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Illuminating and Instructive Clinical Case

Virological Response to Lamivudine and Tenofovir Treatment in a Mono-infected Chronic Hepatitis B Patient with Potential Tenofovir Resistance: A Case Report

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Abstract

Few cases of tenofovir resistance have been reported, and the appropriate treatment for such cases remains unclear. We aimed to share a case of a chronic hepatitis B mono-infected patient with potential tenofovir resistance who required combined lamivudine and tenofovir therapy to achieve adequate viral suppression. The patient's viral load (plasma) was monitored using the cobas® hepatitis B virus Test on the cobas® 6800 system. Hepatitis B antiviral drug resistance (AVDR) mutations were assessed by amplicon-based sequencing. Plasma was extracted using the MagNa Pure 24 system, and polymerase chain reaction targeting the polymerase gene (860bp) was performed. Sequencing was conducted on GridION R10.4.1 flow cells, and the resulting FASTQ files were analyzed using DeepChek®-HBV Software. We describe a female patient in her 60s with chronic hepatitis B who was e-antigen positive. She met treatment criteria in May 2020, when her alanine transaminase levels were 1.5 times above the upper limit of normal. She was initially started on entecavir but had to switch to tenofovir alafenamide in June 2020 due to a rash. Despite three years of tenofovir therapy, her viral load remained unsuppressed. AVDR testing identified two suspected tenofovir resistance mutations (V191I and A317S). Since no mutations associated with lamivudine resistance were detected, the patient was treated with a combination of lamivudine and tenofovir, achieving viral suppression after four months. Although rare, tenofovir resistance should be considered in patients with persistent viremia despite long-term therapy. AVDR sequencing facilitated the detection of potential tenofovir resistance and guided treatment decisions, leading to successful viral suppression in this case.

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Introduction

Chronic hepatitis B (CHB) infection remains a major global health concern, affecting hundreds of millions of individuals worldwide.[1] People with CHB are typically asymptomatic in the early stages but may develop long-term complications such as cirrhosis, hepatocellular carcinoma, and end-stage liver disease.[2] The primary goal of treatment is to suppress the hepatitis B virus (HBV) to prevent these complications, including halting the progression of liver disease, reducing the risk of hepatocellular carcinoma, and decreasing transmission.[2,3] In Canada, eight therapies are approved for the treatment of CHB, with tenofovir and entecavir being the recommended first-line therapies due to their high potency and efficacy in HBV suppression.[3] Since CHB patients often require life-long antiviral therapy, minimizing antiviral resistance is a critical concern. Tenofovir has long been considered to have no associated resistance in HBV mono-infected patients, making it the preferred first-line therapy.[2,4] However, in recent years, rare case reports have documented tenofovir resistance in CHB patients. This resistance is thought to be related to tenofovir resistance-associated mutations (RAMs), although the clinical significance of these mutations remains unclear.[5] Here, we present a case of a CHB patient who, despite being compliant with tenofovir therapy, developed resistance and required the addition of lamivudine to achieve adequate viral suppression.

Case presentation

The patient is a female in her 60s with chronic hepatitis B who is e-Antigen (HBeAg) positive. For many years, she had persistently elevated viral loads but was not initiated on ther-

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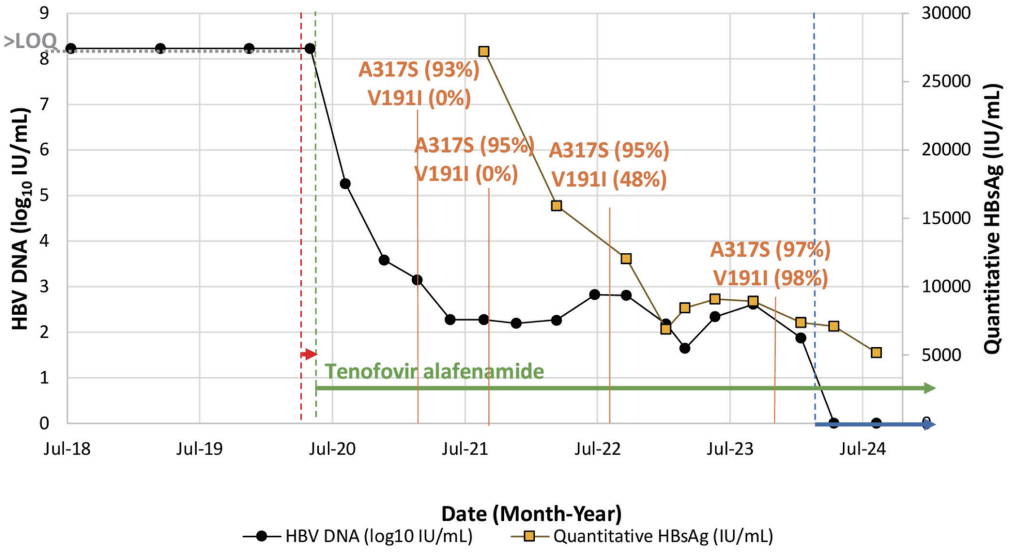

Fig. 1. HBV DNA levels (log_{10} IU/mL, cobas® HBV test) are displayed from July 2018 to April 2024. The upper limit of quantitation of the assay is >170,000,000 IU/mL or >8.23 log_{10} IU/mL (denoted by the gray dashed line, ">LOQ"). HBV AVDR testing was performed for four samples, with the prevalence of mutations A317S and V191I per sample noted in orange (%). AVDR, antiviral drug resistance; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

apy due to minimal fibrosis (F0-F1) on multiple Fibroscan assessments conducted from January 2015 to May 2019, along with relatively normal liver biochemistry. She exhibited only mild elevations in ALT on occasion, which were thought to be related to hepatic steatosis. In 2020, her ALT levels continued to increase, reaching 1.5 times the upper limit of normal (55 U/L), leading to the decision to start antiviral therapy. In May 2020, she was prescribed entecavir as first-line therapy for CHB but developed a rash soon after initiation. It was suspected that the rash was a drug reaction, as it appeared upon starting entecavir and resolved when the medication was discontinued. Due to the rash and the patient's preference, she was switched to Vemlidy (tenofovir alafenamide) in June 2020. Although she developed urticaria with Vemlidy, it was well managed with over-the-counter medications, allowing her to continue treatment. Her ALT levels normalized, and her viral load improved significantly but plateaued (Fig. 1). Hepatitis B antiviral drug resistance (AVDR) testing revealed HBV genotype C with pan-sensitivity to lamivudine, entecavir, tenofovir, and adefovir, with no mutations detected. She continued on tenofovir, although her viral loads remained detectable (range 1.65–2.82log_{10} IU/mL). Notably, throughout treatment with tenofovir, her viral load was never undetectable. Due to clinical suspicion of possible tenofovir resistance, additional HBV AVDR testing was requested (Fig. 1).

Hepatitis B viral load testing was performed on plasma using the cobas® HBV Test on the cobas® 6800 system (Roche Diagnostics). Serology, including quantitative HBsAg, was completed on the Architect platform (Abbott Diagnostics). Amplicon-based sequencing was utilized for AVDR testing on the GridION with R10.4.1 flow cells, using the SQK-NBD114.24 library kit (Oxford Nanopore Technologies).[6] Plasma was extracted with the MagNa Pure 24 system (Roche Diagnostics). PCR targeting an 860 bp region of the polymerase gene was conducted. Sequences were base-called with Guppy (v. 6.4.6), and FASTQ files were analyzed using DeepChek®-HBV Software (ABL SA Group). AVDR mutations were identified based on previously published literature.

Due to the inability to suppress the viral load on tenofovir (Fig. 1), HBV AVDR testing was requested based on the patient's most recent viral load (September 2023). This testing identified mutations associated with tenofovir resistance: V191I (98%) and A317S (97%). No mutations associated with lamivudine, adefovir, telbivudine, or entecavir were identified. Prior to 2023, HBV AVDR testing at our laboratory was performed using a different assay that did not cover A317S. Therefore, archived plasma from previous HBV viral load testing was retrieved from March 2021 [V191I mutation not detected, A317S (93%)], September 2021 [V191I mutation not detected, A317S (95%)], and July 2022 [V191I (48%), A317S (95%)] to be tested on the GridION to determine the presence of A317S. While A317S was consistently identified, the V191I mutation increased in predominance in 2022 and 2023. Lamivudine was added to the HBV therapy in December 2023, and no adverse reactions have been reported by the patient. Since starting treatment with both lamivudine and tenofovir, her liver biochemistry remains normal, and her HBV viral load has been below the limit of detection for eight months after changing therapy. Furthermore, we continue to observe a gradual decline in her quantitative HBsAg since the addition of lamivudine.

Discussion

First-line therapies for CHB include oral nucleos(t)ide analogs (NAs) that inhibit HBV DNA replication. Lamivudine was the first oral agent approved for HBV treatment in Canada. However, 71% of patients treated with lamivudine developed resistance within four years, leading to a decline in its favor as a first-line therapy.[3,4] Tenofovir, a purine nucleotide reverse transcriptase inhibitor, is now one of the most widely used agents due to its efficacy, low side effect profile, and high genetic barrier to resistance. It achieves HBV DNA suppression rates of 76% and 93% in HBeAg-positive and negative patients, respectively, and a 25% rate of HBeAg seroconversion after one year of therapy.[3,7] Tenofovir is also the first-line salvage therapy for patients resistant to lamivudine, entecavir, or telvibudine.[3] Suppression of HBV viremia reduces the risks of hepatitis, cirrhosis, hepatocellular carcinoma, and transmission.

One of the main concerns with long-term antiviral use is the development of resistance in response to selection pressures. Specific RAMs in the HBV polymerase gene have been identified for certain agents. Tenofovir resistance has been rarely reported. A recent systematic review and meta-analysis assessed the risk of HBV resistance in patients treated with tenofovir or entecavir. In the tenofovir group, a total of 30 studies were included (11 with NA-naïve patients and 19 with NA-experienced patients), with a pooled resistance risk of 0.0% at one year, three years, and greater than five years post-initiation of TAF/TDF therapy.[8] Although the pooled resistance risk was 0.0% across all time points, there were three studies that reported a total of five patients with tenofovir resistance, all of whom were NA-experienced.[8] Similarly, a prior longitudinal study of tenofovir therapy showed no resistance after 10 years of treatment.[4]

A recent systematic review generated a list of all polymorphisms reported in association with tenofovir resistance.[5] A total of 37 polymorphism sites were identified from 15 studies. Most studies reported that at least two RAMs were required to result in tenofovir resistance. Only two studies reported a single mutation conferring tenofovir resistance (S78T and A194T).[9,10] The most frequently identified RAMs were L180M, A181T/V, M204I/V, and N236T.[5] Prior NA exposure may also increase the chances of secondary resistance to tenofovir, as L180M, M204I/V, and A181T/V have been associated with resistance to lamivudine, entecavir, and telbivudine. Previous studies indicated that virological breakthroughs occurred between 48 weeks and 48 months after the initiation of tenofovir therapy.[5] The exact mechanisms underlying the development of such resistance remain unknown.

Our case report adds to the current knowledge on RAMs associated with tenofovir resistance. One of the predominant mutations found in our patient was A317S, which was first identified as a secondary mutation in an HBV mono-infected patient with lamivudine resistance.[11] A317S was also one of nine mutation sites in an HBV mono-infected treatment-naïve patient with tenofovir resistance.[12] In our case, it is unclear whether A317S was present at baseline or developed after nine months of tenofovir exposure. Interestingly, V191I was not initially detected in our patient but later emerged as a predominant mutation in line with virologic breakthrough. There may be a lower barrier to the development of the V191I mutation in the presence of A317S. However, it is challenging to determine whether V191I represents a compensatory mutation co-selected due to a primary RAM or if it is a mutation that confers tenofovir resistance. It is unlikely to result in resistance in isolation, as V191I has previously been identified as a variation of potential or secondary resistance.[13] Nevertheless, we suspect that in our patient, who had an HBV viral load of >170 million prior to the initiation of therapy, the addition of tenofovir selected for the low-level resistant strain, resulting in the sustained low-level viremia observed prior to the addition of lamivudine.

Treatment adherence is an important factor for sustained viral suppression. Seventy percent of virologic breakthroughs were associated with non-adherence rather than new resistance mutations.[14] One limitation of our case is the lack of drug concentration testing, as self-reported treatment adherence is subject to bias. Further studies are needed to assess the clinical significance of RAMs. In this case report, the progression of V191I into a predominant mutation corresponded with the stalled suppression of the viral load and suggests a contributory factor to sustained viremia despite tenofovir.

Conclusions

This case report highlights the importance of ongoing monitoring of HBV DNA response when initiating treatment with tenofovir to ensure a reduction in HBV DNA levels and to monitor for potential antiviral resistance. Despite previously reported low or negligible risk of tenofovir resistance, our case demonstrated persistent low-level viremia in a treatment-naïve HBV mono-infected patient treated with tenofovir monotherapy. There remains a paucity of data and guidance on the development and management of tenofovir resistance. Further research is prudent given the widespread use of tenofovir and the clinically significant impacts of drug resistance, which can result in hepatitis flares. Our case uniquely describes virologic suppression following the addition of lamivudine in a patient with tenofovir resistance.

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Conflict of interest

Trana Hussaini is a recipient of unrestricted research grants from Paladin Inc. and has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2018. Eric Yoshida is an investigator in clinical trials sponsored by Gilead Sciences, AbbVie, Merck, Intercept, Madrigal, Pfizer, Novartis, Allergan, and Genfit, and is a recipient of unrestricted research grants from Paladin Inc., and also receives honoraria for CME/Ad Board lectures from Gilead Canada, AbbVie Canada, Merck Canada, Intercept Canada, and Celgene Canada. The other authors have no conflicts of interest related to this publication.

Author contributions

Study concept and design (MD, TT, EMY, CFL), acquisition of data (MD, TT, GR, NM), analysis and interpretation of data (MD, TT, TH, GR, NM, EMY, CFL), drafting of the manuscript (MD, TT, CFL), and study supervision (EMY, CFL). All authors have made significant contributions to this study and have approved the final version and publication of the manuscript.

Ethical statement

The research is in accordance with the Helsinki Declaration. Consent for publication has been obtained from the patient.

Data sharing statement

All data pertaining to the results of this case report are included as part of the article and no additional data is required.

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