The Occurrence of rtA194T Mutant After Long-Term Lamivudine Monotherapy Remains Sensitive to Tenofovir Disoproxil Fumarate: A Case Report

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Abstract: Tenofovir disoproxil fumarate (TDF) is recommended as first-line agents in chronic hepatitis B (CHB) patients for its high antiviral effects and high barrier to resistance. It is controversial whether the rtA194T mutation truly confers resistance against TDF. We present here a 62-year-old CHB patient who occurred rtL180M, rtM204V and rtA194T mutants after lamivudine (LAM) monotherapy for 9 years. TDF was introduced in replacement of LAM and led to Hepatitis B Virus (HBV) DNA undetectable in 1 month, maintained in the follow up of 52 weeks. These observations suggest that rtA194T mutation emerges under LAM monotherapy and remains sensitive to TDF.

Keywords: hepatitis B, tenofovir disoproxil fumarate, resistance, rtA194T mutant, case report

Introduction
Chronic Hepatitis B (CHB) is a major problem for global public health. Antiviral treatment improves liver histology and reduces risks of liver-related fibrosis, hepatocellular carcinoma (HCC), and mortality.¹ Currently, tenofovir disoproxil fumarate (TDF) is recommended as first-line nucleos(t)ide analogue (NA) treatment in patients with CHB for its high antiviral effects and high genetic barriers to drug resistance.² Additionally, TDF is effective in patients harboring lamivudine (LAM)-resistant mutations.³ Although TDF-resistance can be found in anti-HIV treatment,⁴ TDF resistance was not detected in CHB patients after long-term TDF treatment.³ The rtA194T Hepatitis B Virus (HBV) polymerase mutation was firstly identified, along with LAM resistance-associated mutations (rtL180M and rtM204V), in two HIV/ HBV-coinfected patients treated with long-term TDF and LAM therapy. Meanwhile, phenotypic analyses revealed that constructs harboring rtA194T together with rtL180M and rtM204V displayed a 10-fold reduction in TDF susceptibility.⁷ In addition, Samad Amini-Bavil-Olyaee demonstrated that clones harboring rtA194T showed partial resistance (a fivefold to sevenfold increase in the EC50) to TDF in vitro, irrespective of additional mutations.⁸ However, Delaney WE had not found a clear association between rtA194T and viral load by using transfected-HepG2 cell culture.⁹ It appears that the potential impact of rtA194T mutation on TDF susceptibility remains unclear and therefore deserves further

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study. Here, we report a case of a CHB patient who
developed rtM204V, rtL180M and rtA194T mutations in
association with viral breakthrough on LAM monotherapy
and was rescued by TDF.

Case Presentation
A 62-year-old man was diagnosed with HBV-related HCC in
June 2010 and then received LAM antiviral treatment fol-
lowed by surgery. At the time of diagnosis, HBV DNA level
was almost 10,000 copies/mL by self-reports, the patient was
hepatitis B surface antigen (HBsAg) positive, hepatitis B e
antigen (HBeAg) negative, anti-HBe positive, and without
coinfection by hepatitis C virus (HCV) or human immuno-
deficiency virus (HIV). Two months later (August 2010), the
detectable level of HBV DNA was <1000 copies/mL (Roche
Diagnostics, Mannheim, Germany, detection threshold, 1000
copies/mL), HBsAg was 454.12 IU/mL (Roche Diagnostics
GmbH, Mannheim, Germany), serum alanine aminotrans-
ferase (ALT) was 24 IU/L (normal level: 10–64 IU/L),
aspartate aminotransferase (AST) was 24 IU/L (normal
level: 8–40 IU/L), total bilirubin (TBil) was 22.3 μmol/L
(normal level: 4.7–24 μmol/L), direct bilirubin was 4.0
μmol/L (normal level: 0–6.8 μmol/L), indirect bilirubin
(IBil) was 18.3 μmol/L, AFP was 13.05 ng/mL (normal
level: <8.04 ng/mL). No HBV resistance mutations were
detected using direct sequencing of the reverse transcriptase
region of HBV polymerase gene. Ultrasonography (US) of
liver showed that this patient had fatter liver and gallbladder
stone. During follow-up of 8 years, the detectable level of
HBV DNA was <500 IU/mL (Shanghai KEHUA Bio-
engineering Co., LTD; detection threshold, 500 IU/mL),
ALT and AST were normal and IBil mildly elevated
(35.4 μmol/L). In November 2019, the HBV load rebounded
to 4920 IU/mL (Roche COBAS TaqMan HBV test, Roche
Diagnostics, Mannheim, Germany, detection threshold, 20
IU/mL), HBsAg was 20.40 IU/mL, quantification of anti-
HBe (qAnti-HBe) was 3323.12 IU/mL, ALT and AST were
normal, IBil was 23.4 μmol/L, estimated glomerular filtration
rate (eGFR) was 94.1, complete blood count was normal,
AFP was 2.46 ng/mL. Subsequently, TDF was introduced
in replacement of LAM. Further genotypic drug resistance testing
found the emergence of rtL180M, rtM204V and rtA194T
mutations (confirmed by a second sequencing). US showed
that this patient had fatter liver and gallbladder stone.
Transient elastography (FibroScan) revealed that controlled
attenuation parameter (CAP) was 252 dB/m and liver stiffness
measurement (LSM) was 8.8 kPa. After 1 month
(December 2019), HBV DNA was undetectable (Roche
COBAS TaqMan HBV test, Roche Diagnostics,
Mannheim, Germany, detection threshold, 20 IU/mL) and
HBsAg was 18.27 IU/mL, qAnti-HBe was 2801.62 IU/mL.
Sequencing of uridine diphosphate glucuronosyl transferase
1A1 (UGT1A1) revealed that the patient was a heterozygous
individual with a TA insertion in TATA box of promoter
region as A(TA)k(TA)TA(A). In April 2020, HBV DNA
was undetectable (Roche COBAS TaqMan HBV test),
HBsAg was 21.37 IU/mL, qAnti-HBe was 1640.44 IU/mL,
and ALT was 33 IU/L, AST was 27 IU/L, IBil was 28.7 μmol/
L, eGFR was 93.6, complete blood count was normal, AFP
was 2.38 ng/mL. In June 2020, HBV DNA was also unde-
tectable (Roche COBAS TaqMan HBV test), HBsAg was
21.30 IU/mL, qAnti-HBe was 1808.99 IU/mL, and ALT and
AST were normal, IBil was 45.2 μmol/L. In December 2020,
HBV DNA remained undetectable (Roche COBAS TaqMan
HBV test), HBsAg was 20.96 IU/mL, and ALT was 24 IU/L,
IBil was 38.2 μmol/L. The evolution of viral load and HBV
genotypic patterns were presented in Figure 1, and the unified
unit of HBV DNA was IU/mL (1 IU/mL=5.6 copies/mL).10

Discussion
Whether the rtA194T mutation conferring resistance
against TDF is still controversial and more clinical
evidences are needed to reveal the true relevance of the
rtA194T mutation. In this case, we report the occurrence
of rtA194T mutant during the long-term LAM monotherapy
in a CHB patient who remains sensitive to TDF.

Prolonged LAM therapy is associated with the emergen-
cy of LAM-resistant mutations, from 24% in 1 year to
70% in 5 years.11,12 Three mutations associated with LAM
resistance have been mostly described: rtM204V/I in C
domain, rtV173L and rtL180M in B domain.13 In the
present case, no drug-resistant HBV variants were detected
at the initiation of antiviral treatment in this patient. How-
ever, HBV load rebounded to 4920 IU/mL from
<500 IU/mL at the ninth years of LAM monotherapy.
Subsequently, TDF was introduced as rescue therapy to
replace LAM. The HBV load decreased to undetectable
level in 1 month and maintained in follow-up of 52 weeks,
which suggested that the patient was sensitive to TDF.
Interestingly, genotypic drug resistance testing showed
that rtA194T mutation was present in this patient, along
with rtL180M and rtM204V mutations. It is coincided
with an in vitro study that the rtA194T mutation did not
confer to TDF as a single mutation or in a LAM-resistant
viral background, using a HepG2 cell system.9 But these
results do not agree with the previous study that a clear
association between rtA194T and viral load rebound reported by Sheldon. The discrepancy might be explained by differences among clinical data in patients. HBV rtA194T mutant emerged prior TDF treatment in this case, whereas Sheldon reported that rtA194T mutation occurred under the treatment with TDF for 11.2±6.7 months in two HIV/HBV-coinfected patients. Also, the HIV infection may influence the association between rtA194T mutation and TDF resistance. In addition, only one patient had a transient viral increase and the other one had continuous viral decrease after the occurrence of rtA194T mutant under TDF treatment in his study.

Furthermore, previous studies reported that rtA194T mutant can be observed in treatment-naïve patients. Nevertheless, HBV DNA could be detected in a patient again at 3 month after TDF treatment, which can be explained by short follow-up and nonoptimal compliance in this study. Apart from rtL180M, rtM204V and rtA194T mutations, rtM187V and rtV207L were also detected. RtV207L has been previously reported in LAM-resistance patients, but clinical implication needs to be further investigated. Notedly, close virological monitoring is necessary, because CHB patients with HBeAg negative may be at particular risk to rtA194T mutations on account of precore (PC) and basic core promoter (BCP) substitutions enhancing the reduced replicative capacity of rtA194T mutants. PC mutations occur frequently among patients infected with genotype C HBV DNA and BCP mutations are more prevalent in genotype A and C. However, G1896A or A1762T/G1764A mutations were not found in this patient with genotype C HBV DNA sequencing, while A1727T, C1730G and C1799G mutations were found in BCP region, which were reported to be associated significantly with cirrhosis.

The patient was diagnosed as Gilbert syndrome based on mild prolonged indirect hyperbilirubinemia and A(TA)₃TAA genotype. Individuals with Gilbert syndrome may be susceptible to these drugs that require glucuronidation for metabolism, such as menthol, estradiol benzoate, ethinyl estradiol, lamotrigine, tolbutamide, rifamycin SV, acetaminophen, nonsteroidal inflammatory drugs, statins and gemfibrozil human immunodeficiency virus (HIV) protease inhibitors. Indinavir and atazanavir (HIV protease inhibitors) can induce hyperbilirubinemia by inhibiting UGT1A1. Hyperbilirubinemia associated with the use of nucleoside/ nucleotide reverse transcriptase inhibitors is uncommon. TDF as one of nucleotide reverse transcriptase inhibitors, to our knowledge, no previous study has shown that TDF has notable toxicity to patient with Gilbert syndrome. And in our study, the TBil level of patient did not increase obviously after receiving TDF treatment. However, both chronic liver disease and Gilbert syndrome may potentiate the hyperbilirubinemia.
Therefore, the regular clinical and laboratory follow-up is essential.

In conclusion, our case reported that the emergence of rtA194T mutants within the HBV polymerase after LAM treatment is sensitive to TDF rescue therapy. The potential impact of this mutation on TDF susceptibility deserves to be elucidated by long-term observation of large cohorts.

Ethics and Consent Statement
Written informed consent was obtained from the patient for the publication of this case report and the study was approved by the institutional review board of Ruijin Hospital in accordance with the Declaration of Helsinki.

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Disclosure
The authors report no conflicts of interest in this work.

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