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Original Article

Primary partial response with tenofovir monotherapy: two adults and one child

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Abstract

Introduction: Three treatment-naïve cases with chronic hepatitis B (CHB) were reported in terms of a partial response to tenofovir disoproxil (TDF) monotherapy and antiviral-drug resistance.

Methodology: In this retrospective, case series study, patients who were treated for CHB at the departments of infectious diseases and clinical microbiology, University of Medical Science Bakırköy Dr. Sadi Konuk Training and Research Hospital were evaluated.

Results: A 26-year-old female patient and a 59-year-old male patient achieved sustained viral response with TDF (245 mg) or tenofovir alafenamide (TAF, 25 mg) + entekavir (ETV, 1 mg) combination therapy after failure with TDF monotherapy. The son of the female patient who was diagnosed with CHB infection due to a probable mother-to-child transmission did not achieve a complete viral response with interferon alfa-2b therapy for three months followed by lamivudine therapy for 19 months.

Conclusions: A TDF (245 mg) or TAF (25 mg) + ETV (1 mg) combination therapy is effective in the treatment of naïve patients with a partial response to the TDF monotherapy. A combination therapy including tenofovir and entecavir should be initiated to mothers with a primary partial response to the tenofovir monotherapy after the initial 32 weeks of pregnancy, as CHB may cause cirrhosis in the children due to a persistent inflammation in the liver subsequent to a vertical transmission.

Key words: HBV, tenofovir, response, entecavir, combination, therapy.

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Introduction

Hepatitis B virus (HBV), a member of the Hepadnaviridae family and a partially double-stranded DNA virus, replicates only in the liver [1]. The HBV reverse transcriptase does not possess a proofreading feature to resolve erroneously incorporated nucleotides. Therefore, mutations can occur very rapidly. A different swarm of viruses (quasispecies), including single and double mutants potentially related to drug resistance probably exists before treatment. The occurrence of a selected mutation during therapy depends on the capacity of a drug to suppress viral replication [2]. The optimal treatment regime should have antiviral effects targeted at different sites to decrease the risk of selecting out drug-resistant species. If a complete suppression of replication is achieved, resistance would not be a concern. Genetic barriers to the occurrence of mutations, the mechanism of drug resistance in the viral replication site, and several host factors involved in the regulation of the viral replication are associated with the development of the antiviral-drug resistance [2].

Hepatitis Be Antigen (HBe Ag) positivity is related to high viral replication, followed by high levels of HBV-DNA in the liver and blood, and the risk of cirrhosis and hepatocellular carcinoma [3]. HBe Ag seroconversion and viral suppression are key objectives of therapy for cases with HBe Ag-positive chronic hepatitis B (CHB), as they are associated with better long-term clinical outcomes, such as a histologic improvement, the prevention of CHB-related complications and an improved overall survival [4].

Tenofovir disoproxil fumarate (TDF) therapy achieves a sustained viral response and the regression of liver fibrosis and inflammation. TDF resistance has not been detected so far [5]. Tenofovir alafenamide fumarate (TAF) is more stable than TDF in the plasma and provides higher intracellular levels of the active phosphorylated metabolite "TFV-DP" in liver cells [6]. TAF provides less renal toxicity and reductions in the bone mineral density owing to its pharmacological properties compared to the treatment with TDF [7]. A resistance to tenofovir has not been reported yet [8]. A primary non-response is defined by less than 1 $\log_{10}$

decrease of serum HBV DNA value after three months of therapy. The partial virological response is defined as more than 1 $\log_{10}$ IU/mL decrease in the HBV DNA value, but an existence of the detectable HBV DNA after at least 12 months of therapy in compliant patients [9]. Lee *et al* reported two TDF-resistant mutations that include three new substitutions, specifically, rtS106C, rtH126Y, and rtD134E [10].

Treatment-naïve cases with chronic hepatitis B (CHB) were reported in terms of a partial response to the TDF monotherapy and antiviral-drug resistance.

Methodology

In this retrospective, case series-study, treatment naïve patients who were being treated for CHB at the Department of Infectious Diseases and Clinical Microbiology, University of Health Sciences Bakırköy Dr. Sadi Konuk Training and Research Hospital, and achieved a partial response after 12 months of TDF monotherapy were evaluated. Health Sciences University, Bakırköy Dr. Sadi Konuk Training and Research Hospital Ethics Committee approved the study (Approval number 429, 23/09/2019). The diagnosis of cases was based on a physical examination and biochemical parameters (aspartate aminotransferase (AST) and alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, bilirubin, serum albumin and gamma globulins, full blood count and prothrombin time), an abdominal ultrasound (including the liver), HBeAg and anti-HBe tests, the measurement of HBV DNA serum level, co-morbidities, a steatosis or steatohepatitis related to an alcoholic, autoimmune, metabolic liver diseases, and other causes of chronic liver disease should be systematically excluded including co-infections with hepatitis D virus (HDV), hepatitis A and C viruses (HAV, HCV), HIV, and a liver biopsy. The frequency of visit of patients was determined by the course of the disease. The mutation analysis related to antiviral drug-resistance against lamivudine (LAM), adefovir (ADV), entecavir (ETV), tenofovir (TDF), and telbivudin (LdT) was performed in the medical microbiology laboratory of Istanbul Cerrahpaşa School of Medicine by the genafor/arevir–geno2pheno drug resistance tool (Center of Advanced European Studies and Research, Bonn, Germany, http://coreceptor.bioinf.mpi-inf.mpg.de/). A databank was specifically designed for the rapid computer-assisted virtual phenotyping of HBV that admits genome (nucleic acid) sequences as input after quantification of serum HBV DNA levels by means of the real-time polymerase chain reaction (RT-PCR) assay using Cobas Ampliprep/Cobas TaqMan HBV version 2 (Roche Molecular Systems, Pleasanton, California), according to the manufacturer's instructions. The geno2pheno tool searches for HBV drug resistance mutations in the RT domain of the polymerase gene at H124Y, Y135S, and N248H as well as at SHB protein T127P. Patients also underwent a 24-hour urine test and a dual emission X-ray absorbtiometry (DEXA) every year to detect any side effects of TDF on bone and kidneys, such as nephrotoxicity and osteoporosis.

Results

Case 1

In 2015, a 26-year-old female patient was admitted to the infectious disease clinic with a diagnosis of CHB

Table 1. The findings of case 1 with chronic hepatitis B.

Time	ALT (N: 0-40 U/L)	AST (N: 0-40 U/L)	HBV-DNA (IU/mL)	Anti-HDV total	Treatment
One year before the treatment	37	25	773816718	NEGATIVE	Pregnancy
Initiation of treatment at 28th week of pregnancy	17	17	400594927	NEGATIVE	Tenofovir disoproxil (TDF)
3 months (one month after birth)	33	25	23842	NEGATIVE	TDF
6 months	25	19	1795	NEGATIVE	TDF
9 months	34	23	7509	NEGATIVE	TDF
12 months	35	25	730	NEGATIVE	TDF
18 months	34	20	417	NEGATIVE	TDF
21 months	37	24	14743	NEGATIVE	Entecavir (ETV) 1 mg
23 months	34	25	112	NEGATIVE	TDF + ETV 1 mg
26 months	34	24	11	NEGATIVE	TDF + ETV 1 mg
32 months	28	26	8	NEGATIVE	TDF + ETV 1 mg
38 months	27	26	9	NEGATIVE	TDF + ETV 1 mg
44 months	32	27	12	NEGATIVE	TDF + ETV 1 mg
50 months	27	24	8	NEGATIVE	TDF + ETV 1 mg
56 months	29	25	10	NEGATIVE	TDF + ETV 1 mg
68 months	24	27	5	NEGATIVE	TDF + ETV 1 mg

infection (Table 1). She had CHB for two years, but had not visited any clinic. Her mother and brother were also diagnosed with CHB and were being treated. Upon admission, laboratory tests were positive for HBs Ag and HBe Ag and negative for Anti-HDV total test. The HBV-DNA value was 400594927 IU/mL, all other laboratory tests and ultrasonography findings were normal. She did not have a history of comorbid conditions and did not receive any medications. In the liver biopsy, an ISHAK inflammation score of 6/18 and a fibrosis score of 2/6 were reported. She refused the therapy due to her intention of becoming pregnant. TDF treatment (245 mg) was postponed until 32 weeks of pregnancy, and then initiated. HBV-DNA values at month 12 and 18 of TDF therapy were 730 IU/mL and 417 IU/mL, respectively. TDF therapy was continued due to its high-resistance barrier. We could not measure the TDF blood level of the patient, as testing facilities were not available in Turkey. The generic product was chosen for the TDF therapy and the standard dose was given. Drug adherence of the patient was accurate. She refused to receive a combination therapy, and then received only entecavir (ETV) 1 mg treatment for two months. The HBV drug-resistance analysis could not be performed at that time, as it was not available in Turkey. When HBV-DNA values increased to 14743 IU/mL, she agreed to receive the TDF and ETV (1 mg) combination therapy. After five months of combination therapy, the HBV-DNA value decreased to 11 IU/mL. When the HBV drug resistance test was available, TDF was discontinued and ETV 1 mg was continued as a monotherapy to increase the HBV-DNA level. Mutation in the previously mentioned gene regions was not found in the studied gene regions and the HBV-DNA value was 700 IU/mL. After drawing of the blood for the antiviral drug resistance test, the TDF (245 mg) + ETV (1 mg) combination therapy was continued and HBe Ag continued to be positive during the follow-up. She did not have any TDF-related side effect. She continued to receive the TDF + ETV combination therapy with an undetectable HBV-DNA value.

The Hepatitis B vaccine and hyperimmune globulin were administered to her son at the birth, but his laboratory tests indicated HBs Ag (+), HBe Ag (+), total HDV antibody (-), and HBV-DNA: 40689773 IU/mL at 28 months of age (Table 2). He was diagnosed with CHB at two years of age by the pediatric gastroenterologist following laboratory tests and the liver biopsy resulted in an ISHAK grade of 6/18 and a fibrosis stage of 2/6. Interferon alfa-2b 3000000 IU/m^2 subcutaneously three times per week was initiated, but the pediatric gastroenterologist discontinued the therapy due to intolerable side effects after three months of therapy. After those side effects, lamivudine (LAM) was initiated, as the HBV-DNA value was 36883059 IU/mL. During the third month of LAM therapy, the HBV-DNA value decreased to 95967 IU/mL and the LAM therapy continued, as HBe Ag was positive and the total delta antibody was negative. No gene mutation related to HBV drug resistance was found. The HBV-DNA value increased to 116916 IU/mL at month 6 of LAM therapy and was interpreted as a resistance to LAM. He is still being treated at the department of pediatric gastroenterology. Although the viral load decreased to 40261IU/mL in month 11 of his treatment, the viral load increased to 52734977 IU/mL during month 19, and then a primary unresponsiveness was considered. Thereupon, the permission for entecavir therapy was requested from the Ministry of Health (Table 2). At 12 months of entecavir treatment, HBV DNA became negative, and AST and ALT decreased to the normal ranges.

Table 2. The findings of the son of case 1.

Age	ALT (N: 0-40 U/L)	AST (N: 0-40 U/L)	HBV-DNA (IU/mL)	Anti-HDV total	Treatment
12 months	66	58	216342929	NEGATIVE	
18 months	50	37	34425914	NEGATIVE	
24 months	123	79	154648738	NEGATIVE	
28 months	100	73	196762495	NEGATIVE	
29 months	33	25	40689773	NEGATIVE	Interferon alfa-2b initiated
33 months	25	19	36883059	NEGATIVE	Interferon alfa-2b discontinued and lamivudine (LAM) initiated
36 months	34	23	95967	NEGATIVE	Month 3 of LAM therapy
39 months	44	52	116916	NEGATIVE	Month 6 of LAM therapy
44 months	42	47	40261	NEGATIVE	Month 11 of LAM therapy
52 months	146	88	52734977	NEGATIVE	Month 19 of LAM therapy
56 months	126	82	21326811	NEGATIVE	Entecavir (ETV) therapy
59 months	64	48	75458	NEGATIVE	Month 3 of ETV therapy
62 months	45	48	82700	NEGATIVE	Month 6 of ETV therapy
68 months	24	22	NEGATIVE	NEGATIVE	Month 12 of ETV therapy

Case 2

A 59-year-old male patient was admitted to the infectious disease clinic for the examination of HBV infection after his wife had an acute Hepatitis B infection. In 2013, he was diagnosed with CHB infection without a history of comorbid diseases and medications. His test results were: HBs Ag (+), HBe Ag (+), Anti-HDV total (-), HBV-DNA value of 287243345 IU/mL, and the liver biopsy resulted in an ISHAK score of 4/18 and a fibrosis score of 2/6. The TDF monotherapy was initiated and HBV-DNA values were 7910 IU/mL at month 12 and 12439 at month 24, respectively. We could not measure the TDF blood levels of this case, as testing facilities were not available in Turkey. The generic product was chosen for the TDF therapy to be sure that the standard dose was given and the drug adherence of the patient was accurate. The patient received the TDF and ETV (0.5 mg) combination therapy and his HBV-DNA titer decreased to 63 IU/mL at month 24 of the combination therapy. When he requested to discontinue the combination therapy, he received only ETV (1 mg) treatment for three months. The HBV-DNA titer increased to be 10449 IU/mL, and then he agreed to receive the TDF and ETV (1 mg) combination therapy once again. After six months of TDF + ETV combination therapy, HBV-DNA titer decreased to 6 IU/mL. He underwent the 24-hour urine test and dual emission X-ray absorptiometry (DEXA) every year. Although his renal function was normal, osteoporosis was diagnosed, and then a calcium plus vitamin-D supplementation was initiated. The TDF therapy was switched to the TAF (25 mg) therapy at month 63 of CHB treatment due to osteoporosis, when TAF was available in the markets of Turkey in 2019 (Table 3). When the HBV drug resistance test was available, TAF was discontinued and ETV 1mg was continued as a monotherapy to increase the HBV-DNA level. Any mutation in aforementioned gene regions was not found and the HBV-DNA value was 500 IU/mL. After the antiviral drug resistance test, the TAF (25 mg) + ETV (1 mg) combination therapy was continued and HBV-DNA became undetectable after one month of the combination therapy. HBe Ag continued to be positive during the follow-up.

Discussion

The TDF monotherapy did not achieve a virological response in two naïve cases and HBV-DNA levels decreased to undetectable levels after the ETV-TDF combination therapy. The partial response suggests an antiviral drug resistance or an inadequate bioavailability of TDF. We could not measure the TDF blood levels, as testing facilities were not available in Turkey. The generic product was chosen for the TDF

Table 3. The findings of case 2 with chronic hepatitis B.

Time	ALT (N: 0-40 U/L)	AST (N: 0-40 U/L)	HBV-DNA (IU/mL)	Anti-HDV total	Treatment
Initiation of treatment	79	45	287243345	NEGATIVE	TDF
1 month	70	44	915959	NEGATIVE	TDF
3 month	63	39	58413	NEGATIVE	TDF
6 month	69	37	10033	NEGATIVE	TDF
12 month	87	41	7910	NEGATIVE	TDF
15 month	71	38	15999	NEGATIVE	TDF
16 month	65	32	12573	NEGATIVE	TDF
18 month	54	31	15951	NEGATIVE	TDF
21 month	48	29	12439	NEGATIVE	TDF + ETV 0.5 mg
26 month	36	22	185	NEGATIVE	TDF + ETV 0.5 mg
33 month	20	16	74	NEGATIVE	TDF + ETV 0.5 mg
36 month	34	16	245	NEGATIVE	TDF + ETV 0.5 mg
43 month	18.2	14	74	NEGATIVE	TDF + ETV 0.5 mg
45 month	24	20	63	NEGATIVE	TDF + ETV 0.5 mg
48 month	22	18	140	NEGATIVE	ETV 1 mg
49 month	25	23	9473	NEGATIVE	ETV 1 mg
50 month	16	16	10449	NEGATIVE	ETV 1 mg
53 month	25	20	109	NEGATIVE	TDF + ETV 1 mg
57 month	21	18	6	NEGATIVE	TDF + ETV 1 mg
63 month	18	22	5	NEGATIVE	Tenofovir alafenamide + ETV 1 mg
69 month	21	18	4	NEGATIVE	Tenofovir alafenamide + ETV 1 mg
74 month	25	21	6	NEGATIVE	Tenofovir alafenamide + ETV 1 mg
80 month	28	25	7	NEGATIVE	Tenofovir alafenamide + ETV 1 mg
96 month	25	22	4	NEGATIVE	Tenofovir alafenamide + ETV 1 mg

TDF: Tenofovir disoproxil; ETV: Entecavir.

therapy to be sure about that the standard dose was given and the drug adherence of the patient was accurate. Higher TDF plasma concentrations were reported in patients with HIV in relation to ABCC2 and ABCC4 polymorphisms that cause renal toxicity [11,12]. Although the examination of HBV drug resistance performed in the RT domain of the polymerase gene at H124Y, Y135S, and N248H as well as at SHB protein T127P in a reference laboratory of Turkey resulted in negative result, it is more reasonable that a resistance gene area exists that was not detected. There are some reported viral resistance gene areas in addition to those that we examined in the samples. Marhoon *et al.* reported A194T mutation associated with the TDF-resistance, and L180M, A181T/V, M204V/I/S and N236T mutations associated with the multi–drug resistance in 20 patients who had CHB with high viral loads after six months of the TDF-ETV combination therapy [13]. The rtA194T mutation causes a decrease in TDF sensitivity by increasing the IC50 value in vitro analysis, as it developed neither TDF-resistance in vivo, nor a partial TDF drug-resistance [14-16]. Park *et al*. reported two chronic hepatitis B cases with TDF-resistance with the seven common mutations, including rtS106C [C], rtH126Y [Y], rtD134E [E], rtV173L [L], rtL180M [M], rtM204I/V [V], and rtL269I [I] that could be associated with the TDF-resistance. These results indicated that the CYE mutation reduces TDF-susceptibility (by 3.7-fold) and the CYEI mutation provides complete resistance (by 15.3-fold) to TDF. The TDF-resistance with the CYEI mutation (i.e., CYELMVI) is boosted by the ETV-resistance along with a LMV resistance mutation. In their study, a 57-year-old woman with a history of LAM, ETV, adefovir (ADV), LAM+ TDF, and ETV+TDF treatments and a 66-year-old man with a history of lamivudine (LAM), ETV, LAM+ ADV, TAF+ telbivudine (LdT), and TDF treatments were reported. An undetectable viral level was reported in both cases and one case died of hepatocellular carcinoma [17].

Van Bömmel *et al*. analyzed 113 patients who received the TDF monotherapy and 21 patients with a history of ADV resistance. All patients without ADV-resistant variants achieved HBV DNA values less than 400 copies/mL, as only 52% of the patients with prior ADV-resistance reached below that level [18]. Lee *et al*. reported nine mutation sites associated with resistances against LAM, ETC and ADV in patients who developed virological and enzymatic breakthroughs after the TDF treatment due to failures with LAM, ETV, and LAM+ADV treatments, respectively [19]. The fact that both of our cases were treatment naïve, is the main difference between our cases and the previously reported ones. Therefore, primary resistance could be an issue in our cases, rather than cross-resistance.

The percentage of cases, who were HBV resistant to multiple drugs, including lamivudine, ETV, LdT or ADV, and whose HBV-DNA values were under 15 IU/mL at week 48, were reported to be 68% with the TDF monotherapy and 69.5% with the TDF-EDV combination therapy in the study by Lim *et al* [20]. At week 144 of the treatment, 81% of patients with TDF monotherapy and 77% of patients with TDF-EDV combination therapy had HBV-DNA < 15 IU/mL, as six of the nineteen patients, who had HBV DNA levels > 60 IU/mL, continued to have some baseline resistance mutations [20]. A virological breakthrough was reported in six patients, who had poor drug adherence and comprised of four patients with the TDF-monotherapy and two patients with TDF-EDV combination therapy.

AST and ALT continued to be normal from the beginning of the treatment in Case 1and after 26 months of treatment in Case 2. The inflammatory process during CHB and the virological response to antiviral therapy are the main factors in the development of long-term complications of CHB. We switched to TAF (25 mg) therapy in Case 2 due to osteoporosis, and then biochemical and virological tests continued to be within normal ranges. On the other hand, Agarwal *et al.* reported that the short-term safety of 120 mg/day TAF, which is 4.8-fold higher than the standard dose, might not be an optimal salvage therapy for patients who have TDF-resistance. Since the IC50 and IC90 values of the CYEI mutant were 15.3- and 26.3-fold higher than those of wild-type HBV, respectively [21].

The son of Case 1 was most likely infected with HBV by a mother-to-child transmission. LAM did not achieve an undetectable viral load at month six of therapy; moreover, the HBV-DNA value at month six of treatment was higher than that at month three. ETV achieved a viral response at month 12 of therapy. Pan *et al.* reported that the rates of mother-to-child transmission under TDF treatment were significantly lower than the cases without any antiviral therapy [22]. Although TDF was initiated at week 28 of the pregnancy and the HBV-DNA level of Case 1 decreased, her son was infected with HBV despite administering vaccines and a hyperimmunoglobulin after birth. The vertical transmission of HBV was reported in spite of postnatal active and passive immunizations in 9-39% of infants of highly viremic

mothers (≥ 8 log copies/mL), and there was a relationship between the transmission and maternal serum HBV-DNA levels [23].

Conclusions

A TDF or TAF+ETV 1 mg combination therapy is effective in the treatment of naïve patients with partial response to the TDF monotherapy. Pregnant women who have CHB and high viral loads (≥ 8 log copies/mL) should be evaluated in terms of an initiation of the antiviral treatment prior to 28 weeks of the pregnancy to prevent mother-to-child transmission. A combination therapy including tenofovir and entecavir should be initiated to mothers with a primary partial response to the tenofovir monotherapy prior to 32 weeks of the pregnancy, as CHB may cause cirrhosis in the children, due to the persistent inflammation in the liver subsequent to a vertical transmission.

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