultant selection of lamivudine-resistant HBV and HIV has been a main concern [14, 27], the impact of HDV coinfection on the emergence of lamivudine-resistant HBV has not, to our knowledge,

been investigated before in patients with HBV-HIV coinfection. Among our patients, who received lamivudine 300 mg daily for both HIV and HBV infection for 3 years, 50% developed YMDD mutation, a rate similar to that in another cohort of HIV-uninfected patients receiving lamivudine 100 mg daily, in which YMDD variant HBV emerged in 57% of the patients [28]. In this study, we found that our patients with HDV infection, with or without viremia, tended to have lower HBV loads at baseline. This virological benefit is also supported by our findings that HBV genotypic resistance developed in none of the case patients after a median duration of 3 years of lamivudine-containing HAART.

Because the HBsAg carriers permit a continuous replication of HDV, HDV may play a role in the development of fulminant hepatitis and accelerate the progression of chronic liver damage in both HIV-uninfected [6, 25] and HIV-infected patients [17, 18, 23] with chronic HBV infection. Despite the fact that HDV coinfection conferred virological benefit by suppression of HBV replication at baseline and reduced the appearance of lamivudine-resistant HBV mutants, our study was not able to demonstrate its clinical benefit in HDV-HIV–coinfected patients. Instead, we found that patients with HDV coinfection remained at a higher risk for complications of chronic HBV infection. The findings imply that HDV coinfection has a much more important effect than HBV or YMDD variants on clinical hepatic events in patients who are receiving lamivudine-containing HAART.

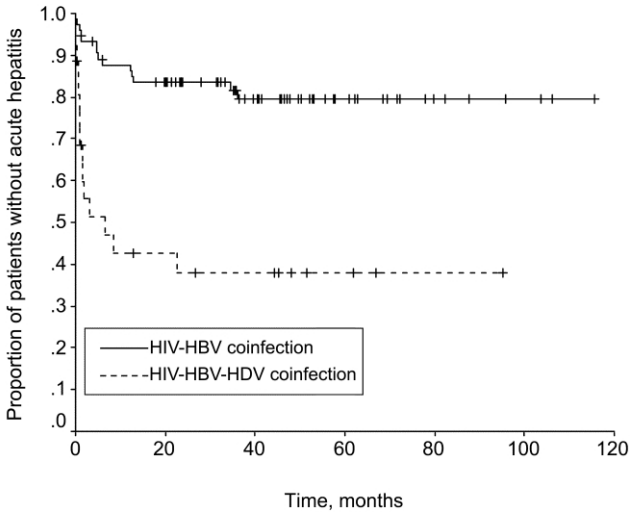


**Figure 1.** Kaplan-Meier estimates of hepatitis flares in patients with HIV, hepatitis B virus (HBV), and hepatitis D virus (HDV) coinfection and patients with HIV-HBV coinfection. $P = .001$, by log-rank test.

Anti-HDV antibody is not, in itself, diagnostic of persistent HDV infection, because it may also represent a serologic marker of previous HDV infection in HBV carriers. The rate of HDV

**Table 3. Hepatic, immunologic, virologic, and final outcomes for patients with HIV, hepatitis B virus (HBV), and hepatitis D virus (HDV) coinfection and patients with HIV-HBV coinfection.**

| Characteristic | HIV-HBV-HDV coinfected ($n$ = 26) | HIV-HBV coinfected ($n$ = 78) | Adjusted OR or HR[a] (95% CI) | $P$ |
|---|---|---|---|---|
| Hepatitis flares | 15 (57.7) | 18 (23.1) | 5.88 (1.96–17.54) | .002 |
| Hyperbilirubinemia | 9 (34.6) | 11 (14.1) | 3.40 (1.06–10.71) | .04 |
| Cirrhosis | 7 (26.9) | 4 (5.1) | 12.8 (1.78–72.89) | .009 |
| Hepatic decompensation | 6 (23.1) | 4 (5.1) | 9.68 (2.21–42.44) | .007 |
| Hepatocellular carcinoma | 1 (3.8) | 2 (2.6) | 1.57 (0.13–37.11) | .58 |
| Increase in $CD4^+$ cell count | | | | |
| Median cells/$\mu$L(range) | 201 (4–768) | 237 (2–835) | … | .69 |
| $\geqslant$100 cells/$\mu$L | 20 (76.9) | 63 (80.8) | 0.69 (0.23–2.04) | .50 |
| $\geqslant$200 cells/$\mu$L | 13 (50) | 45 (57.7) | 0.70 (0.28–1.79) | .45 |
| New OI | 7 (26.9) | 10 (12.8) | 1.93 (0.45–8.19) | .38 |
| Undetectable HIV-PVL <400 copies/mL | 17 (65.4) | 69 (88.5) | 0.37 (0.12–1.18) | .09 |
| Virological failure[b] | 6 (23.1) | 12 (15.4) | 2.45 (0.67–8.89) | .17 |
| Death | | | | |
| Any cause | 6 (23.1) | 4 (5.1) | 5.41 (1.39–23.85) | .02 |
| Liver related | 4 (15.4) | 2 (2.6) | 6.49 (1.16–6.85) | .03 |

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. HR, hazard ratio; OI, AIDS-defining opportunistic illnesses; PVL, plasma viral load.

[a] Adjustment for risk behavior associated with HIV infection, baseline OI, baseline HIV-PVL $\geqslant 5$ $\log_{10}$ copies/mL, use and duration of lamivudine therapy and HAART, HBV genotypes, baseline HBV load $\geqslant 5$ $\log_{10}$ copies/mL, and HBV genotypic resistance to lamivudine.

[b] Antiretroviral-naive patients who initiated HAART at baseline and had at least 1 HIV-PVL $\geqslant$400 copies/mL during 6 months of follow-up; missing data equaled treatment failure.

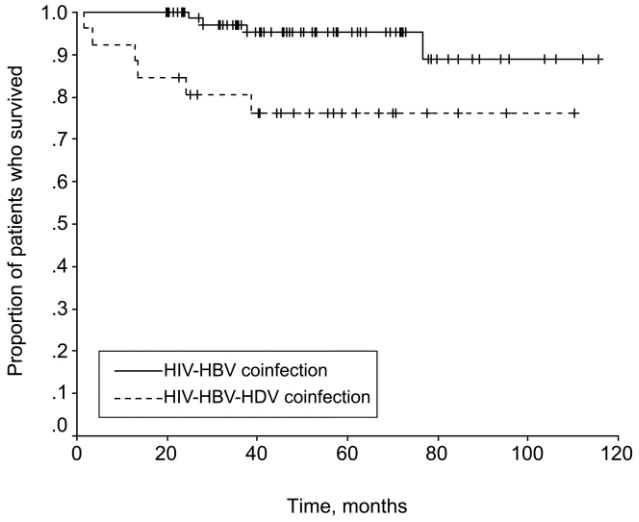


**Figure 2.** Kaplan-Meier survival estimates of mortality for patients with HIV, hepatitis B virus (HBV), and hepatitis D virus (HDV) coinfection and patients with HIV-HBV coinfection. $P = .02$, by log-rank test.

RNA detection among anti-HDV–positive patients in our study was 36.8%, which is compatible with the findings of the study by Lu et al. [29]. Despite the small number of cases included in the study, patients with detectable HDV RNA at enrollment had an increased risk of hepatitis flares and death, compared with control subjects. Because there is no concomitant HCV infection and HBV replication is suppressed in patients with HDV coinfection, the worse hepatic outcomes in patients with HDV viremia are likely to be attributable to ongoing HDV replication.

Our study is limited by the small number of cases included and the fact that HDV RNA testing and liver biopsy were not performed for each patient at baseline. Exclusion of patients with known cirrhosis or decompensated liver disease may underestimate the impact of HDV on the outcomes. The proportion of injection drug users was low, and we did not collect clinical information on the use of substances or over-the-counter medications associated with potential hepatotoxicity and drug-drug interactions with HAART. Furthermore, our patients received only lamivudine. Whether combination therapy with lamivudine and newer agents with more potent anti-HBV activities would have any impact on the interactions remains to be studied. Therefore, caution should be exercised in generalizing our study results.

In conclusion, our data suggest that HDV infection does not increase the risk of HIV progression among patients with HBV-HIV coinfection and may confer protection against the emergence of lamivudine-resistant HBV. However, HDV infection increases risk of hepatic complications and death in patients with HBV-HIV coinfection.

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## APPENDIX

The primer pairs of hepatitis D virus (HDV) used in the PCR amplification were designed on the basis of the consensus sequences of HDV, and the locations of the primers were individually indicated in the parentheses. The first primer pair used was HDV F1: 5′-CGGATGCCCAGGTCGGACC′3’ (850–868) and HDV R1: 5′-GGAGCWCCCCCGGCGAAGA-3′ (1379–1397). The second primer pair used was HDV F2: 5′-AGGTGGAGATGCCATGCCGAC-3′ (875–895) and HDV R2: 5′-GGAYCACCGAAGAAGGAAGGCC′3′ (1275–1296).

The first primer pair of hepatitis B virus (HBV) used was HBV F1: 5′-CCGATCCATACTGCGGAAC-3′ (1261–1279) and HBV R1: 5′-GCAGAGGTGAAGCGAAGTGCA -3′ (1600–1580) with anchor probe: 5′-TCTGTGCCTTCTCATCTGCCGGACC-3′ (1552–1576) and sensor probe: 5′-TCTTTACGCGGACTCCCC-3′ (1533–1550). The second primer pair used was HBV F2: 5′-GCATGCGTGGAACCTTTGTG-3′ (1232–1251) and HBV R2: 5′-CAGAGGTGAAGCGAAGTGC-3′ (1599–1581) with anchor probe 5′-CGGCGCTGAATCCCGCGGAC-3′(1436–1455) and sensor probe 5′-ACGTCCTTTGTCTACGTCCCG-3′ (1414–1434).

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