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ORIGINAL ARTICLE

Higher rate of hepatitis events in patients with human immunodeficiency virus, hepatitis B, and hepatitis D genotype II infection: A cohort study in a medical center in southern Taiwan

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Hepatitis B;
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Background: The epidemiology and impact of hepatitis δ virus (HDV) on hepatic outcomes and virological and immunological responses to highly active antiretroviral therapy (HAART) in human immunodeficiency virus (HIV) patients coinfected with hepatitis B virus (HBV) in northern Taiwan have been reported. However, the epidemiology and impact of HDV infection in HIV–HBV coinfection patients in southern Taiwan remains uncertain.
Methods: In this cohort study, a total of 64 HIV patients coinfected with HBV were identified between January 1, 2009 and May 30, 2012. The seroprevalence of anti-HDV antibodies, HDV genotyping, clinical manifestations and hepatic outcomes were compared between the patients with and without HDV coinfection, and laboratory examinations and hepatic outcomes were recorded.

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Results: Among the 64 HIV patients coinfected with HBV, seven were seropositive for HDV (10.9%). There were no statistically significant differences in risk factors for acquiring HIV infection. During a median observation period of 27.8 months, the adjusted hazard ratio of HDV and HBV genotype (type B vs. non-type B) on hepatitis flare-ups were 62.132 ($p = 0.04$) and 0.028 ($p = 0.01$), respectively. All seven patients had genotype II and were HDV viremic. The phylogenetic tree analysis and clinical history evaluation did not identify any clusters of HDV infection.
Conclusion: HDV infection resulted in higher rate of hepatitis flare-ups, but it did not have a statistical significance on HIV progression and immunological response to HAART. Whether higher rate of HDV viremia has worse impact on the hepatic outcomes requires further investigation.

Introduction

Viral hepatitis is a disease that contributes to abnormalities of liver function in HIV-infected patients. In the era of highly active antiretroviral therapy (HAART), liver damage caused by chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections is the most common non—AIDS-related cause of death in HIV-infected patients, especially those with a lower CD4 cell count.[1] However, in hepatitis B surface antigen (HBsAg)-positive HIV-infected patients, coinfection with hepatitis δ virus (HDV) further leads to exacerbation and rapid progression of chronic liver disease, and consequently worse hepatic outcomes.[2—4]

The prevalence of chronic HBV infection in general population is 15—20% in Taiwan,[5] and the prevalence of HDV coinfection in HBV-infected patients is estimated to be 5—8%.[6] In comparison, the prevalence of HBV infection in HIV-infected patients is estimated to be 11.5—21.7%.[7,8] However, HIV-infected patients are at a higher risk of acquiring HDV due to parenteral and sexual routes of transmission, and the overall prevalence of HDV infection in HIV—HBV-coinfected patients has been reported to range from 14.5% to 43.9% in Taiwan.[3,4,7,9]

Sheng et al[3] reported the epidemiology and impact of HDV on hepatic outcomes, and virologic and immunological responses to HAART in HIV—HBV-coinfected patients in northern Taiwan. However, the epidemiology and impact of HDV infection in HIV—HBV-coinfected patients in southern Taiwan remains uncertain. We therefore conducted this prospective cohort study to investigate the epidemiology, hepatic events, and virologic responses to HAART in HBV—HIV coinfected patients in a medical center in southern Taiwan.

Methods

Patients

This prospective cohort study was conducted in Kaohsiung Veterans General Hospital from January 2009 to May 2012. During this period, all of the HIV-infected patients who were seropositive for HBsAg were included. The minimum follow-up time was 6 months. Each patient underwent tests for hepatitis A IgG, hepatitis B e-antigen, antibodies to hepatitis B e-antigen, and antibodies to hepatitis C and hepatitis D when they were enrolled.

Serologic tests for hepatotropic viruses, CD4, and viral load measurement

HBsAg and anti-HDV antibodies were determined using an HBsAg radioimmunoassay, and an AUSAB-EIA kit (ARCHITECT i2000SR; Abbott Laboratories, Abbott Park, IL, USA), respectively. Anti-HCV antibodies were determined using an anti-HCV EIA kit (ARCHITECT i1000SR; Abbott Laboratories).

The plasma HIV RNA load and CD4 cell counts were quantified using a Cobas Amplicor HIV-1 monitor test, version 1.5 (Roche Diagnostics Corporation, Indianapolis, IN, USA) and FACSFlow (Becton Dickinson and Company, Franklin Lakes, NJ, USA), respectively.

Polymerase chain reaction

HBV

The first primer pair used for polymerase chain reaction (PCR) for HBV was POLHB1F (5′-CCT GCT GGT GGC TCC AGT T-3′) and POLHB2R (5′-CRT CAG CAA ACA CTT GRC-3′). The amplification conditions were 40 cycles at 94°C for 1 minute, 60°C for 1 minute, 72°C for 2 minutes, and a final extension at 72°C for 10 minutes. A 2-μL aliquot of the first-round PCR product was used for the second-round PCR, for which the conditions were the same as the first round. The second primer pair used was POLHB3F (5′-CTC GTG GTG GAC TTC TCT C-3′) and POLHB4R (5′-GCA AAN CCC MAA AGR CCC AC-3′). The expected size of the PCR product was 729 bp, and the PCR results were visualized by gel electrophoresis.

HDV

The first primer pair used was HDV850 (5′-CGG ATG CCC AGG TCG GAC C-3′) and HDV1380 (5′-GGA GCW CCC CCG GCG AAG A-3′). The amplification conditions were 30 cycles at 94°C for 30 seconds, 55°C for 1 minute, 72°C for 2 minutes, and a final extension at 72°C for 7 minutes. A 1-μL aliquot of the first-round PCR product was used for the second-round PCR, for which the conditions were the same as the first round. The second primer pair used was HDV856 (5′-AGG TGG AGA TGC CAT GCC GAC-3′) and HDV1275 (5′-

GGA YCA CCG AAG AAG GAA GGC C-3′). The expected size of the PCR product was 419 bp and the PCR results were visualized by gel electrophoresis.

Sequence analysis

HBV

Sequencing of HBV was performed on the automated ABI Prism 3130 instrument, by using BigDye terminator cycle sequencing kit (Applied Biosystems, Warrington, Cheshire, UK). The primers used for the sequencing reaction were the same as those used for the second round of PCR; the sequencing cycling conditions were denaturation 2 minutes at 95°C, then 25 cycles of 10 seconds at 96°C, 5 minutes at 55°C, 3 minutes at 60°C.[10] To define HBV genotype, the *pol* sequences obtained by sequencing are compared to GenBank reference sequences by Basic Local Alignment Search Tool (BLAST) search analysis.

HDV

The sequences were aligned with the Clustal W program in the Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 (Manufacturer: Megasoftware.net, Arizona, USA), with minor manual adjustments.

The phylogenetic trees were constructed by the neighbor-joining method based on the Kimura 2-parameter distance matrix in the MEGA software. Bootstrap values >700 of 1000 replicates were considered significant.

Clinical follow-up and assessment of hepatic outcomes

Serum VDRL, aminotransferase, bilirubin levels, CD4 cell count, and HIV-plasma viral load were determined regularly every 3—6 months. Abdominal sonography was performed every 6—12 months during follow-up or when clinically indicated at the discretion of the in-charge physician. Those who had abnormal findings on abdominal sonography were further investigated with abdominal computed tomography (CT). The virologic response to antiretroviral therapy was assessed by the HIV-plasma viral load (PVL) every 3—4 months during follow-up and within 3 months of the end of the study. Virologic failure was defined as failure to achieve an undetectable HIV-PVL after 6 months of HAART. The immunological response was assessed by the increase in CD4 cell count from baseline to within 3 months of the end of the study.

Hepatitis flare was defined as 2-fold elevation in serum aspartate and alanine aminotransferase level without any other etiologies. Liver cirrhosis was considered positive if coarse echogenicity, an irregular liver surface, and splenomegaly were all detected in abdominal sonography or abdominal CT. A diagnosis of hepatocellular carcinoma was based on typical findings on dynamic liver CT or magnetic resonance imaging scanning with or without α-fetoprotein >200 ng/mL.

Statistical analysis

All statistical analyses were performed using SPSS software, version 12.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were compared using χ^2 or Fisher exact test, and noncategorical variables were compared using the Mann—Whitney *U* test. All tests were two-tailed, and $p < 0.05$ was considered significant.

The survival probabilities were estimated using the Kaplan—Meier method.

A Cox proportional hazard model was used to compare the difference in hepatitis flare-up rate between the HDV-negative and HDV-positive groups.

Results

Patient profile

Over the 41-month study period, 7 of 64 (10.9%) HIV—HBV coinfected patients had serological evidence of HDV infection. The baseline demographic and clinical characteristic data are summarized in Table 1. Men who have sex with men (MSM) and heterosexual behavior were the major risk factors for HIV in both the HDV-positive and HDV-negative groups (85.6% and 94.6%, respectively). There were no statistically significant differences regarding mean age, sex, risk factor for HIV, underlying disease, $CD4^+$ count, and HIV-PVL at baseline.

HBV genotype, baseline hepatitis B viral load, and hepatitis virus markers

The characteristics of HBV genotype, baseline hepatitis B viral load, and hepatitis virus markers are summarized in Table 2. The distribution of HBV genotype in the HDV-positive patients was B (1/2, 50%) and C (1/2, 50%). The distribution of HBV genotype in the HDV-negative patients was as follows: B (38/41, 92.6%), C (2/41, 4.8%), and D (1/41, 2.4%). There were no statistically significant differences in HBV viral load at baseline ($p = 0.11$) or in the other hepatitis viral markers. There were also no statistically significant differences in HBV viral load stratified by genotype at baseline (type B and non-type B, $p = 0.28$ and $p = 0.65$, respectively). All seropositive HDV patients exhibited detectable HDV viremia.

Hepatic outcomes and HBV viral load at end of the study

The HDV-positive patients had higher rates of hepatitis flare-ups and cirrhosis at the end of the study (Table 3 and Fig. 1) compared with the HDV-negative group. The adjusted hazard ratio of HDV and HBV genotype (type B vs. non-type B) on hepatitis flares were 62.132 (95% confidence interval, 1.14—3382.17, $p = 0.04$) and 0.028 (95% confidence interval, 0.002—0.329, $p = 0.01$), respectively (Table 4). There were no statistically significant differences in hepatocellular carcinoma ($p = 1.0$), aspartate aminotransferase ($p = 0.16$), alanine aminotransferase ($p = 0.49$), total bilirubin ($p = 0.81$), α-fetoprotein ($p = 0.17$), and HBV viral load at the end of the study ($p = 0.80$). There were also no statistically significant differences in HBV viral load stratified by genotype at the end of the study (type B and non-type B, $p = 0.59$ and $p = 0.18$, respectively).

Table 1 Demographic and clinical characteristics of the patients with and without hepatitis δ virus (HDV) infection in 64 human immunodeficiency virus (HIV) and hepatitis B virus (HBV) coinfected patients

Characteristics	All *N* = 64	HDV+ *N* = 7	HDV− *N* = 57	*p*
Age, y	39 (23−64)	45 (29−63)	39 (23−64)	0.19
Male sex	63 (98.4)	7 (100)	56 (98.2)	1.0
Duration of follow-up, mo	27.8 (6.2−40)	30.8 (12−40)	26.2 (6.2−39)	0.70
Risk factor for HIV infection				0.51
MSM	51 (79.7)	4 (57.1)	47 (82.4)	
IDU	4 (6.3)	1 (14.2)	3 (5.2)	
Heterosexual	9 (14.1)	2 (28.5)	7 (12.2)	
Underlying diseases				
Diabetes mellitus	3 (4.7)	1 (14.2)	2 (3.5)	0.29
Hypertension	1 (1.6)	0 (0)	1 (1.7)	1.0
History of syphilis at baseline	29 (45.3)	5 (71.4)	24 (42.1)	0.23
Cerebral vascular accident	1 (1.6)	0 (0)	1 (1.7)	1.0
Chronic obstructive pulmonary disease	1 (1.6)	0 (0)	1 (1.7)	1.0
Pulmonary tuberculosis	2 (3.1)	0 (0)	2 (3.5)	1.0
CD4 count at baseline cells/μL	379 (3−1042)	350 (136−679)	383 (3−1042)	0.59
<100 cells/mL	4 (6.3)	0 (0)	4 (7.0)	1.0
100−199	9 (14.0)	2 (28.5)	7 (12.3)	0.25
200−349	21 (32.8)	3 (42.9)	18 (31.5)	0.67
>350	30 (46.9)	2 (28.5)	28 (49.1)	0.43
HIV−PVL at baseline, $\log_{10}$ copies/mL	3.12 (1.60−5.67)	2.74 (1.60−3.90)	3.17 (1.60−5.67)	0.19
OI at baseline	14 (21.8)	3 (42.8)	11 (19.2)	0.17
AST at baseline, IU/L	28 (13−85)	37 (15−85)	27 (13−67)	0.41
ALT at baseline, IU/L	38 (13−178)	56 (17−178)	35 (13−87)	0.73
Total bilirubin at baseline, mg/dL	0.7 (0.2−3.4)	0.7 (0.3−1.3)	0.7 (0.2−3.4)	0.61

Data are presented as *n* (%) or median (range).
ALT = alanine aminotransferase; ART = antiretroviral therapy; AST = aspartate aminotransferase; IDU = intravenous drug user; MSM = men who have sex with men; OI = AIDS-defining opportunistic illness; PVL = plasma viral load.

Table 2 Characteristics of the genotype, baseline hepatitis B viral load, and hepatitis markers of patients with and without hepatitis δ virus (HDV) infection in 64 human immunodeficiency virus (HIV) and hepatitis B virus (HBV) coinfected patients

Characteristics	All *N* = 64	HDV+ *N* = 7	HDV− *N* = 57	*p*
HBV genotype[a]				
Genotype B	39	1	38	0.17
Genotype C	3	1	2	0.13
Genotype D	1	0	1	1.0
HBV viral load at baseline, $\log_{10}$ copies/mL	2.35 (0.60−8.88)	1.06 (0.60−1.94)	2.51 (0.60−8.88)	0.11
Genotype B[b]	3.13 (0.60−8.88)	8.88	3.04 (0.60−8.07)	0.28
Genotype non-B[b]	2.48 (0.60−4.35)	1.07	2.95 (0.60−4.35)	0.65
Viral hepatitis markers				
HBeAg positive at baseline[c]	6 (14.8)	0 (0)	6 (15)	0.57
HAV IgG at baseline[d]	19 (33.3)	3 (60)	16 (30.7)	0.32
HCV Ab at baseline[e]	7 (11.1)	1 (14.2)	6 (10.7)	0.58

[a] HBV genotype data were available for two of the HIV−HBV−HDV-coinfected patients and 41 of the HIV−HBV-coinfected patients during the study period.
[b] Of the 43 patients who had available HBV genotype data, 39 had genotype B.
[c] HBeAg data at baseline were available for seven of the HIV−HBV−HDV-coinfected patients and 40 of the HIV−HBV-coinfected patients.
[d] HAV IgG data at baseline were available for five of the HIV−HBV−HDV-coinfected patients and 52 of the HIV−HBV-coinfected patients.
[e] HCV Ab data at baseline were available for seven of the HIV−HBV−HDV-coinfected patients and 56 of the HIV−HBV-coinfected patients.
Data are presented as *n* (%) or median (range).
HAV = hepatitis A virus; HBeAg = hepatitis B e-antigen.

Table 3 Characteristics of hepatic outcomes and HBV viral load at the end of the study for patients with and without hepatitis δ virus (HDV) infection in 64 human immunodeficiency virus (HIV) and hepatitis B virus (HBV) coinfected patients

Characteristics	All *N* = 64	HDV+ *N* = 7	HDV− *N* = 57	*p*
Hepatic outcome				
Hepatitis flares, times (range)	0.42 (0−4)	1.29 (0−4)	0.32 (0−3)	<0.01
Hepatitis flare, positive	20 (31.2)	5 (71.4)	15 (26.3)	0.02
Cirrhosis at the end of the study	9 (14.0)	4 (57.1)	5 (8.7)	<0.01
Hepatocellular carcinoma at the end of the study[a]	1 (1.6)	0 (0)	1 (1.7)	1.0
HBV viral load at the end of the study[b]	2.12 (0.6−9.0)	2.18 (1.0−6.68)	2.12 (0.60−9.0)	0.80
Genotype B[c]	2.53 (0.60−9.0)	1.14	2.58 (0.60−9.0)	0.59
Genotype non-B	3.44 (1.00−6.68)	6.68	2.35 (1.00−3.07)	0.18
Total bilirubin at the end of the study, median mg/dL (range)	0.9 (0.3−3.9)	0.8 (0.4−2.3)	0.9 (0.3−3.9)	0.81
α-fetoprotein at the end of the study (range)[d]	4.67 (3−38)	10.9 (3−38)	3.6 (3−10.4)	0.17

[a] Image studies of hepatocellular carcinoma at the end of the study were available for five of the HIV−HBV−HDV-coinfected patients and 57 of the HIV−HBV-coinfected patients.
[b] HBV viral load data at the end of the study were available for five of the HIV−HBV−HDV-coinfected patients and 49 of the HIV−HBV-coinfected patients.
[c] Of the 54 patients who had available data for HBV viral load at the end of the study, 33 patients had HBV genotype B.
[d] a-FP data at the end of the study were available for five of the HIV−HBV−HDV-coinfected patients and 29 of the HIV−HBV-coinfected patients.
Data are presented as *n* (%), unless otherwise indicated.
ALT = alanine aminotransferase; ART = antiretroviral therapy; AST = aspartate aminotransferase.

Immunologic and virologic responses to HAART

Fifty-two patients received antiretroviral therapy during follow-up. The immunological and virologic responses to antiretroviral therapy are summarized in Table 5. At the end of the study, hepatitis D virus had no statistically significant impact on the immunological and virologic responses to HAART. The median increase of $CD4^+$ cell count was 8 cells/μL and 118 cells/μL for the HDV-positive and HDV-negative groups, respectively ($p = 0.17$). The virologic failure rates were 33.3% and 17.3% for the HDV-positive and HDV-negative groups, respectively ($p = 0.61$); 71% of the HDV-positive and 58% of the HDV-negative patients had undetectable HIV-PVL ($p = 0.69$) in the end of the study.

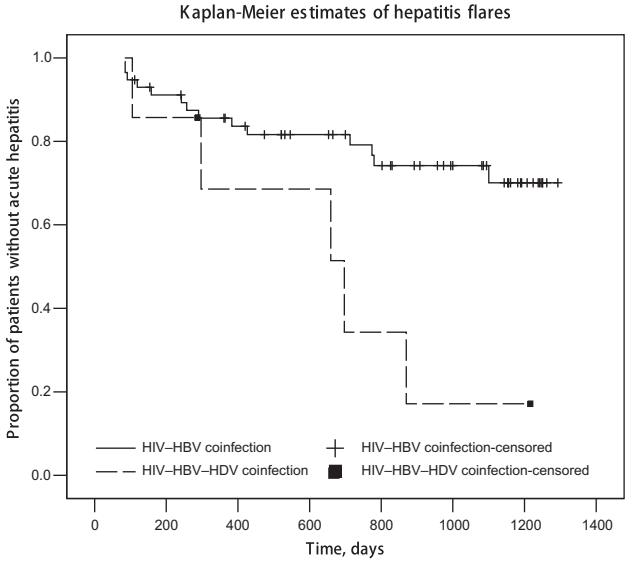

Figure 1. Kaplan−Meier estimates of hepatitis flares in patients with human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis δ virus (HDV) coinfection, and patients with HIV−HBV coinfection; $p < 0.01$, by the log-rank test.

Mortality

Four patients died during follow-up. There was no statistically significant difference in mortality rate between the HDV-positive and HDV-negative groups (Table 5 and Fig. 2). One HDV-positive patient died of multiple myeloma and the other three HDV-negative patients died from complications of liver cirrhosis.

HDV phylogenetic analysis

HDV RNA was detected in all of the seven patients who were tested positive for anti-HDV and had genotype II. The phylogenetic tree analysis did not identify any clusters (Fig. 3).

Discussion

In this prospective study, we found a lower seroprevalence (7/64, 10.9%) of HDV infection in HIV-HBV coinfected patients, and all of the HDV seropositive patients had genotype II, detectable HDV-RNA, and higher rate of hepatitis flare-ups compared with HDV seronegative patients.

Table 4 Predictor of progression to hepatitis flare-up

Variable	Progression to hepatitis flare-up			
	HR	95% CI		*p*
Male vs. female	0.874	0.002	363.976	0.96
Age	1.168	0.998	1.367	0.53
IDU vs. Non-IDU	137.397	1.179	16014.946	0.04
Liver cirrhosis, + vs. −	0.061	0.001	5.593	0.22
ALT at baseline (IU/L)	1.110	1.026	1.201	0.01
Total bilirubin at baseline (mg/dL)	2.006	0.116	34.817	0.63
HDV infection, + vs. −	62.132	1.141	3382.179	0.04
CD 4 count at baseline	0.995	0.987	1.003	0.24
HIV-PVL at baseline (log_{10} copies/mL)	3.458	0.737	16.239	0.116
HBV viral load at baseline (log_{10} copies/mL)	1.660	1.070	2.576	0.02
HBV genotype, type B vs. non−type B	0.028	0.002	0.329	0.01
Opportunistic infection history, + vs. −	1.684	0.014	196.655	0.83
On ART, + vs. −	0.035	0.003	0.385	0.01

ALT = alanine aminotransferase; ART = antiretroviral therapy; AST = aspartate aminotransferase; HBV = hepatitis B virus; HDV = hepatitis D virus; HIV = human immunodeficiency virus; HR = hazard ratio; IDU = intravenous drug user; PVL = plasma viral load; + = positive; − = negative.

HDV can be transmitted via the parenteral route, sexual contact, and, rarely perinatal, transmission.[11] A very small inoculum parenterally is sufficient to transmit infection,[12] and parenteral route through exposure to infected blood is a more effective way of transmitting HDV infection than sexual contact whether with or without HIV infection.[4,9,13–17] In this study, MSM and heterosexual behavior are the major risk factors for acquiring HIV, with intravenous drug users (IDUs) only 6.3%, and it may result in lower seroprevalence of HDV infection in HIV−HBV coinfected patients in our study (10.9%) compared with previous studies (14.5−43.9%).[3–5,8]

HDV has eight genotypes, and the distribution of genotypes has been found to vary across geographic regions. HDV genotype I is prevalent worldwide, whereas genotype II (previously termed genotype-2a) is found in the Far East.[18] The genotype of HDV has changed in Taiwan in the past 2 decades. Early studies reported that genotype II accounted for 85.4% of patients with HBV-HDV coinfection in 1995,[19] whereas Su et al[20] in 2006 reported that genotype I and genotype II accounted for 26.3% and 38.1% of cases, respectively. Chang et al[9] reported that genotype II (41%) and genotype IV (55.7%) were the two major genotypes in HIV-infected patients. In our study, HDV genotype II was the only genotype isolated. This difference in distribution of HDV genotype may result from the different populations studied. In further phylogenetic tree analysis and clinical history evaluation through detailed questionnaire, we did not identify any clusters of HDV infection, excluding the possibility of laboratory contamination and virus spreading among our patients. Different HDV genotypes may have an impact on the manifestation of disease progression, with HDV genotype II presenting with higher remission rate and milder outcomes than patients infected with HDV genotype I.[19,20]

There are three patterns of HDV infection: coinfection, superinfection, and a minor pattern−helper independent latent infection, which has been reported in the liver transplant setting.[11] Coinfection often leads to eradication of both agents, whereas superinfection mostly evolves to

Table 5 Immunological and virological responses to antiretroviral therapy[a] and final outcomes of the patients with and without hepatitis δ virus (HDV) infection in 64 human immunodeficiency virus (HIV) and hepatitis B virus (HBV) coinfected patients

Characteristics	All *N* = 64	HDV + *N* = 7	HDV − *N* = 57	*p*
CD4 count at end of study, cells/μL[a]	454 (20−1576)	374 (20−679)	464 (34−1576)	0.67
Increase in CD4 count, cells/μL[a]	105 (−472−823)	8 (−116−189)	118 (−472−823)	0.17
HIV−PVL at end of study, log_{10} copies/mL[a]	1.98 (1.60−4.33)	2.02 (1.60−4.11)	1.97 (1.60−4.33)	0.70
Virologic failure[a]	10 (19.2)	2 (33.3)	8 (17.3)	0.61
New OI[b]	4 (6.2)	2 (28.5)	2 (3.5)	0.05
Death[b]	4 (6.25)	1 (14.2)	3 (5.2)	0.37

[a] Among the 52 patients initiating highly active antiretroviral therapy during follow-up, six were HIV−HBV−HDV-coinfected patients and 46 were HIV−HBV-coinfected patients.
[b] Among the 64 HIV and HBV coinfected patients.
Data are presented as *n* (%) or median (range).
OI = AIDS-defining opportunistic illness; PVL = plasma virus load.

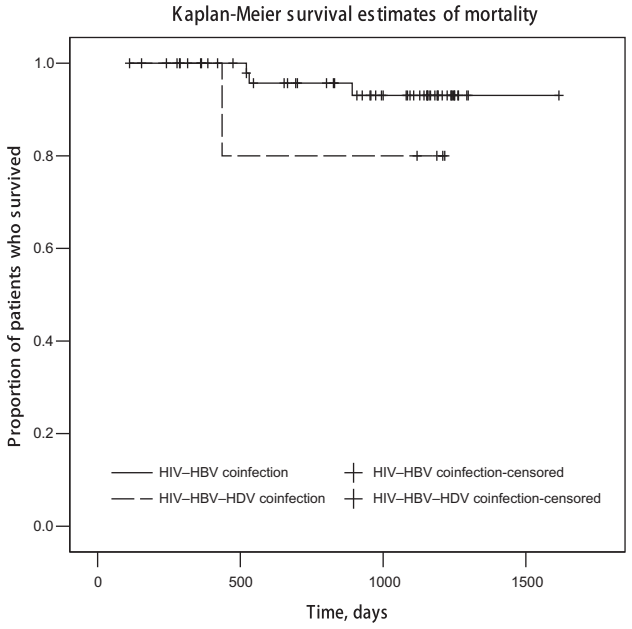

Figure 2. Kaplan—Meier survival estimates of mortality for patients with human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis δ virus (HDV) coinfection and patients with HIV—HBV coinfection; $p = 0.25$, by the log-rank test.

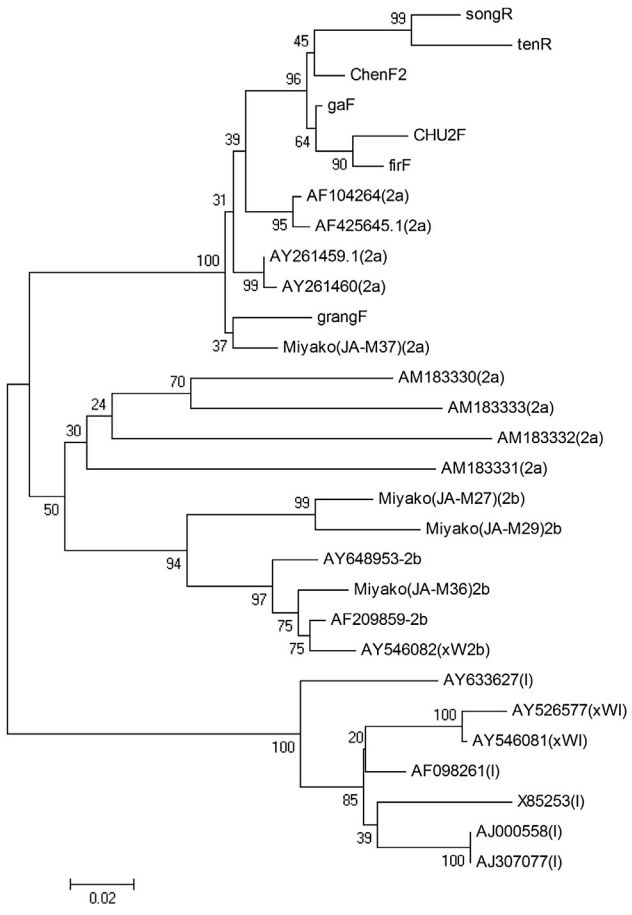

Figure 3. Phylogenetic analysis of hepatitis D virus.

HDV chronicity. Acute HBV/HDV coinfection is characterized by the presence of a high titer of IgM antibody to hepatitis B core antigen (IgM anti-HBc), antibodies that disappear in chronic HBV infection.[11] None of the seven HDV seropositive patients in our study were positive for IgM anti-HBc, and therefore they were all cases of superinfection. Superinfection of an individual with chronic HBV can cause severe acute hepatitis and up to 80% of infected patients progress to chronicity, which causes more rapid progression to liver cirrhosis and decompensated liver than HBV infection alone.[18,21] In the present study, the finding of increased rate of hepatitis events in the HDV-positive group compared to the HDV-negative group is compatible with previous reports in HIV-infected patients.[3,4] Different HBV genotypes has an impact on the clinical and therapeutic outcome. In HBV-monoinfected patients, HBV genotype C is more closely associated with active liver disease and increased risk for HCC than infection with genotype B.[22] In comparison, in HIV-infected patients, HBV genotype B has a higher risk of hepatitis flares and liver-related death than HBV genotype C.[23] In our study, risk of hepatitis flare-ups was lower in HBV genotype B than in HBV genotype non-type B (hazard ratio, 0.028; $p = 0.01$). However, only four of the 43 (9.3%) individuals were HBV genotype non-type B in our cohort study. Whether HBV genotype B in HIV-infected patients is associated with lower hepatitis flare-ups needs further investigation.

Anti-HDV antibody is not, in itself, diagnostic of persistent HDV infection, and it may represent a serological marker of previous HDV. Viremia is representative of persistent viral replication, which results in worse hepatic outcomes. The reported incidence of HDV viremia in HDV seropositive HIV-infected patients is 36.8—86.8%.[3,4,9] A higher risk of hepatitis flare-ups and death in patients with HDV viremia was reported by Sheng et al[3] in 2007. Schaper et al[24] reported that levels of HDV-RNA and HBV-DNA fluctuate, and longitudinal testing revealed circulating serum HDV-RNA in most anti-HDV patients. Although all of the HDV-positive patients in our study were HDV viremic, further quantitative longitudinal testing of the HDV-positive patient in our study may be needed to determine the impact of viremia on disease progression and hepatic outcomes.

There are several limitations to our study. First, only a small numbers of patients were included, especially in the HDV seropositive group. Second, only four of 64 individuals were IDU patients, and therefore our result cannot not be applied to the IDU population. Third, no patients underwent a liver biopsy and the diagnosis of liver cirrhosis depended on imaging findings rather than histological change. Therefore, the incidence of liver cirrhosis may have been underestimated. Fourth, among 14 patients who ever received tenofovir-based regimen during follow-up, 11 received non—tenofovir-based regimen initially and were then switched to the tenofovir-based regimen due to adverse effect of the initial regimen or virologic failure or an episode of hepatitis. Due to the small sample size and limitation of study design, further comparison of hepatitis events between tenofovir-based and non—tenofovir-based regimen may have bias. Finally, a median follow-up period of 27.8 months is too short to demonstrate the difference in mortality between HDV seropositive and HDV seronegative patients. However, our cohort disclosed a statistically

significant difference in rate of hepatitis flare-ups even through a short period of follow-up.

In conclusion, in our prospective study conducted in a medical center in southern Taiwan, HDV infection resulted in higher rate of hepatitis flare-ups. However, it did not have a statistical significance on HIV progression and immunological response to HAART. Lower HDV seroprevalence rate and changing genotype of HDV with only genotype II isolated were also noted. Whether a higher rate of viremia has a worse impact on the hepatic outcome requires further investigation.

Conflicts of interest

The authors have no conflicts of interest to declare.

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References

1. Weber R, Sabin CA, Friis-Møller N, Reiss P, El-Sadr WM, Kirk O, et al. Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. *Arch Intern Med* 2006;**166**:1632—41.
2. Novick DM, Farci P, Croxson TS, Taylor MB, Schneebaum CW, Lai ME, et al. Hepatitis D virus and human immunodeficiency virus antibodies in parenteral drug abusers who are hepatitis B surface antigen positive. *J Infect Dis* 1988;**158**:795—803.
3. Sheng WH, Hung CC, Kao JH, Chang SY, Chen MY, Hsieh SM, et al. 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. *Clin Infect Dis* 2007;**44**:988—95.
4. Soriano V, Grint D, d'Arminio Monforte A, Horban A, Leen C, Poveda E, et al. Hepatitis delta in HIV-infected individuals in Europe. *AIDS* 2011;**25**:1987—92.
5. Chen CJ, Wang LY, Yu MW. Epidemiology of hepatitis B virus infection in the Asia-Pacific region. *J Gastroenterol Hepatol* 2000;**15**(Suppl.):E3—6.
6. Chen DS, Lai MY, Sung JL. Delta agent infection in patients with chronic liver diseases and hepatocellular carcinoma—an infrequent finding in Taiwan. *Hepatology* 1984;**4**:502—3.
7. Chu FY, Chiang SC, Su FH, Chang YY, Cheng SH. Prevalence of human immunodeficiency virus and its association with hepatitis B, C, and D virus infections among incarcerated male substance abusers in Taiwan. *J Med Virol* 2009;**81**:973—8.
8. Sheng WH, Chen MY, Hsieh SM, Hsiao CF, Wang JT, Hung CC, et al. Impact of chronic hepatitis B virus (HBV) infection on outcomes of patients infected with HIV in an area where HBV infection is hyperendemic. *Clin Infect Dis* 2004;**38**:1471—7.
9. Chang SY, Yang CL, Ko WS, Liu WC, Lin CY, Wu CH, et al. Molecular epidemiology of hepatitis D virus infection among injecting drug users with and without human immunodeficiency virus infection in Taiwan. *J Clin Microbiol* 2011;**49**:1083—9.
10. Vincenti D, Solmone M, Garbuglia AR, Iacomi F, Capobianchi MR. A sensitive direct sequencing assay based on nested PCR for the detection of HBV polymerase and surface glycoprotein mutations. *J Virol Methods* 2009;**159**:53—7.
11. Pascarella S, Negro F. Hepatitis D virus: an update. *Liver Int* 2011;**31**:7—21.
12. Ponzetto A, Hoyer BH, Popper H, Engle R, Purcell RH, Gerin JL. Titration of the infectivity of hepatitis D virus in chimpanzees. *J Infect Dis* 1987;**155**:72—8.
13. Kucirka LM, Farzadegan H, Feld JJ, Mehta SH, Winters M, Glenn JS, et al. Prevalence, correlates, and viral dynamics of hepatitis delta among injection drug users. *J Infect Dis* 2010;**202**:845—52.
14. Chung DC, Ko YC, Chen CJ, Chen ER, Wu CC, Wu PS. Seroepidemiology of hepatitis B virus, hepatitis D virus, and human immunodeficiency virus infections among parenteral drug abusers in southern Taiwan. *J Med Virol* 1989;**28**:215—8.
15. Lee SD, Wang JY, Wu JC, Chiang YT, Tsai YT, Lo KJ. Hepatitis B and D virus infection among drug abusers in Taiwan. *J Med Virol* 1986;**20**:247—52.
16. Chen PJ, Chen DS, Chen CR, Chen YY, Chen HM, Lai MY, et al. Delta infection in asymptomatic carriers of hepatitis B surface antigen: low prevalence of delta activity and effective suppression of hepatitis B virus replication. *Hepatology* 1988;**8**:1121—4.
17. Hsu HM, Wang YF, Lo SH, Sun HC, Yip KK, Chen JS, et al. Hepatitis D virus infection among intravenous drug abusers in Taiwan: analysis of risk factors and liver function tests. *J Med Virol* 1990;**31**:76—81.
18. Hughes SA, Wedemeyer H, Harrison PM. Hepatitis delta virus. *Lancet* 2011;**378**:73—85.
19. Wu JC, Choo KB, Chen CM, Chen TZ, Huo TI, Lee SD. Genotyping of hepatitis D virus by restriction-fragment length polymorphism and relation to outcome of hepatitis D. *Lancet* 1995;**346**:939—41.
20. Su CW, Huang YH, Huo TI, Shih HH, Sheen IJ, Chen SW, et al. Genotypes and viremia of hepatitis B and D viruses are associated with outcomes of chronic hepatitis D patients. *Gastroenterology* 2006;**130**:1625—35.
21. Smedile A, Farci P, Verme G, Caredda F, Cargnel A, Caporaso N, et al. Influence of delta infection on severity of hepatitis B. *Lancet* 1982;**2**:945—7.
22. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. *Gastroenterology* 2000;**118**:554—9.
23. Sheng WH, Hung CC, Chang SY, Liu CJ, Chen MY, Hsieh SM, et al. Differential clinical and virologic impact of hepatitis B virus genotypes B and C on HIV-coinfected patients receiving lamivudine-containing highly active antiretroviral therapy. *Clin Infect Dis* 2012;**54**:548—55.
24. Schaper M, Rodriguez-Frias F, Jardi R, Tabernero D, Homs M, Ruiz G, et al. Quantitative longitudinal evaluations of hepatitis delta virus RNA and hepatitis B virus DNA shows a dynamic, complex replicative profile in chronic hepatitis B and D. *J Hepatol* 2010;**52**:658—64.