Impact of tenofovir on hepatitis delta virus replication in the Swiss HIV Cohort Study

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Abstract
We analyzed changes in hepatitis B virus (HBV) and hepatitis delta virus (HDV) viral loads 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 viral load within five years.

Keywords: coinfection, hepatitis delta virus, human immunodeficiency virus, replication, tenofovir

Introduction
In 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 eight times more likely to die from liver-related complications than HDV-uninfected ones(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 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.

Patients and methods
All SHCS (www.shcs.ch) participants with a positive HBsAg test between January 1988 and December 2014 were considered(13). HDV serology was assessed in all patients as described before(4). For this analysis, we included all patients with an HDV RNA >300 cp/ml at TDF initiation and who had a follow-up sample after at least one year of TDF. All routine data were collected prospectively within the framework of the SHCS. Local Ethical Committees of all participating study sites have approved the study and written consent has been 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 by PCR Amplification, Sanger sequencing and subsequent in silico analysis by 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, USA).

Demographic and clinical characteristics at TDF start were described using absolute numbers and proportions, or medians and interquartile ranges (IQR). Individual follow-up started at initiation of TDF and ended on the date of death, loss to follow-up, TDF interruption or database closure (31.12.2014), whichever happened first. The proportion of patients reaching HDV RNA suppression or experiencing a viral load drop ≥2 log were described and their main characteristics compared with those of the other patients using Fisher’s exact and Mann-Whitney tests. Changes in HBV DNA 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 a year and follow-up samples were available in 21 of them. At baseline, patients were predominantly middle-aged (median: 40 years [IQR 35-45]) males (19/21 [90.5%]) from north-western Europe (15/21 [71.4%]). Only 2/21 (9.5%) patients were from sub-Saharan Africa (Supplementary Table). Thirteen of the 21 (61.9%) were persons who currently or previously injected drugs (PWID). Most individuals had already been on another ART regimen at 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-HCV serology (66.7%) whereas only 2/21 (9.5%) patients had detectable HCV RNA. Transaminases were elevated [ACTG (AIDS Clinical Trials Group) grade 1-4] in 15/21 (75%) of the 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, one from HCC and one from decompensated cirrhosis, and no patients were lost to follow-up. Median HDV RNA was 7.0 log₁₀ cp/ml (IQR 5.7-8.1) before TDF initiation and 6.7 log₁₀ cp/ml (IQR 4.6-7.2) at the last follow-up visit (Figure). 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 log₁₀ IU/ml at TDF initiation and 3.6 log₁₀ IU/ml (IQR 3.1-3.9) at the last follow-up visit. Six (28.6%) patients experienced >2 log₁₀ HDV RNA reduction during follow-up, and HDV RNA became undetectable in three 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 six 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] log₁₀ cp/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] log₁₀ cp/ml vs. 7.4 [IQR 6.0-8.1], p=0.01) and lower qHBsAg (2.1 [IQR -0.7-3.5] log₁₀ cp/ml vs. 4.0 [IQR 3.8-4.1], p=0.02) at baseline and none of them was pre-treated with an HBV-active drug. HBsAg loss was only observed in one 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 three individuals 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 time 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 three studies, concentrations of HBsAg 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 towards a potential role of immunological recovery in the suppression of HDV infection. In the present study, the three 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 efficient in suppressing HIV and HBV replication in patients coinfected with HDV. However, in the majority of these patients TDF did 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.
NOTES:

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Conflict of interest

The authors who have taken part in this study declared their conflicts of interest using the
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References


Figure:
Hepatitis D virus (HDV) RNA, Hepatitis B virus (HBV) DNA, quantitatively Hepatitis B surface antigen (HBsAg) and alanine aminotransferase (ALT) trajectories during tenofovir (TDF)-containing ART.