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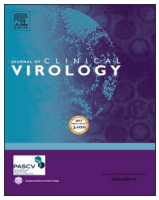

Full Length Article

Hepatitis B virus (HBV) viremia despite tenofovir disoproxil fumarate-containing antiretroviral therapy in persons with HBV/HIV coinfection

Patrick Ryan [a], Elizabeth Odegard [a], Heidi Meeds [a], Margaret Lartey [b,c], Vincent J. Ganu [c], Kenneth Tachi [b,c], Hongmei Yang [d], Oluwayemisi Ojewale [e], Isaac Boamah [f], Adjoa Obo-Akwa [b], Kenneth Antwi [c], Peter L. Anderson [g], Jason T. Blackard [a], Awewura Kwara [e,h,*]

[a] Division of Digestive Diseases, University of Cincinnati College of Medicine, Cincinnati, OH, USA
[b] Department of Medicine and Therapeutics, University of Ghana Medical School, Accra, Ghana
[c] Department of Medicine, Korle-Bu Teaching Hospital, Accra, Ghana
[d] Department of Biostatistics and Computational Biology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA
[e] Department of Medicine, University of Florida College of Medicine, Gainesville, Florida, USA
[f] Department of Microbiology, School of Biomedical and Allied Health Sciences, Accra, Ghana
[g] Colorado Antiviral Pharmacology Laboratory and Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado-Anschutz Medical Campus, Aurora, Colorado, USA
[h] Medical Service, North Florida South Georgia Veterans Health System, Gainesville, Florida, USA

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ABSTRACT

Background: The goal of treatment of hepatitis B virus (HBV) and human immunodeficiency virus (HIV) coinfection is suppression of both viruses; yet incomplete HBV suppression on tenofovir (TFV) disoproxil fumarate (TDF)-based antiretroviral therapy (ART) is common. This study investigated TFV resistance-associated mutations (RAMs) in individuals with HBV/HIV coinfection with viremia on TDF/lamivudine (3TC)-containing ART.
Methods: Samples from individuals with HBV DNA levels ≥20 IU/mL in a cross-sectional study of 138 persons with HBV/HIV coinfection in Ghana were analyzed in the present study. HBV was sequenced for RAM analysis. TFV-diphosphate (TFV-DP) concentration in peripheral blood mononuclear cells (PBMCs) was used to assess ART adherence level.
Results: Nine of 138 participants (6.5 %) had detectable HBV DNA levels ≥20 IU/mL while on ART. Seven of the nine participants had TFV-DP concentrations commensurate with 7 doses per week, and six had suppressed HIV RNA. Phylogenetic analysis revealed that eight sequences were HBV genotype E, with one genotype E/A recombinant. Ten previously-reported TFV RAMs were present in the study samples; eight were wild-type for HBV genotype E. The non-genotype-E-wild-type point mutations M267L and K333Q were found in two and one patients, respectively. No 3TC RAMs were found.
Conclusion: HBV viremia despite high adherence to TDF/3TC-based ART may be associated with the presence of TFV RAMs. These findings highlight the need for enhanced resistance monitoring and further research to examine the clinical significance of reported TFV RAMs. Individuals with HBV/HIV coinfection and TFV resistance on TDF-based ART may need alternative treatment strategies.

1. Introduction

HIV/HBV coinfection is common in areas that are endemic for both viruses. In 2015, ~2.7 million (7.4 %) of 36.7 million people living with HIV worldwide were infected with HBV, with 71 % (1.96 million) living in sub-Saharan Africa [1]. Complications of chronic HBV infection like cirrhosis, hepatocellular carcinoma, and death [2-5] are associated with HBV replication [6,7]. Thus, it is important to achieve HBV suppression in individuals with HIV/HBV coinfection as those with detectable HBV viremia on ART remain at increased risk for hepatocellular cancer and

* Corresponding author at: University of Florida College of Medicine, 2055 Mowry Road, P.O. Box 103600, Gainesville, FL 32610, USA.
E-mail address: awewura.kwara@medicine.ufl.edu (A. Kwara).

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Table 1
Primers used for HBV PCR.
Primers sets used for full- or partial-length DNA segment PCR amplification of HBV DNA from study samples.

Primer set	Primer direction	Primer name	Primer sequence	Binding site (nucleotides from *Eco*RI Site)	Fragment length
Full-Length	Forward	P1 [Günther et al., 1995]	5′- CCG GAA AGC TTG AGC TCT TCT TTT TCA CCT CTG CCT AAT CA - 3′	1821–1841	3.2 Kbp
	Reverse	P2 [Günther et al., 1995]	5′-CCG GAA AGC TTG AGC TCT TCA AAA AGT TGC ATG GTG CTG G - 3′	1825–1806	
A	Forward	CoreF [Powell et al., 2015]	5′- GTG TGG ATT CGC ACT CCT - 3′	2269–2287	2.3 Kbp
	Reverse	FengR [Feng et al., 2008]	5′- CCG ATG AGC TTT GCT CCA GAC C - 3′	1328–1307	
B	Forward	CoreF [Powell et al., 2015]	5′- GTG TGG ATT CGC ACT CCT - 3′	2269–2287	2.1 Kbp
	Reverse	Werle AS [Werle et al., 2004]	5′- CGT CAG CAA ACA CTT GGC - 3′	1175–1192	
C	Forward	P6 [Günther et al., 1995]	5′- GGG CAG GTC CCC TAG AAG AAG AAC T - 3′	2363–2386	2.2 Kbp
	Reverse	FengR [Feng et al., 2008]	5′- CCG ATG AGC TTT GCT CCA GAC C - 3′	1328–1307	

HBV, hepatitis B virus; PCR, polymerase chain reaction; Kbp, kilobase-pair.

mortality [8-10].

Tenofovir (TFV) disoproxil fumarate (TDF), TFV alafenamide (TAF), emtricitabine (FTC), and 3TC are antiretrovirals with activity against HBV. The World Health Organization recommends dolutegravir in combination with the nucleoside reverse-transcriptase inhibitors TDF plus 3TC (or FTC) as first-line ART regimen for people with HBV/HIV coinfection [11]. Despite the high barrier to TFV resistance [12-14], incomplete suppression of HBV on TDF-based ART is common [9,12, 15-20]. Baseline HBV DNA level, positive HBV e-antigen (HBeAg), prior 3TC therapy, less than 95 % ART adherence, $CD4^+$ count <200 cells/mm^3, and detectable HIV RNA have been associated with HBV viremia on TDF-based therapy [16-18,21]. HBV viremia despite suppressed HIV (suggestive of good adherence) on TDF-containing ART is also common [12,16-18] but the underlying reason(s) is not fully understood. The prevailing hypothesis is that levels of medication adherence and/or TFV-DP concentrations in cells must be higher to suppress HBV DNA below the level of detection compared to HIV RNA [15,18, 22]; however, there may be reduced susceptibility of HBV to TFV in some individuals. Emerging evidence shows some polymorphisms in HBV reverse transcriptase may reduce TFV susceptibility; however, the clinical significance of these mutations is not well established [23-27]. Therefore, we investigated whether detectable HBV viremia is associated with the presence of TFV and/or 3TC RAMs individuals with HBV/HIV coinfection on TDF/3TC-containing ART.

2. Methods

Study population. In a cross-sectional study, 138 individuals with HIV infection aged 18 years or older and on TDF/3TC-based ART with a positive HBsAg were enrolled at Korle-Bu Hospital in Ghana from November 2020 to November 2021 as previously reported [28]. Persons with HIV-2 or HIV-1/2 dual-infection were excluded because HIV-2 viral load could not be measured with available assays.

Demographic and clinical data, baseline $CD4^+$ counts, and HIV viral loads were collected. Blood samples were collected for confirmatory HBsAg status, liver function, and viral load testing. Plasma HIV viral load was measured by real-time PCR by an ISO-certified laboratory service provider in Ghana [28]. HIV RNA ≥20 copies/mL was considered unsuppressed. Plasma HBV DNA quantification was performed using a COBAS® TaqMan® Analyzer (Roche Diagnostics GmbH, Mannheim, Germany) with a range of 20–170,000,000 IU/mL. HBV DNA ≥20 IU/mL was considered unsuppressed. Nine samples with unsuppressed HBV DNA were evaluated in the current analysis.

For pharmacokinetic (PK) determination of antiviral activity and adherence, 4 mL of blood were collected in EDTA vacutainers, and PBMC concentrations of TFV-DP and 3TC-triphosphate from the samples were quantified at the University of Colorado Antiviral Pharmacology Laboratory [28-30]. As obtaining hepatic tissue is challenging clinically, TFV-DP in PBMCs was used as a surrogate of intrahepatic concentration as it has been used *in vitro-in vivo* translation of antiviral activity of TFV prodrugs [31]. TFV-DP concentrations in PBMCs were used to categorize antiviral activity and PK-determined adherence: TFV-DP concentrations in PBMCs ≥52 fmol/10^6 cells were considered commensurate with ingestion of seven doses per week, 30–51 fmol/10^6 cells 4–6 doses per week, 15–29 fmol/10^6 cells 2–3 doses per week, and <15 fmol/10^6 cells <2 doses per week [32].

Ethical Considerations. The Institutional Review Boards of Korle-Bu Teaching Hospital (KBTH/MD/G3/20), University of Ghana College of Health Sciences (EPRC/MAR/2020) and University of Florida (IRB202000688) reviewed and approved the study.

Sample processing, amplification, and sequencing. HBV DNA from nine study samples was extracted from 300 μL of plasma stored at -80 °C using the QIAmp UltraSens Virus Kit (QIAGEN Inc., Valencia, CA, USA). Completion-ligation reactions were performed by incubating the partially double-stranded HBV genome with a T4 ligase and polymerase at 30 °C for 30 minutes and then 20 minutes at 75 °C to inactivate the enzymes [33]. The samples, as well as negative controls, then underwent polymerase chain reaction (PCR) using the PicoMaxx High Fidelity PCR system (Agilent, Santa Clara, CA, USA) to amplify the full-length genome of 3.2 kilobase-pairs (Kbp) with forward primer P1 and reverse primer P2 [34]. Amplicons were isolated using 1 % agarose gel electrophoresis. For samples that did not amplify initially, PCR was attempted using different, previously-published primer sets A, B, and C (Table 1) targeting 2.1–2.3 Kbp of the polymerase open reading frame containing the entire reverse transcriptase (RT) gene [34-37]. For samples which still did not amplify, a nested PCR was undertaken by performing partial-length PCR on the amplicon of the initial full-length PCR. Samples 056, 106, and 150 were amplified using the full-length primer set. Use of primer set C achieved amplification of samples 066, 070, and 168. A nested PCR with full-length PCR followed by partial-length PCR with primer set B achieved amplification of samples 045 and 049. HBV DNA in sample 051 was amplified using a nested PCR with primer set C. The PCR protocol consisted of two minutes at 94 °C followed by ten cycles of 94 °C, 60 °C, and 68 °C for 40 seconds, 90 seconds, and three minutes, respectively. The samples were then held at 68 °C for two minutes before 30 cycles of 94 °C, 60 °C, and 68 °C for 40 seconds, 90 seconds, and five minutes, respectively. The protocol finished with eight minutes at 68 °C. PCR amplicons were separated by gel electrophoresis and purified with the QIAEX II Gel Extraction Kit (QIAGEN) and submitted for next-generation sequencing (NGS).

Table 2
Analysis for presence of previously-reported TDF resistance mutations.
Results of assessment for presence of various TDF resistance mutations in the reverse transcriptase (RT) protein, along with a reference source for each reported mutation [23-26]. Instances where the amino acid sequence of the study samples were positive for a reported mutation are labeled with an asterisk (*) Instance where the mutation is not present in HBV genotype E wild-type sequences are labeled with two asterisks (**). Point mutations that comprise a portion of a larger combination of amino acid mutations are reported as such in the "Notes" column.

Amino Acid # in RT	Genotype E Wild-Type amino acid	Mutation amino acid	Mutation Name	Sample 45	Sample 49	Sample 51	Sample 66	Sample 70	Sample 56	Sample 106	Sample 150	Sample 168	Notes	Reference
9	H	H	Y9H	H*	H*	H*	H*	H*	H*	H*	H*	H*		Mokaya 2020
35	H	N	H35N	-	-	-	-	-	-	-	-	-		Winckelmann 2022
35	H	Q	H35Q	-	-	-	-	-	-	-	-	-		Winckelmann 2022
63	V	V	I63V	V*	V*	V*	V*	V*	V*	V*	V*	V*		Mokaya 2020
78	S	T	S78T	-	-	-	-	-	-	-	-	-		Liu 2022
80	L	M	L80M	-	-	-	-	-	-	-	-	-		Mokaya 2020
91	L	I	L91I	-	-	-	-	-	-	-	-	-		Mokaya 2020
106	S	C	S106C	-	-	-	-	-	-	-	-	-	Part of CYEI mutation	Park 2019
106	S	G	S106G	-	-	-	-	-	-	-	-	-		Mokaya 2020
118	N	C	N118C	-	-	-	-	-	-	-	-	-		Mokaya 2020
118	N	G	N118G	-	-	-	-	-	-	-	-	-		Mokaya 2020
122	I	L	I122L	-	-	-	-	-	-	-	-	-		Mokaya 2020
126	Y	Y	H126Y	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Part of CYEI mutation	Park 2019
130	P	S	P130S	-	-	-	-	-	-	-	-	-		Mokaya 2020
134	D	E	D134E	-	-	-	-	-	-	-	-	-	Part of CYEI mutation	Park 2019
134	D	E	D134E	-	-	-	-	-	-	-	-	-		Mokaya 2020
153	R	W	R153W	-	-	-	-	-	-	-	-	-		Mokaya 2020
153	R	Q	R153Q	-	-	-	-	-	-	-	-	-		Mokaya 2020
177	P	G	P177G	-	-	-	-	-	-	-	-	-		Liu 2022
180	L	M	L180M	-	-	-	-	-	-	-	-	-	Part of MLVV mutation	Liu 2022
184	T	L	T184L	-	-	-	-	-	-	-	-	-	Part of MLVV mutation	Liu 2022
191	V	I	V191I	-	-	-	-	-	-	-	-	-		Mokaya 2020
192	R	P	R192P	-	-	-	-	-	-	-	-	-		Mokaya 2020
194	A	T	A194T	-	-	-	-	-	-	-	-	-		Liu 2020
200	A	V	A200V	-	-	-	-	-	-	-	-	-	Part of MLVV mutation	Liu 2022
204	M	V	M204V	-	-	-	-	-	-	-	-	-	Part of MLVV mutation	Liu 2022
207	V	L	V207L	-	-	-	-	-	-	-	-	-		Mokaya 2020
217	L	R	L217R	-	-	-	-	-	-	-	-	-		Mokaya 2020
221	Y	Y	F221Y	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*	Y*		Mokaya 2020
223	S	A	S223A	-	-	-	-	-	-	-	-	-		Mokaya 2020
229	L	V	L229V	-	-	-	-	-	-	-	-	-		Mokaya 2020
229	L	W	L229W	-	-	-	-	-	-	-	-	-		Mokaya 2020
249	F	A	F249A	-	-	-	-	-	-	-	-	-		Mokaya 2020
256	S	S	C256S	S*	S*	S*	S*	S*	S*	S*	S*	-		Mokaya 2020
263	E	E	D263E	E*	E*	E*	E*	-	E*	E*	-	E*		Mokaya 2020
267	M	L	M267L	L**	-	R	L**	-	-	-	-	-		Mokaya 2020
269	I	I	L269I	I*	I*	I*	I*	I*	I*	I*	I*	I*	Part of CYEI mutation	Park 2019
269	I	L	I269L	-	-	-	-	-	-	-	-	-		Mokaya 2020
278	V	I	V278I	-	-	-	-	-	-	-	-	-		Mokaya 2020
317	S	S	A317S	S*	-	S*	S*	S*	S*	S*	S*	S*		Mokaya 2020
333	K	Q	K333Q	N	-	Q**	N	-	-	T	-	-		Mokaya 2020
337	N	H	N337H	-	T	-	-	T	-	-	-	-		Mokaya 2020

CYEI: mutation containing combination of rtS106C, rtH126Y, rtD134E and rtL269I; MLVV: mutation containing combination of rtL180M, rtT184L, rtA200V, and rtM204V; RT, reverse transcriptase protein; TDF, tenofovir disoproxil fumarate.

Table 3
Demographics, clinical characteristics, and antiretroviral adherence levels in individuals with HIV/HBV coinfection with viremia at the time of the study visit.

Characteristic	Median (IQR) or n (Percent)
Age (years)	40.0 (32.0 – 51.0)
Weight (kg)	61.0 (55.0 – 88.0)
BMI (kg/m2)	21.1 (20.3 – 29.1)
Known duration of HIV (months)	103.0 (60.0 – 154.0)
Duration of ART (months)	89.0 (59.0 – 101.0)
Duration of TDF-based ART (months)	77.0 (49.0 – 89.0)
Sex	
Female	5 (55.6 %)
Male	4 (44.4 %)
Current CD4 cell count (cells/μL)*	206 (118 – 504)
Current CD4 count (cells/μL)*	
< 200	4 (44.4 %)
200 – 350	2 (22.2 %)
351 - 500	1 (11.1 %)
> 500	2 (22.2 %)
HBV DNA (IU/mL)	
20 to 2000	4 (44.4 %)
2001 to 20, 000	2 (22.2 %)
> 20, 000	3 (33.3 %)
HIV suppressed	
Yes	6 (66.7 %)
No	3 (33.3 %)
HIV suppressed / HBV unsuppressed	6 (66.7 %)
HIV unsuppressed / HBV unsuppressed	3 (33.3 %)
HBe antigen positive	3 (33.3 %)
HBe antibody positive	1 (11.1 %)
Current ART	
TDF/3TC/EFV	2 (22.2 %)
TDF/3TC/DTG	7 (77.8 %)
Number of times medications not taken in the last week?	
0	8 (88.9 %)
1 to 2	0 (0.0 %)
3 – 4	1 (11.1 %)
≥5	0 (0.0 %)
Tenofovir diphosphate in PBMCs (fmol/10^6 cells)	
≥ 52 (commensurate with 7 doses/week)	7 (77.8 %)
30 – 51 (commensurate with 4–6 doses/week)	1 (11.1 %)
15 – 29 (commensurate with 2–3 doses/week)	0 (0 %)
< 15 (commensurate with < 2 doses/week)	1 (11.1 %)
TFV-DP in PBMCs fmol (10^6 cells)	76.5 (57.0 – 93.6)
3TC-TP in PBMCs pmol (10^6 cells)	9.5 (7.4 – 13.5)

* Last known $CD4^+$ count in medical records closest to study visit (n = 119); BMI, body mass index; HIV, human immunodeficiency virus; ART, antiretroviral therapy; TDF, tenofovir disoproxil fumarate; 3TC lamivudine; FTC, emtricitabine; ART, antiretroviral therapy; PBMCs, peripheral blood mononuclear cells; TFV-DP, tenofovir diphosphate; 3TC-TP, lamivudine triphosphate.

Sequence analysis. NGS for each of the nine samples was performed using an Illumina HiSeq 1000 sequencer (Illumina Inc., San Diego, CA, USA). NGS 51 base-pair short-read sequences were aligned, and a consensus nucleotide sequence for each of the nine samples was generated using UGENE (Unipro, Novosibirsk, Russia) software.

References for HBV genotypes A to H were retrieved from the Hepatitis Virus Diversity Research database [38]. Genotype E references were available from several African countries, including Ghana (15), Central African Republic (7), Nigeria (7), Guinea (6), Liberia (6), Burkina Faso (5), Cameroon (5), Cape Verde (5), Niger (5), Namibia (3), Egypt (2), South Africa (2), Ethiopia (1), Madagascar (1), and Somalia (1), as well as Belgium (6), United Kingdom (3), Argentina (2), Cuba (2), Saudi Arabia (2), Japan (1), and Mexico (1). Consensus study sequences and references were aligned using Clustal X v2.1 [39] and trimmed using AliView [40] to contain the 2.1 Kbp segment of the viral genome that was amplified from all study samples, and visualized in FigTree.

Intergenotypic recombination was evaluated using the jumping profile Hidden Markov Model (jpHMM) program [41]. Consensus HBV sequences are available in GenBank as accession numbers PP818629 – PP818637.

Mutation identification. Aligned patient nucleotide sequences were translated into amino acid sequences using the Sequence Manipulation Suite online tool [42] and examined manually for known TDF RAMs previously reported in literature (Table 2). A WebLogo was generated, depicting amino acids at locations of selected TFV RAMs in RT protein sequences among the samples sequenced in this study and other HBV genotype E references from Ghana [43].

3. Results

Of the 138 enrolled individuals with HBV/HIV coinfection, ten (7.2 %) had HIV RNA ≥20 copies/mL, and nine (6.5 %) had HBV DNA ≥20 IU/mL. As previously reported, HBeAg status, HIV non-suppression status, and lower TFV-DP concentrations in PBMCs were associated with HBV non-suppression [28]. Demographic, clinical, and laboratory characteristics of the participants with HBV non-suppression included in the current study are shown in Table 3. Four of the nine participants were male with a median age of 40 years. Seven participants were on TDF/3TC/DTG, while the remaining two patients were on TDF/3TC/efavirenz (EFV). Median alanine transaminase (ALT) and aspartate transaminase (AST) levels were 30 and 45 U/L, respectively. Three participants had elevated ALT and six had elevated AST. Seven of nine participants had TFV-DP concentration ≥52 fmol/10^6 cells (commensurate with seven doses/week), a threshold that was established in HIV-negative adult [32]. The median duration of continuous TDF/3TC-based ART among the nine patients was 77 months. Three patients were positive for HBeAg, and one for HBe-antibody. Only participant 066 had received TDF/3TC-based ART for less than two years before the study visit. However, this individual was on TDF/3TC/EFV for 20 months, zidovudine/3TC/lopinavir/ritonavir for 12 months, and then TDF/3TC/DTG for 19 months before the study visit. Of the three participants with HIV viremia at the study visit, all had HIV RNA <400 copies/mL at last testing (range 17–38 months) before the study visit (Table 4). One of the participants with unsuppressed HIV had undetectable TFV-DP and 3TC-TP concentrations.

PCR amplification of HBV DNA was positive for all nine study samples. NGS reads ranged from 4,776 to 4,916,478 with an average of 2,269,004 reads per sample. Eight patients were identified as HBV genotype E—the dominant genotype in western Africa—while one sequence was a genotype E/A recombinant. A phylogenetic tree comparing the RT gene nucleotide sequences of the study samples to HBV references is presented in Fig. 1. Ten distinct, previously-reported TFV RAMs within the HBV RT gene were present in at least one of the samples. Eight mutations (rtY9H, rtI63V, rtH126Y, rtF221Y, rtC256S, rtD263E, rtL269I, and rtA317S) are wild-type for HBV genotype E. The point mutations rtM267L and rtK333Q—which are not wild-type for genotype E—were found in two and one samples, respectively. The amino acids at the studied mutation positions in genotype E HBV samples from this study and other references from Ghana are presented in Fig. 2 [44-46].

None of the 3TC RAMs considered in this study (rtL80V, rtL80I, rtI169T, rtV173L, rtL180M, rtA181T, rtA181V, rtT184S, rtS202G, rtM204V, rtM204I, rtM204S, rtV214A, rtQ215S, rtN236T, and rtN238D) were present in any of the nine samples. The presence or absence of reported TFV RAMs in the study samples investigated are shown in Table 2.

4. Discussion

This study provides valuable insights into the underlying factors associated with HBV non-suppression in persons with HIV/HBV coinfection on TDF/3TC-based ART. First, the high prevalence of HBV genotype E in our study is consistent with the known epidemiology of HBV in western Africa [47]. This is notable because genotype E has been associated with a distinct clinical course and response to therapy [48]. The identification of a case of HBV genotype E/A recombinant suggests

Table 4
Antiretroviral regimen, viral loads, and phosphate anabolites concentrations of the nucleoside analogs in peripheral blood mononuclear cells for each participant.

PID	HB eAg status	Months on TDF+3TC-based ART	Previous HIV RNA (copies/mL)	ART at study visit**	Visit HIV RNA (copies/mL)	Visit HBV DNA (IU/mL)	TFV-DP conc. (fmol 10^6 cells)	3TC-TP conc. (pmol 10^6 cells)
045*	Negative	99	397	TDF/3TC/EFV	92,872	212	BLQ	BLQ
049	Negative	87	65	TDF/3TC/EFV	110,497	740	98.6	13.4
051	Negative	49	< 20	TDF/3TC/DTG	24,136	351	55.0	8.8
056	Positive	86	TND	TDF/3TC/DTG	< 20	47,853	88.5	18.9
066*	Negative	19	209	TDF/3TC/DTG	< 20	6590	71.7	13.6
070*	Negative	70	19,658	TDF/3TC/DTG	< 20	288	143	7.2
106*	Positive	58	< 20	TDF/3TC/DTG	< 20	571,754	59.0	7.6
150*	Positive	43	162,515	TDF/3TC/DTG	< 20	17,887	81.4	4.6
168*	Negative	143	27	TDF/3TC/DTG	< 20	572,059	46.0	10.3

PID, participant ID.
* had elevated aspartate or alanine transaminase; TDF, tenofovir disoproxil fumarate; 3TC, lamivudine; EFV, efavirenz; DTG, dolutegravir.
** all except participant 045 and 049 were switched to TDF/3TC/DTG fixed-dose combination between the previous viral load test and study visit; HIV, human immunodeficiency virus; HBV, hepatitis B virus; TFV-DP, tenofovir diphosphate; 3TC-TP, lamivudine triphosphate; BLQ, below limit of quantification; TND, target not detected.

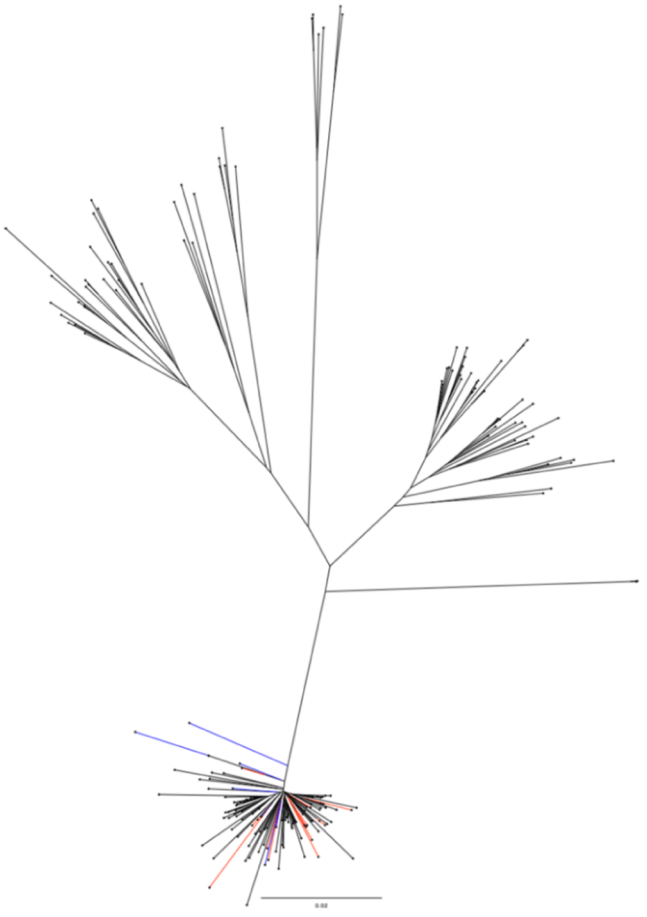

Fig. 1. Phylogenetic analysis of 9 partial HBV genomes from Ghana. Study sequences are shown in blue, while other genotype E sequences from Ghana are highlighted in red. The scale bar indicates nucleotide substitutions per site.

that recombination events could play a role in virological behavior and drug-resistance profiles of HBV in this region. Second, the detection of HBV viremia at the study visit despite seven of nine participants displaying adherence levels commensurate with ingestion of seven doses of TDF per week and as evidenced by HIV suppression in six of the nine patients is concerning. Although there are no established therapeutic TFV-DP concentrations for HBV suppression, it is possible that factors beyond adherence, such as viral resistance, may contribute to the observed incomplete HBV suppression. Also, past ART non-adherence may have contributed to the detectable HIV and HBV viremia and possible emergence of viral resistance, as the one-time TFV-DP concentration at the study visit may not reflect remote treatment non-adherence. Furthermore, Patient ID 045 had PBMC TFV-DP and 3TC concentrations below the lower limit of quantification, so the detectable viremia in this participant is likely due in-part to medication non-adherence. Although HIV drug resistance testing was not performed in this study, participants IDs 049 and 051 may have HIV drug resistance since HIV viremia was present despite having achieved therapeutic drug concentrations.

Sequence analysis identified several previously-reported TDF RAMs in the HBV RT gene in samples within this study. This could be concerning for development of TFV RAMs in patients taking TDF/3TC-based ART. Eight TFV RAMs identified in the study population are wild-type for HBV genotype E. Yet, two mutations—rtM267L in Patient IDs 045 and 066 and rtK333Q in Patient ID 051—are not wild-type for genotype E and could be associated with reduced susceptibility to TFV or represent drivers of increased viral fitness in persons on TDF-based ART. The fact that several TFV RAMs are wild-type for HBV genotype E may suggest a reduced barrier to resistance against TFV in this patient population. The importance of the mutations identified in our study or other mutations reported in a South African cohort [24] regarding their impact on HBV fitness and antiviral activity requires further investigation. As shown in Fig. 2, considerable heterogeneity at TFV RAM positions in the RT protein sequence exists in HBV genotype E samples from Ghana. These variations indicate a complex polymorphism landscape and underscore the potential for resistance patterns that could undermine the effectiveness of TDF-based regimens. It is also notable that no 3TC RAMs considered in this study were present in any study samples. The nine patients in the study had been on prolonged TDF/3TC therapy; thus, TDF use may have prevented the development of 3TC RAMs.

Fig. 2. A WebLogo chart depicting the frequencies of amino acids at select locations in the sequence of the hepatitis B virus reverse transcriptase protein among 68 HBV genotype E samples from Ghana, including from the present study, Archampong 2016, Zahn 2008, and Huy 2006. Amino acid position is shown at the bottom of the chart and abbreviations for amino acid found at that position in the included samples are listed vertically in decreasing order of prevalence, with letter height corresponding to proportion of samples with that amino acid at that location in the RT protein sequence.

HBeAg positivity was associated with HBV viremia in our study [28]. This finding is consistent with previous studies that have shown HBeAg positivity as a risk factor for incomplete HBV suppression on TDF-based therapy [16,17,49,50]. Three participants with HIV and HBV non-suppression at the study visit previously had fairly-controlled HIV suppression, which might suggest interval ART non-adherence or emergence of HIV resistance. Ongoing HIV replication in these individuals may have led to reduced immune status, lowering the genetic barrier to HBV resistance. In the parent study, lower median $CD4^+$ count was associated with HBV non-suppression [28], which is consistent with what others have observed [17]. The detectable concentrations of TFV-DP and 3TC-TP in PBMCs in those with suppressed HIV but unsuppressed HBV suggest that intracellular concentrations of these drugs needed to suppress HIV may not be adequate to prevent HBV treatment failure. This hypothesis requires further investigation to define the therapeutic concentrations of TFV-DP needed for maximum HBV suppression.

Limitations of this study include small sample size, lack of prospective data, and imperfect means of determining treatment adherence. The cross-sectional design makes drawing conclusions about the causality of RAMs in participants with viremia impossible. Larger longitudinal cohort studies to understand the dynamics of resistance development and use of objective measures of drug adherence may better-establish relationships between mutations and incomplete HBV suppression. In-vitro testing for changes in 50% inhibitory concentration of tenofovir would help to demonstrate that the mutations detected in this or other studies indeed confer resistance to TFV. These would provide more direct evidence of the clinical relevance of the reported mutations.

In conclusion, our findings underscore the complexity of managing HBV/HIV coinfection. The clinical relevance of the HBV RAMs in our study population with HBV viremia on TDF-based ART requires further investigations. Formulations that achieve higher TFV-DP concentrations or newer antiviral agents with different mechanisms of action may be needed to suppress HBV in those with reduced susceptibility to TFV. Our findings provide several leads and directions for future research to better understand the contribution of TFV resistance to incomplete HBV suppression during long-term TDF-based ART.

CRediT authorship contribution statement

Patrick Ryan: Writing – review & editing, Writing – original draft, Validation, Visualization, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Elizabeth Odegard:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Methodology, Data curation. **Heidi Meeds:** Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation. **Margaret Lartey:** Methodology, Investigation, Conceptualization. **Vincent J. Ganu:** Methodology, Investigation, Conceptualization. **Kenneth Tachi:** Methodology, Investigation, Conceptualization. **Hongmei Yang:** Methodology, Investigation, Conceptualization. **Oluwayemisi Ojewale:** Methodology, Investigation, Conceptualization. **Isaac Boamah:** Methodology, Investigation, Conceptualization. **Adjoa Obo-Akwa:** Methodology, Investigation, Conceptualization. **Kenneth Antwi:** Methodology, Investigation, Conceptualization. **Peter L. Anderson:** Methodology, Investigation, Conceptualization. **Jason T. Blackard:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Awewura Kwara:** Writing – review & editing, Validation, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors report no conflicts of interest related to this manuscript.

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