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BRIEF REPORT

Impact of Tenofovir on Hepatitis Delta Virus Replication in the Swiss Human Immunodeficiency Virus Cohort Study

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We analyzed changes in hepatitis B virus and hepatitis delta virus (HDV) viral loads (VL) during tenofovir-containing antiretroviral therapy among patients with a replicating HDV infection in the Swiss HIV Cohort Study. Only 28.6% experienced a ≥2.0 log reduction in HDV RNA, and 14.3% had undetectable HDV VL within 5 years.

Keywords. coinfection; hepatitis delta virus; human immunodeficiency virus; replication; tenofovir.

In human immunodeficiency virus (HIV)–infected patients, hepatitis delta virus (HDV) coinfection is associated with a higher incidence of hepatic flares and decompensation, an increased incidence of hepatocellular carcinoma (HCC), as well as higher mortality [1–3]. In the Swiss HIV Cohort Study (SHCS), HDV-infected patients were 8 times more likely to die from liver-related complications than HDV-uninfected patients [4]. Currently, pegylated interferon (IFN)-alpha remains the mainstay of HDV therapy, despite its limited success [5–7]. The addition of nucleos(t)ide reverse transcriptase inhibitors (NRTIs) to IFN did not improve virological outcomes in large trials [8–10]. The impact of long-term treatment with NRTIs on HDV suppression in HIV/hepatitis B virus (HBV)-coinfected patients has been a matter of debate. While Soriano et al showed a reduction in HDV replication and liver stiffness after a median of 4.8 years of tenofovir (TDF)-containing antiretroviral therapy (ART) in HIV/HBV-coinfected individuals in Spain [11], Boyd et al failed to show a similar impact of TDF in a French cohort [12]. As TDF is now available as a component of first-line ART throughout the world, more data are needed on its impact on long-term virological outcomes. We assessed the impact of long-term TDF therapy on HBV and HDV replication in a nationwide HIV cohort.

METHODS

All SHCS (www.shcs.ch) participants with a positive hepatitis B surface antigen (HBsAg) test between January 1988 and December 2014 were considered [13]. HDV serology was assessed in all patients as described previously [4]. For this analysis, we included all patients with an HDV RNA >300 copies/mL at TDF initiation and who had a follow-up sample after at least 1 year of TDF. All routine data were collected prospectively within the framework of the SHCS. Local ethics committees of all participating study sites approved the study, and written consent was obtained from all participants.

For HDV amplification, total nucleic acids were purified from 200 µL plasma (Qiagen EZ1 DSP kit), and cDNA (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems) was subjected to real-time polymerase chain reaction (PCR) according to Ferns et al [14]. HDV genotype was assessed by sequencing and phylogenetic analysis of a 260-base pair cDNA fragment encompassing the 3' end of the hepatitis D antigen coding region. HBV DNA was quantified using the COBAS AmpliPrep/TaqMan48 system (Roche Diagnostics International AG, Switzerland). HBV genotyping was performed using PCR amplification, Sanger sequencing, and subsequent in silico analysis with the geno2pheno tool (http://hbv.geno2pheno.org/index.php) as described previously [15]. HBV suppression was defined as HBV DNA <20 IU/mL and low-level viremia as HBV DNA 20–2000 IU/mL. Quantitative HBsAg (qHBsAg) was analyzed with a fully automated chemiluminescent microparticle immunoassay (Architect, Abbott Diagnostics).

Demographic and clinical characteristics at TDF start were described using either absolute numbers and proportions or medians and interquartile ranges (IQRs). Individual follow-up started at initiation of TDF and ended on the date of death, loss to follow-up, TDF interruption, or database closure (31 December 2014), whichever happened first. The proportion of patients who reached HDV RNA suppression or experienced a viral load drop ≥2 log were described and their main characteristics compared with those of the other patients using Fisher exact and Mann-Whitney tests. Changes in HBV DNA

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and qHBsAg levels were also described. Statistical analyses were performed using Stata version 13.1.

RESULTS

Study Population

Of 73 patients with replicating HDV in the SHCS, 34 (46%) had been on TDF for more than 1 year, and follow-up samples were available for 21 of them. At baseline, patients were predominantly middle-aged (median, 40 years [IQR, 35–45]) males (19/21 [90.5%]) from northwestern Europe (15/21 [71.4%]). Only 2/21 (9.5%) patients were from sub-Saharan Africa (see the Supplementary Table). Thirteen of the 21 (61.9%) were persons who currently or previously injected drugs. Most individuals had already been on another ART regimen at the time of TDF start (80.9%) for a median of 6.3 years, and 70.6% of them had a suppressed HIV viral load. Prior lamivudine treatment was present in 8/21 (38.1%) patients. Two-thirds had a CD4 count <350 cells/μL. At TDF start, one half of the patients had either suppressed (5/21, 23.8%) or low-level (6/21, 28.6%) HBV DNA. Of 11 patients with available results from HBV sequencing, 8 were infected with genotype D and 3 with genotype A. All patients were infected with HDV genotype 1. Median qHBsAg was 3.9 $\log_{10}$ IU/mL (IQR, 3.4–4.1). Two-thirds had a positive anti-Hepatitis C Virus (HCV) serology (66.7%), whereas only 2/21 (9.5%) patients had detectable HCV RNA. Transaminases were elevated (AIDS Clinical Trials Group grade 1–4) in 15/21 (75%) patients.

HBV and HDV Follow-up Parameters

Patients were followed for a median of 4.9 years (IQR, 2.4–7.7) on TDF. Two of the 21 patients died during follow-up, 1 from HCC and 1 from decompensated cirrhosis; no patients were lost to follow-up. Median HDV RNA was 7.0 $\log_{10}$ copies/mL (IQR, 5.7–8.1) before TDF initiation and 6.7 $\log_{10}$ copies/mL (IQR, 4.6–7.2) at the last follow-up visit (Figure 1). Sixteen of the 21 patients (76%) reached HBV suppression (<20 IU/mL) after a median of 2.9 years (IQR, 2.0–3.1), and the 5 remaining patients had low-level viremia. qHBsAg was 3.9 $\log_{10}$ IU/mL at TDF initiation and 3.6 $\log_{10}$ IU/mL (IQR, 3.1–3.9) at the last follow-up visit. Six (28.6%) patients experienced >2 $\log_{10}$ HDV RNA reduction during follow-up, and HDV RNA became undetectable in 3 of them (14.3%). No differences in HBV DNA levels, qHBsAg, liver enzymes, or HIV-1–related characteristics were noted between individuals with and without HDV RNA reduction during TDF treatment. These 6 patients were all men, were slightly younger (37 years [IQR, 35–40] vs 43 [IQR, 36–46]), had a lower baseline HDV RNA (5.8 [IQR, 3.2–7.7] $\log_{10}$ copies/mL vs 7.3 [IQR, 5.9–8.1]), and were less likely to be on an HBV-active treatment at TDF start (1/6 [16.7%] vs 7/15 [46.7%]), but none of these results were statistically significant. The 3 patients who achieved HDV suppression during follow-up had lower HDV RNA (3.2 [IQR, 3.1–5.5] $\log_{10}$ copies/mL vs 7.4 [IQR, 6.0–8.1], $P = .01$) and lower qHBsAg (2.1 [IQR, −0.7–3.5] log10 copies/mL vs 4.0 [IQR, 3.8–4.1], $P = .02$)

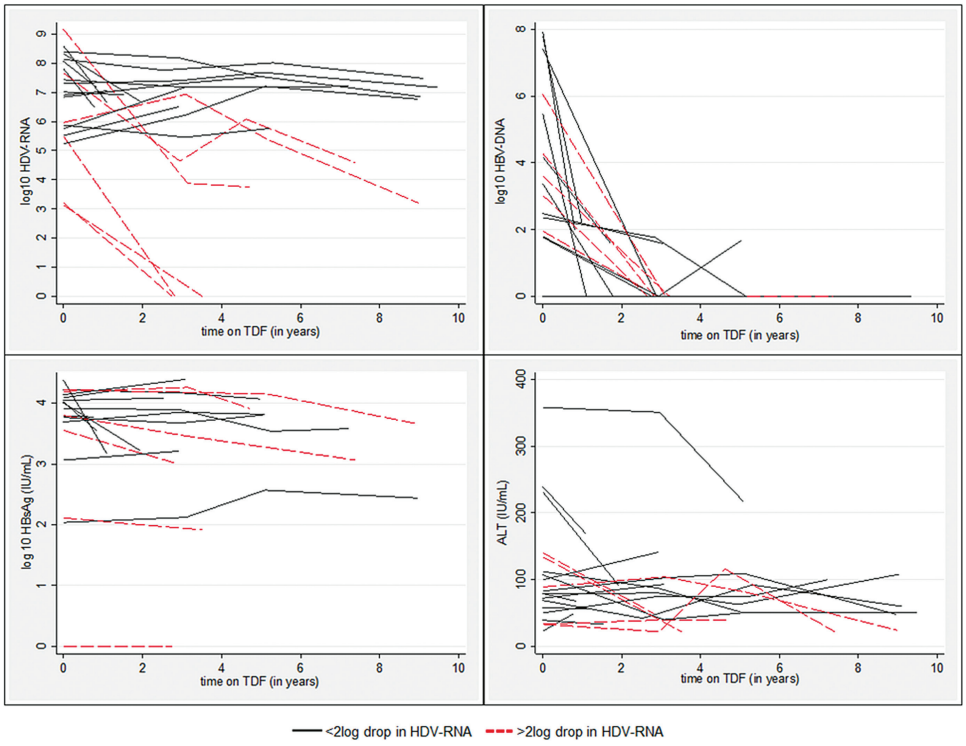

Figure 1. Hepatitis delta virus RNA, hepatitis B virus DNA, quantitative hepatitis B surface antigen, and alanine aminotransferase trajectories during tenofovir-containing antiretroviral therapy. Abbreviations: ALT, alanine aminotransferase; qHBsAg, quantitative hepatitis B surface antigen; HBV, hepatitis B virus; HDV, hepatitis delta virus; TDF, tenofovir.

at baseline and none were pretreated with an HBV-active drug. HBsAg loss was only observed in 1 patient who initiated TDF with a very low baseline qHBsAg.

DISCUSSION

Among 21 HIV/HBV-coinfected individuals with a replicating HDV infection at TDF initiation, only a minority experienced a reduction of at least 2 log in HDV viral load during a median of 5 years of therapy and only 3 reached HDV RNA suppression despite successful HBV therapy. In most patients, TDF therapy was not associated with a decrease in HDV RNA levels, which underlines the need for alternative therapies in order to control HDV in this population at high risk of liver-related events.

In line with the results from a cohort of 17 patients in France, we found that HDV RNA decreased minimally during TDF treatment and that only a minority of patients achieved full HDV suppression [12]. Our results contrast with the findings from a Spanish cohort in which HDV RNA dropped significantly in all 19 participants after a median of 54 months [11]. The main reasons for these differences across studies cannot be explained by major differences in demographic and HIV-related characteristics. In all 3 studies, concentrations of qHBsAg seemed not to be affected by TDF [11, 12, 16]. Despite the limited success of TDF in treating HDV infection, positive outcomes seem to be possible in selected individuals, as reported previously in HIV-uninfected individuals [17, 18]. We recently reported the case of an HIV/HBV/HDV-coinfected patient with an uncontrolled hepatitis delta after initial suppression on TDF [19]. In this case, the observation that initial HDV suppression was achieved when the patient had a low CD4 cell count pointed to a potential role of immunological recovery in the suppression of HDV infection. In the present study, the 3 patients who achieved HDV suppression did not have a severe impairment of cellular immunity but had lower HDV-RNA and qHBsAg at baseline compared to the other patients. Interestingly, they were not treated with HBV-active drugs before TDF initiation and might have benefitted from the initial impact on HBV infection from this drug. It is uncertain if these patients would have cleared HDV without TDF treatment.

We studied the impact of TDF on HDV replication in a nationwide cohort of HIV/HBV-coinfected individuals. Detailed virological analyses allowed the thorough assessment of the long-term impact of TDF-containing ART on HDV outcomes. Unfortunately, we did not have data on liver fibrosis at different time points for most patients, which limited the robustness of our results regarding clinical outcomes. Furthermore, 13 patients had to be excluded from our analyses because they did not have a stored serum or plasma sample available or were lost to follow-up.

In conclusion, TDF is highly efficacious in suppressing HIV and HBV replication in patients coinfected with HDV. However, in the majority of these patients, treatment with TDF does not result in a reduction of HDV RNA or qHBsAg. As replicating HDV infection is strongly associated with liver-related mortality, there is an urgent need for new treatment options.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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