

Efficacy and Safety of Tenofovir Disoproxil Fumarate for Patients with Lamivudine-Resistant Hepatitis B Virus Infection

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ABSTRACT

AIM: Tenofovir disoproxil fumarate (TDF) has potent antiviral efficacy and lack of resistance during long-term use in chronic hepatitis B (CHB) patients. The purpose of this study was to evaluate antiviral efficacy and safety of TDF in lamivudine-resistant CHB patients.

METHODS: We performed a retrospective study of consecutive CHB patients who had detectable HBV DNA ($> 50\text{IU/mL}$) and documented lamivudine-resistant mutations during antiviral treatment. Patients who had adefovir or entecavir-resistant HBV infection were excluded. They were treated with TDF monotherapy or combination with lamivudine more than 6 months. We analyzed virologic response (HBV DNA $< 20\text{ IU/mL}$), biochemical and serologic responses to the TDF treatment and adverse events.

RESULTS: A total of 101 CHB patients (HBeAg-positive 86%, mean baseline HBV DNA $3.29\text{ log}_{10}\text{IU/mL}$) were enrolled. They were treated with TDF ($n = 74$) or combination with lamivudine ($n = 27$) for median duration of 20 months. The proportion of patients achieving virologic response at 6 months and 12 months was 80.2%,

and 89.7%, respectively. The mean change from baseline in HBV DNA was $-2.05\text{ log}_{10}\text{IU/mL}$, and $-2.14\text{ log}_{10}\text{IU/mL}$, respectively. Multivariate Cox regression analysis showed that baseline HBV DNA level was a significant predictive factor of virologic response at 12 months. (HR = 0.645; 95% CI 0.504-0.826; $p = 0.001$). Two patients (2.4%) showed HBeAg loss, and no patient lost HBsAg during the treatment period. Serious adverse events or renal impairment was not observed.

CONCLUSION: TDF is safe and effective for complete viral suppression in patients with lamivudine-resistant HBV infection.

Key words: Chronic hepatitis B; Lamivudine; Resistance; Tenofovir

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INTRODUCTION

Long-term goal of antiviral treatment for patients with chronic hepatitis B is to prevent serious long-term consequences of cirrhosis, hepatocellular carcinoma, and death. Lamivudine (LAM) was the most commonly prescribed first line agent for treatment of chronic hepatitis B in many Asian countries which were highly prevalent in HBV infection^[1]. LAM therapy is effective in reduction of hepatitis B virus DNA (HBV DNA) and normalization of alanine aminotransferase (ALT)^[2,3]. However, long-term administration of LAM has resulted in the emergence of lamivudine-resistant mutations in up to 70% after 5 years of treatment^[4,5]. The LAM resistance increases the risk of progression of liver disease and antiviral resistance to secondary antiviral agents^[6]. Mutations in the YMDD catalytic motif in the C domain of HBV polymerase (rtM204V/I) with or without rtL180M

are responsible for LAM resistance^[7].

Adefovir dipivoxil (ADV) has an antiviral efficacy in wild-type and LAM-resistant HBV, however, suboptimal viral response is frequent^[8]. ADV-resistant mutations occur more frequently in patients with LAM-resistant HBV infection than in treatment-naïve patients^[9]. ADV plus LAM combination appears to reduce the risk of genotypic resistance to ADV and virologic breakthrough^[10]. Clinical guidelines for antiviral resistant HBV have recommended that ADV plus LAM combination is more preferable strategy than ADV monotherapy for LAM resistance to decrease the rate of resistance to ADV. LAM plus ADV combination therapy resulted in suboptimal virologic response particularly in patients with high viral load and resistance to both drugs^[11].

Entecavir (ETV) shows viral load reduction and ALT normalization compared with LAM continuation in patients with LAM resistance^[12]. However, resistance to ETV frequently develops in pre-existing LAM-resistant HBV despite administration of ETV at a daily dose of 1mg rather than 0.5 mg recommended for naive-patients. ETV therapy is not sufficient for the rescue therapy in LAM-resistant patients^[13].

Tenofovir (TDF) has a potent antiviral activity against both wild-type or LAM-resistant HBV^[4]. TDF demonstrated superior antiviral efficacy compared to ADV at 48 weeks and long-term suppression of HBV DNA through 5 years with absence of long-term resistance^[14,15]. TDF is currently recommended for the first-line agents for chronic hepatitis B patients^[16,17]. Randomized controlled trial of TDF therapy in LAM-resistant HBV was recently demonstrated that TDF achieved excellent viral suppression at a rate of 89% of undetectable HBV DNA at 96 weeks^[18,19]. Several studies in small number of patients showed efficacy of TDF-based therapy for LAM-resistant patients, however, real life data of TDF in the patients is currently limited.

This study aimed to evaluate the antiviral efficacy and safety of TDF or TDF plus LAM combination in patients with documented genotypic resistance to LAM.

METHODS

Patients

This was a retrospective cohort study conducted in adult chronic hepatitis B patients with LAM-resistant chronic HBV infection treated with TDF more than one year. They were enrolled between January 2012 and December 2013 at Konkuk University Hospital and Korea University Guro Hospital in Seoul, Korea.

Inclusion criteria were documented genotypic resistance mutations to LAM (rtM204V/I ± rtL180M) and a serum HBV DNA level above 50 IU/mL (300 copies/mL) while currently receiving antiviral treatment. Patients were excluded if they had hepatocellular carcinoma; evidence of coinfection with hepatitis C, hepatitis C, or human immunodeficiency virus; a history of systemic corticosteroids or other immunosuppressive agents; renal impairment (serum creatinine > 1.5 mg/dL); other malignancy or decompensated liver disease; a history of prior treatment with TDF. Patients who had adefovir or entecavir-resistant HBV infection during antiviral treatments were excluded. Patients were treated with 300 mg TDF daily or TDF plus 100mg LAM daily for more than 12 months.

The study protocol was approved by the Institutional Review Board of Konkuk University Hospital and was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Study design

All patients included were monitored at baseline of TDF treatment

and every three months during TDF therapy. They were followed up every three months during the therapy for the clinical assessment of tolerability, a physical examination, blood chemistry, and HBV status. Serum HBeAg and anti-HBe (Cobas e immunoassay, Roche, Switzerland), and serum HBV DNA level (COBAS AmpliCor PCR assay, which has a lower limit of detection of 20 IU/mL, Roche Molecular Systems, Branchburg, NJ) were measured. Restriction fragment mass polymorphism (RFMP) assays of the HBV genome were performed to detect LAM, ADV, and ETV mutations at baseline and at the time of virologic breakthrough^[20].

We analyzed the proportion of patients who achieved complete virologic response (serum HBV DNA concentration of < 20 IU/mL) at 12 month of TDF therapy as a primary endpoint. Changes in serum HBV DNA level, the proportion of patients with HBeAg loss, HBeAg seroconversion, and normalization of ALT were analyzed through the treatment period. Viral breakthrough was defined as a ≥ 1 log₁₀ increase in HBV DNA from nadir on two consecutive occasions after an initial decline in HBV DNA by > 2 log₁₀ copies/mL.

Regarding adverse events, we evaluated changes in serum creatinine level and proportion of the patients who developed renal impairment defined as an increase in serum creatinine level of > 0.5 mg/dL from baseline, or decrease in serum phosphate level to < 2 mg/dL.

Statistical analysis

HBV DNA levels were logarithmically transformed for analysis. Continuous variables were expressed as the mean ± standard deviation (SD) and categorical variables were expressed as absolute and relative frequencies. All statistical data were analyzed using SPSS Inc. for Windows, ver. 17.0. Categorical variables were analyzed using the Chi-squared test. Continuous variables were evaluated using the Student's *t*-test. The Kaplan-Meier survival analysis was used to estimate proportion of complete virologic response and normalization of ALT level. Cox regression analysis was applied to identify factors that were independently associated with complete virologic response to TDF therapy. For all statistical tests, a two-sided *p* value of < 0.05 was considered significant.

RESULTS

A total of 101 patients were included in this study. Table 1 shows the patients characteristics at baseline. Mean age was 50.1 years, and 68 were male. Median HBV DNA level was 3.3 log₁₀ IU/mL, and 86% of the patients enrolled were HBeAg-positive. All the patients were treated with LAM as the first line agent for HBV. ADV was most commonly used for the second line agent for treatment of LAM-resistance. All the patients were confirmed to have LAM-resistant mutations of rtL180M or rtM204I and/or V of the HBV polymerase gene prior to start of TDF therapy. No other mutations of ADV or ETV resistance were detected by RFMP assay. TDF monotherapy (*n* = 74) or TDF plus LAM (*n* = 27) were administered for the treatment of LAM-resistant HBV. Mean follow-up duration of TDF therapy was 23 months.

The proportion of complete virologic response was 80.2% and 89.7% at 6 months and 12 months after TDF treatment, respectively (Figure 1).

The response rate increased to 91.9% at 24 months. Viral breakthrough was not observed during the follow-up period. The decrease in HBV DNA level was -2.05 log₁₀ IU/mL from the baseline level 3.24 log₁₀ IU/mL at 6 month, and -2.14 log₁₀ IU/mL at 12 month (Figure 2).

Virologic breakthrough was not observed. Two patients (2.6%) among 77 HBeAg-positive patients achieved HBeAg loss at 12

month. No patient had HBsAg loss during the follow-up period.

Seven patients among 10 patients with detectable level of HBV DNA (> 20 IU/mL) at 12 months were tested genotypic analysis for drug resistant mutation. Genotyping was available in one patient and were not successful in the others due to low level of HBV DNA. There was no change in mutation pattern in one patient who had M204I with high viral load (1×10^6 IU/L) at baseline.

Baseline mean ALT level was 69.7 IU/L, mean ALT level decreased to 30.9 IU/L and 25.9 IU/L at 6 months and 12 months of TDF treatment, respectively (Figure 3). There was no ALT flare (ALT elevation > 5 × UNL) during the treatment period.

Table 2 shows the Cox regression analysis for the predictive factors of virologic response. Baseline HBV DNA level was significantly associated with complete virologic response at 12 months of TDF treatment (HR = 0.487, 95% CI 0.649-0.953, $p = 0.014$). Combination treatment with LAM was not a significant factor for the virologic response.

There were no significant adverse events during the TDF treatment. Renal impairment (increase in creatinine level of > 0.5 mg/dL from baseline or decrease in serum phosphate level to < 2 mg/dL) was not observed during the treatment period. The change in creatinine level was not significant during the therapy (0.81 ± 0.17 at baseline vs 0.81 ± 0.18 at 12 months).

DISCUSSION

LAM has been popularly used for the first line treatment of chronic hepatitis B in Korea^[1,2]. However, the long-term treatment of LAM is associated with a high risk of drug resistance of HBV. LAM

resistance results in virologic relapse with biochemical relapse and deterioration of hepatic function^[4,5,21]. A combination treatment of LAM and ADV was recommended for the rescue therapy to prevent the development of ADV resistance during the second-line ADV monotherapy in patient with LAM-resistant HBV infection^[10,22]. The antiviral efficacy of the combination of ADV and LAM was not favorable although the ADV resistance was very rare. Recent guidelines recommend TDF based treatment for the treatment of LAM resistance^[11,16,17,23].

TDF is a potent antiviral agent against wild-type and LAM-resistant HBV. It was well demonstrated that TDF was more effective in achieving virologic response in patients with LAM-resistant HBV infection as well as treatment naïve patients compared with ADV^[24]. A randomized controlled trial of TDF monotherapy or combination with emtricitabine through 96 weeks demonstrated that TDF monotherapy achieves potent viral suppression (89% of patients having HBV DNA < 69 IU/mL at week 96) in LAM-resistant CHB

Table 1 Patients characteristics at baseline ($n = 101$).

Variables	Results
Sex (Male/Female)	68 / 33
Age (years)†	52.2 ± 10.9
Liver cirrhosis	22 (23.2%)
HBeAg positive	82 (86%)
HBV DNA (log ₁₀ IU/mL) †	3.3 ± 1.62
ALT (U/L) †	69.7 ± 138
Serum creatinine (mg/dL) †	0.81 ± 0.17
Prior exposed antiviral agents	
LAM	8
LAM, ADV	46
LAM, ETV	8
LAM, LdT, ADV	11
LAM, ADV, ETV	22
LAM-resistant mutations	
rtM204I	33
rtM204I + rtL180M	19
rtM204V + rtL180M	36
rtM204I/V + rtL180M	13
Follow up duration with TDF (months) †	23 ± 5.5

†Data represents mean ± standard deviation. LAM: lamivudine; ADV: adefovir dipvoxil; ETV: entecavir; LdT: telbivudine; TDF: tenofovir disoproxil fumarate.

Table 2 Cox regression analysis for complete virologic response at 12 month of tenofovir therapy in lamivudine-resistant hepatitis B patients.

Factors	Univariate			Multivariate		
	HR	CI (95%)	P	HR	CI (95%)	P
Gender (male)	0.993	0.641-1.540	0.977			
Age	0.992	0.974-1.011	0.992			
Liver cirrhosis	0.038	0.642-1.679	0.879			
TDF vs. TDF+LAM	1.170	0.727-1.885	0.517	1.080	0.662-1.763	0.757
Baseline HBV DNA	0.786	0.664-0.929	0.005	0.787	0.649-0.953	0.014
Baseline HBeAg+	0.749	0.419-1.331	0.322	1.304	0.202-1.403	0.202
Baseline ALT	0.999	0.997-1.001	0.226	1.000	0.998-1.002	0.970

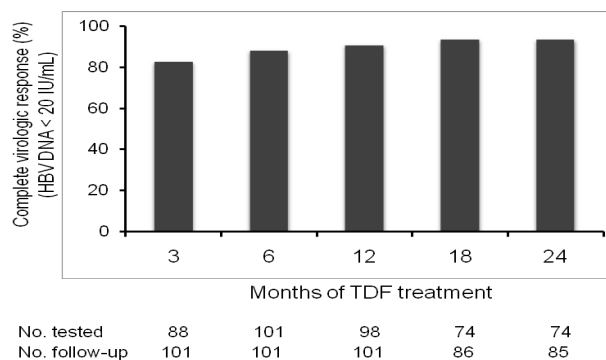


Figure 1 Proportion of patients achieving complete virologic response after TDF treatment. TDF: Tenofovir disoproxil fumarate.

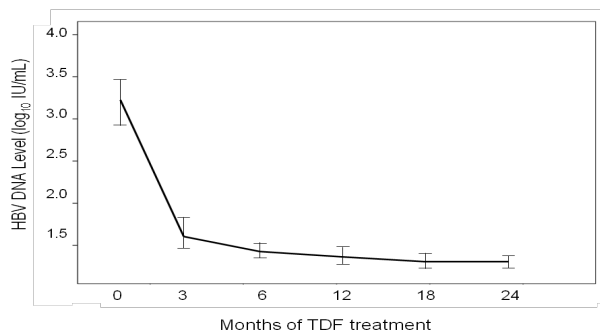


Figure 2 Changes in HBV DNA level after TDF treatment. TDF: Tenofovir disoproxil fumarate.

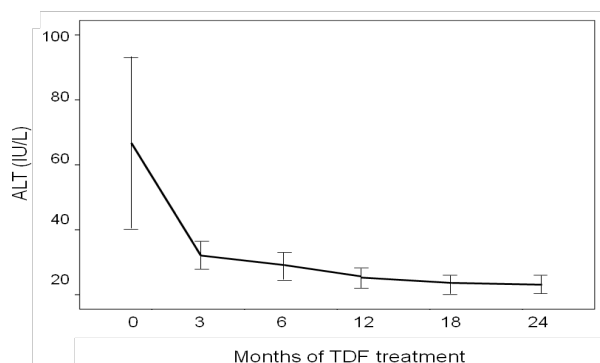


Figure 3 Changes in ALT level level after TDF treatment. TDF: Tenofovir disoproxil fumarate.

patients^[18]. They showed that addition of emtricitabine to TDF did not increase the virologic response. Current guidelines recommend TDF monotherapy or combination with nucleoside analogues for LAM resistant HBV. However, long-term efficacy and safety of TDF or combination with other agents in real-life data is still limited.

This study was a retrospective cohort study to evaluate antiviral efficacy and safety in CHB patients with documented LAM-resistance who failed to achieve virologic response during the antiviral treatments. We included the patients with detectable HBV DNA above 50 IU/mL during antiviral treatment and genotypic resistance mutation to LAM tested by RFMP. All the patients were treated with LAM for the first line therapy of CHB. Most cases were exposed to ADV for salvage therapy for LAM resistance and showed suboptimal response to for secondary antiviral agents. We excluded the patients with detectable genotypic mutations to ADV or ETV at baseline or during the previous antiviral treatment were excluded. Seventy four patients among the 101 patients with LAM resistance were treated with TDF monotherapy, and the other 27 patients were treated with TDF and LAM combination. This study demonstrated that the TDF-based treatment through two years is effective and safe in the patients with LAM-resistant HBV. Virologic response was achieved 89.7% at 12 months after TDF treatment, and the rate increased up to 92% at 24 months. This result is comparable to the previous results in treatment-naïve patients^[14,25]. The response rates are also comparable with previous clinical trial data by Fung *et al.*, even though higher proportion of patients in our study was HBeAg positive and more cases were exposed to ADV compared with the previous study^[18]. The baseline HBV DNA level of the patients was 3.3 log₁₀ IU/mL which was lower than that in the clinical trial. Most patients in our study had suboptimal response to previous antiviral agents. On the other hand, HBeAg loss or seroconversion rate was very low (2.6%) during the observational period compared with previous studies. This result might be due to long-standing infection of HBV by vertical transmission and a particular pattern of HBV genotype (genotype C) in Korea even though we did not analyze the genotype^[26].

In this study, virologic breakthrough was not observed, and ten patients did not show complete virologic response during the treatment with TDF. However, genotypic analysis was not available in 9 patients due to very low level of HBV DNA. Genotyping was successful in one case who had high viral load at baseline, however, additional resistant mutations were not observed.

Baseline HBV DNA level was a significant predictive factor for the virologic response in the LAM-resistant patients. HBeAg positivity which was related to poor response to TDF in previous studies was not significant for the prediction of virologic response. This study included relatively higher proportion of HBeAg-positive patients compared with the previous studies. Twenty seven patients among the 101 patients were treated with combination of TDF plus LAM, and the others were treated TDF monotherapy in the current study. Antiviral efficacy of the TDF monotherapy was comparable to that in the combination treatment. This result is in agreement with a randomized controlled trial comparing TDF/emtricitabine combination with TDF monotherapy for LAM-resistant HBV. The randomized study showed that 86.3% and 89.4% of virologic response (< 69 IU/mL) at 96 week of the treatment in each group, respectively. Real-life data also showed that TDF monotherapy has an excellent antiviral efficacy in LAM-resistant patients^[19,27]. However, several studies have suggested that antiviral efficacy of TDF is reduced in patients with adefovir-resistant HBV^[28,29]. TDF/entecavir combination is recommended for the multi-drug resistant

patients^[16,17]. In this study, patients who had an evidence of genotypic resistance to adefovir or entecavir were excluded. Taken together, TDF monotherapy is effective to achieve the complete virologic response and pretreatment HBV DNA level is an important predictive factor for the virologic response in LAM-resistant CHB patients. Monitoring of HBV DNA level and drug adherence is important for achieving complete suppression of HBV DNA, particularly in patients with high viral load.

In terms of biochemical response, decrease in ALT level was significant after TDF treatment. There was no ALT flare during the TDF treatment. Regarding safety profile, TDF was well tolerated without renal impairment during the follow up period. It has been suggested that long-term use of TDF results in renal impairment and proximal tubular dysfunction^[8,18,30]. The risk of renal dysfunction is higher in patients with decompensated liver disease, hypertension, diabetes mellitus, and organ transplantation^[8,18]. Our results showed that TDF was safe in LAM-resistant patients. However, the follow-up period in the current study was short to evaluate the safety of TDF. The long-term study is needed for the evaluation of renal safety and bone mineral changes.

In conclusion, TDF treatment shows high rate of complete virologic response in CHB patients with LAM-resistance. TDF is tolerable and safe during the 96 weeks of treatment period. Pretreatment HBV DNA level is a predictive factor for antiviral response.

CONFLICTS OF INTEREST

All authors have none to declare.

REFERENCES

- Liaw YF, Leung N, Guan R, Lau GK, Merican I. Asian-Pacific Consensus Working Parties on Hepatitis B. Asian-Pacific consensus statement on the management of chronic hepatitis B: an update. *J Gastroenterol Hepatol.* 2003; **18**: 239-245.
- Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, Crowther L, Condreay LD, Woessner M, Rubin M, Brown NA. Lamivudine as initial treatment for chronic hepatitis B in the United States. *N Engl J Med.* 1999; **341**: 1256-1263.
- Hadziyannis SJ, Papatheodoridis GV, Dimou E, Laras A, Papatheodoridis C. Efficacy of long-term lamivudine monotherapy in patients with hepatitis B e antigen-negative chronic hepatitis B. *Hepatology.* 2000; **32**: 847-851.
- Lok AS, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, Dienstag JL, Heathcote EJ, Little NR, Griffiths DA, Gardner SD, Castiglia M. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. *Gastroenterology.* 2003; **125**: 1714-1722.
- Lau DT, Khokhar MF, Doo E, Ghany MG, Herion D, Park Y, Kleiner DE, Schmid P, Condreay LD, Gauthier J, Kuhns MC, Liang TJ, Hoofnagle JH. Long-term therapy of chronic hepatitis B with lamivudine. *Hepatology.* 2000; **32**: 828-834.
- Kim YJ, Kim BG, Jung JO, Yoon JH, Lee HS. High rates of progressive hepatic functional deterioration whether lamivudine therapy is continued or discontinued after emergence of a lamivudine-resistant mutant: a prospective randomized controlled study. *J Gastroenterol.* 2006; **41**: 240-249.
- Ono-Nita SK, Kato N, Shiratori Y, Masaki T, Lan KH, Carrilho FJ, Omata M. YMDD motif in hepatitis B virus DNA polymerase influences on replication and lamivudine resistance: A study by in vitro full-length viral DNA transfection. *Hepatology.* 1999; **29**: 939-945.
- Lampertico P, Viganò M, Manenti E, Iavarone M, Lunghi G, Colombo M. Adefovir rapidly suppresses hepatitis B in HBeAg-

- negative patients developing genotypic resistance to lamivudine. *Hepatology*. 2005; **42**: 1414-1419.
- 9 Lee YS, Suh DJ, Lim YS, Jung SW, Kim KM, Lee HC, Chung YH, Lee YS, Yoo W, Kim SO.. Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. *Hepatology*. 2006; **43**: 1385-1391.
 - 10 van der Poorten D, Prakoso E, Khoo TL, Ngu MC, McCaughan GW, Strasser SI, Lee AU. Combination adefovir-lamivudine prevents emergence of adefovir resistance in lamivudine-resistant hepatitis B. *J Gastroenterol Hepatol*. 2007; **22**: 1500-1505.
 - 11 Yatsuji H, Suzuki F, Sezaki H, Akuta N, Suzuki Y, Kawamura Y, Hosaka T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Watahiki S, Iwasaki S, Kobayashi M, Kumada H. Low risk of adefovir resistance in lamivudine-resistant chronic hepatitis B patients treated with adefovir plus lamivudine combination therapy: two-year follow-up. *J Hepatol*. 2008; **48**: 923-931.
 - 12 Karino Y, Toyota J, Kumada H, Katano Y, Izumi N, Kobashi H, Sata M, Moriyama M, Imazeki F, Kage M, Ishikawa H, Masaki N, Seriu T, Omata M. Efficacy and resistance of entecavir following 3 years of treatment of Japanese patients with lamivudine-refractory chronic hepatitis B. *Hepatol Int*. 2010; **4**: 414-422.
 - 13 Tenney DJ, Rose RE, Baldick CJ, Levine SM, Pokornowski KA, Walsh AW, Fang J, Yu CF, Zhang S, Mazzucco CE, Eggers B, Hsu M, Plym MJ, Poundstone P, Yang J, Colonna RJ. Two-year assessment of entecavir resistance in Lamivudine-refractory hepatitis B virus patients reveals different clinical outcomes depending on the resistance substitutions present. *Antimicrob Agents Chemother*. 2007; **51**: 902-911.
 - 14 Baran B, Soyer OM, Ormeci AC, Gokturk S, Evirgen S, Bozbey HU, Akyuz F, Karaca C, Demir K, Besisik F, Onel D, Gulluoglu M, Badur S, Kaymakoglu S. Efficacy of tenofovir in patients with Lamivudine failure is not different from that in nucleoside/nucleotide analogue-naïve patients with chronic hepatitis B. *Antimicrob Agents Chemother*. 2013; **57**: 1790-1796.
 - 15 Snow-Lampart A, Chappell B, Curtis M, Zhu Y, Myrick F, Schawwalder J, Kitrinis K, Svarovskaia ES, Miller MD, Sorbel J, Heathcote J, Marcellin P, Borroto-Esoda K. No resistance to tenofovir disoproxil fumarate detected after up to 144 weeks of therapy in patients monoinfected with chronic hepatitis B virus. *Hepatology*. 2011; **53**: 763-773.
 - 16 Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, Chen DS, Chen HL, Chen PJ, Chien RN, Dokmeci AK, Gane E, Hou JL, Jafri W, Jia J, Kim JH, Lai CL, Lee HC, Lim SG, Liu CJ, Locarnini S, Al Mahtab M, Mohamed R, Omata M, Park J, Piratvisuth T, Sharma BC, Sollano J, Wang FS, Wei L, Yuen MF, Zheng SS, Kao JH. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int*. 2016; **10**: 1-98.
 - 17 Korean Association for the Study of the Liver (KASL). KASL clinical practice guidelines: management of chronic hepatitis B. *Clin Mol Hepatol*. 2016; **22**: 18-75.
 - 18 Fung S, Kwan P, Fabri M, Horban A, Pelemis M, Hann HW, Gurel S, Caruntu FA, Flaherty JF, Massetto B, Dinh P, Corsa A, Subramanian GM, McHutchison JG, Husa P, Gane E. Randomized comparison of tenofovir disoproxil fumarate vs emtricitabine and tenofovir disoproxil fumarate in patients with lamivudine-resistant chronic hepatitis B. *Gastroenterology*. 2014; **146**: 980-988.
 - 19 Park JH, Jung SW, Park NH, Park BR, Kim MH, Kim CJ, Lee BU, Jeong ID, Kim BG, Bang SJ, Shin JW.. Efficacy of tenofovir-based rescue therapy in lamivudine-resistant chronic hepatitis B patients with failure of lamivudine and adefovir combination. *Clin Ther*. 2015; **37**: 1433-1442.
 - 20 Kim HS, Han KH, Ahn SH, Kim EO, Chang HY, Moon MS, Chung HJ, Yoo W, Kim SO, Hong SP. Evaluation of methods for monitoring drug resistance in chronic hepatitis B patients during lamivudine therapy based on mass spectrometry and reverse hybridization. *Antivir Ther*. 2005; **10**: 441-449.
 - 21 Yoon SK, Jang JW, Kim CW, Bae SH, Choi JY, Choi SW, Lee YS, Lee CD, Chung KW, Sun HS, Kim BS. Long-term results of lamivudine monotherapy in Korean patients with HBsAg-positive chronic hepatitis B: response and relapse rates, and factors related to durability of HBsAg seroconversion. *Intervirology*. 2005; **48**: 341-349.
 - 22 Seto WK, Liu K, Fung J, Wong DK, Yuen JC, Hung IF, Lai CL, Yuen MF. Outcome of lamivudine-resistant chronic hepatitis B after up to 5 years of combination therapy with adefovir. *Antivir Ther*. 2012; **17**: 1255-1262.
 - 23 European Association For The Study Of The Liver. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol*. 2012; **57**: 167-185.
 - 24 Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, Germanidis G, Lee SS, Flisiak R, Kaita K, Manns M, Kotzev I, Tchernev K, Buggisch P, Weibert F, Kurdas OO, Shiffman ML, Trinh H, Washington MK, Sorbel J, Anderson J, Snow-Lampart A, Mondou E, Quinn J, Rousseau F. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. *N Engl J Med*. 2008; **359**: 2442-2455.
 - 25 Yu HM, Kwon SY, Kim J, Chung HA, Kwon SW, Jeong TG, An SH, Jeong GW, Yun SU, Min JK, Kim JH, Choe WH. Virologic response and safety of tenofovir versus entecavir in treatment-naïve chronic Hepatitis B patients. *Saudi J Gastroenterol*. 2015; **21**: 146-151.
 - 26 Bae SH, Yoon SK, Jang JW, Kim CW, Nam SW, Choi JY, Kim BS, Park YM, Suzuki S, Sugauchi F, Mizokami M. Hepatitis B virus genotype C reverts among chronic carriers of the virus in Korea. *J Korean Med Sci*. 2005; **20**: 816-820.
 - 27 Yang DH, Xie YJ, Zhao NF, Pan HY, Li MW, Huang HJ. Tenofovir disoproxil fumarate is superior to lamivudine plus adefovir in lamivudine-resistant chronic hepatitis B patients. *World J Gastroenterol*. 2015; **21**: 2746-2753.
 - 28 Villet S, Pichoud C, Billioud G, Barraud L, Durantel S, Trépo C, Zoulim F. Impact of hepatitis B virus rtA181V/T mutants on hepatitis B treatment failure. *J Hepatol*. 2008; **48**: 747-755.
 - 29 Qi X, Xiong S, Yang H, Miller M, Delaney WE 4th. In vitro susceptibility of adefovir-associated hepatitis B virus polymerase mutations to other antiviral agents. *Antivir Ther*. 2007; **12**: 355-362.
 - 30 Cho H, Cho Y, Cho EJ, Lee JH, Yu SJ, Oh KH, Lee K, Mustika S, Yoon JH, Kim YJ. Tenofovir-associated nephrotoxicity in patients with chronic hepatitis B: two cases. *Clin Mol Hepatol*. 2016; **22**: 286-291.

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