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Telbivudine protects renal function in patients with chronic hepatitis B infection in conjunction with adefovir-based combination therapy treatment

– Renal protective effect of telbivudine in adefovir-based combination therapy –

만성 B 형 간염 환자에서
아데포비어 병합 요법시
텔비부딘이 신기능에 미치는 영향

– 아데포비어 병합 요법시 텔비부딘이
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Telbivudine protects renal function in patients with chronic hepatitis B infection in conjunction with adefovir-based combination therapy

by

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A thesis submitted to the Department of Clinical Medical Sciences in partial fulfillment of the requirements for the Degree of Master of Science in Clinical Medical Science at Seoul National University College of Medicine

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논문 제목: Telbivudine protects renal function in patients with chronic hepatitis B infection in conjunction with adefovir-based combination therapy treatment

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ABSTRACT

Introduction: Previous studies have demonstrated that the treatment of chronic hepatitis B (CHB) infection with adefovir (ADV) can impair renal function. In contrast, treatment with telbivudine (LdT) improves renal function in CHB patients. The aim of this study was to evaluate the renoprotective effect of LdT in CHB patients receiving ADV-based combination therapy.

Methods: The effects of treatment with ADV + LdT on renal function were compared to those resulting from treatment with ADV + entecavir (ETV), ADV + lamivudine (LAM), ADV alone and ETV alone. The consecutive cohort analysis included 831 CHB patients who received ADV + LdT, ADV + LAM, ADV + ETV, ADV alone or ETV alone for 96 weeks. Alterations in estimated glomerular filtration rate (eGFR) were compared between the five groups using a linear mixed-effects model. HBV DNA levels were also compared between the five groups during the 96-week period.

Results: Among the five treatment groups, significant improvements in eGFR were observed in the ADV + LdT and ADV + LAM groups over time ($P<0.001$ for each group compared to baseline eGFR). In patients with a baseline eGFR between 50–90 ml/min, the change in eGFR was the most significant in the ADV + LdT group (+0.641 ml/min; $P<0.001$). Age, gender, baseline eGFR and treatment option were

significant predictive factors for eGFR changes.

Conclusions: In conclusion, our results suggest that the combination therapy of LdT and ADV is significantly associated with renoprotective effects in CHB patients when compared with other ADV-based combination or single therapies.

Keywords: chronic hepatitis B; renal function; adefovir dipivoxil; telbivudine

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LIST OF ABBREVIATIONS

ADV	Adefovir
LdT	Telbivudine
ETV	Entecavir
LAM	Lamivudine
CHB	Chronic hepatitis B
eGFR	Estimated glomerular filtration rate
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
MDRD	Modification of Diet in Renal Disease

INTRODUCTION

The primary goal of antiviral therapy in patients with chronic hepatitis B (CHB) infection is to maintain undetectable levels of serum hepatitis B virus (HBV) DNA (1, 2). To achieve disease remission and/or serological therapeutic goals, long-term treatment with oral nucleos(t)ide analogues is required for some patients with CHB virus infection (3). However, 5-year cumulative resistance rates of 70% were reported for low genetic barrier drugs in patients treated with LAM (4), and rates of 29% were reported in patients treated with ADV (5). Four-year cumulative resistance rates were 10% in patients treated with LdT (6).

In previous studies, antiviral effects of ADV-based combination treatments such as ADV plus LAM, ADV plus LdT, and ADV plus ETV were well-elucidated for second-line therapy for patients demonstrating LAM-resistance, or the primary failure of LAM therapy (7-11). Although combination antiviral therapy can be an effective rescue therapy, long-term treatment against resistant HBV is also recommended for patients with chronic HBV infection or advanced liver disease, as well as for those treated with chronic immunosuppressive therapy (12). Through the use of this long-term combination strategy, the number of patients undergoing long-term combination treatment has increased substantially over the last several decades (3).

With a higher number of patients undergoing long-term treatment, rare but serious adverse events such as myopathy, neuropathy, lactic acidosis, and

renal dysfunction have been reported during post-marketing surveillance (3). Among these conditions, renal dysfunction is one area of particular clinical concern. The pattern of renal dysfunction in patients treated with ADV is characterized by slight increases in serum creatinine with low serum phosphate levels during one year of treatment with 10 mg of ADV (13); however, long-term adverse renal effects were not assessed following ADV therapy lasting more than 1 year. Furthermore, few studies have analyzed changes in eGFR when ADV treatment was combined with other nucleoside anti-viral drugs.

Although rare cases of lactic acidosis and Fanconi-like syndrome have been reported following LAM treatment of patients with HBV and HIV coinfection (4, 14), dose-adjusted lamivudine treatment is considered safe in patients with CHB and renal insufficiency (15, 16). The safety of ETV, in terms of renal insufficiency, has not been well studied. However, until now, there have been no studies reporting clinically noticeable nephrotoxicity caused by ETV, and renal toxicity induced by this drug may therefore be negligible (17, 18). Treatment with the nucleoside thymidine analogue telbivudine (LdT) has been associated with a significant improvement in renal function, compared to treatment with LAM and ADV (6, 19-24). Although previous studies have investigated the effects of individual anti-viral agents on renal function, there is little safety data available regarding the nephrotoxicity of ADV-based combination therapy in CHB patients. It may be particularly difficult to predict changes in the renal function of CHB patients treated with ADV and LdT because ADV is capable of impairing renal function while LdT improves

renal function.

The present study evaluated the treatment efficacy and changes in renal function following long-term combination therapy consisting of ADV and other anti-viral drugs including LdT, LAM, and ETV in CHB patients with LAM resistance. The aim of the study was to determine if nucleoside/nucleotide combination therapy improves renal function and affects HBV DNA concentrations.

MATERIALS AND METHODS

1. Study population

This was a retrospective study involving 1,043 consecutive CHB patients who were treated with ADV, LdT, LAM, ETV or a combination of these drugs between March 2005 and January 2013. The eligible population was divided into the following five groups: three ADV-based combination groups treated with ADV plus LdT, ADV plus LAM, and ADV plus ETV, as well as groups treated with ADV alone and ETV alone. The ADV-based combination groups consisted of patients who were prescribed 10 mg of ADV combined with 1 mg of ETV, 100 mg of LAM, or 600 mg of LdT. Patients in these groups demonstrated baseline eGFR ≥50 ml/min.

In patients treated with ADV, ADV plus LdT, and ADV plus LAM, they received LAM therapy as the former treatment. All patients showed LAM-resistance. In patients treated with ADV and ETV, the previous treatment was sequential LAM and ETV therapy. They were enrolled when ADV was added to ETV therapy for partial virological response or viral breakthrough in spite of ETV therapy. The definition of partial virological response was a decrease in serum HBV DNA of more than 1 $\log_{10}$IU/mL but detectable HBV DNA after at least 6 months of therapy (3). The definition of virological breakthrough was an increase in serum HBV DNA levels of more than 1 $\log_{10}$IU/mL from the nadir in a patient who had an initial virological response (3).

The patients treated with ETV were defined as the control group, which consisted of patients who were treatment-naive and initially treated with ETV and had baseline eGFR ≥50 ml/min (Fig. 1). Patients who were not treated with any anti-HBV therapy were not chosen for the control group because HBV-associated glomerulonephritis and/or vasculitis can be aggravated by high levels of HBV DNA (25, 26).

Patients who had a history of ADV treatment and/or impaired renal function of a baseline eGFR <50 ml/min were excluded. CHB patients co-infected with HIV and/or HCV were also excluded. The cut-off eGFR value of 50 ml/min was chosen because dose and/or interval adjustments are required for patients with eGFR <50 ml/min. In this study, the eGFR *Gray zone* was defined as eGFR values between 50 and 90 ml/min.

The date of entry was determined by the date at which treatment was initiated, and the date of exit was defined by the termination of either the treatment or the study. This study was approved by the Seoul National University Hospital Institutional Review Board.

2. Evaluation of renal function

We used the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation to calculate eGFR. This equation was recently validated and is considered more accurate than the Modification of Diet in Renal Disease (MDRD) study equation in patients without renal impairment (27). The CKD-EPI formula was as follows: for females with creatinine levels ≤0.7 mg/dl: eGFR = 144 × (creatinine/0.7) exp − 0.329 × (0.993) exp age; for

females with creatinine levels >0.7 mg/dl: eGFR = 144 × (creatinine /0.7) exp − 1.209 × (0.993) exp age; for males with creatinine levels ≤0.9 mg/dl: eGFR = 141 × (creatinine/0.9) exp − 0.411 × (0.993) exp age; for males with creatinine levels >0.9 mg/dl: eGFR = 141 × (creatinine/0.9) exp − 1.209 × (0.993) exp age.

3. Statistical Analyses

To assess the differences in demographic and clinical variables among the five treatment groups, the χ^2-test or Fisher's exact test for categorical variables was applied. All continuous variables were tested by one-way analysis of variance (ANOVA) or by the Kruskal-Wallis test with assumptions of normality. Spearman's rank correlation test was used to examine the correlation between the baseline eGFR and logarithm-transformed serum HBV DNA concentrations.

To evaluate the association between several variables and eGFR changes over time, we employed a linear mixed-effects model for repeated measures (28), which uses all available data and provides valid results in the presence of missing data under the assumption that missing data are missing at random (29, 30) . The model considered the baseline eGFR, age (in years), sex, comorbidity of hypertension and diabetes mellitus, treatment group, time, and group-by-time interaction as fixed effects and incorporated random effects for individual subjects, such as a random intercept and a random slope (with respect to time). Because the repeated measures from the same subject

are correlated, we also investigated which correlation structure was well delineated among the responses. The final results were selected based on the likelihood ratio test (LRT), Akaike information criterion (AIC) (31) and Bayesian information criterion (BIC) (32). For the correction of multiple comparisons, the false discovery rate (FDR) method was applied (33).

We divided the dataset into two subsets to investigate the changes in eGFR based on treatment options and baseline renal functions. One patient group had baseline eGFR ≥90 ml/min, and the other group had baseline eGFR values in the *Gray Zone* (between 50 and 90 ml/min). Identical models were applied for two subgroup analyses. To compare the changes in eGFR among the five groups, we included the baseline eGFR as a fixed effect and considered random effects to account for patient variability in the model.

The five groups were compared for differences in serum HBV DNA concentrations at each time point using the linear mixed-effects model. Additionally, the correlation between eGFR changes and serum concentrations of HBV DNA was examined with the linear mixed-effects model.

All statistical analyses were performed with SAS version 9.2 (SAS Institute Inc., NC, USA) and R version 2.15.2 (http://www.r-project.org) software. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Baseline patient characteristics

A total of 539 consecutive CHB patients were enrolled and evaluated for 2 years. The treatment groups included patients receiving ADV alone, ADV plus LdT, ADV plus LAM, and ADV plus ETV. The control group consisted of 292 consecutive CHB patients prescribed ETV alone for 2 years (Figure. 1).

Baseline characteristics for the five groups are summarized in Table 1. There were no differences among the five groups in terms of age, sex, or comorbidity of hypertension and diabetes mellitus. However, the distributions of eGFR, HBV DNA levels, and hepatitis B e antigen-positive rates were significantly different among the five groups.

The mean baseline eGFR was highest (80.76±16.49 ml/min) in CHB patients treated with ADV plus ETV. The mean baseline eGFR was lowest (68.70±13.58 ml/min) in patients prescribed ADV alone. The baseline median logarithm-transformed serum HBV DNA level was highest (5.35) in the group treated with ETV, while the lowest median value (3.53) was obtained from patients treated with ADV plus ETV. Of the patients treated with ADV plus ETV, 62.55% were positive for hepatitis B e antigen, while 40.85% of the patients treated with ETV alone were positive.

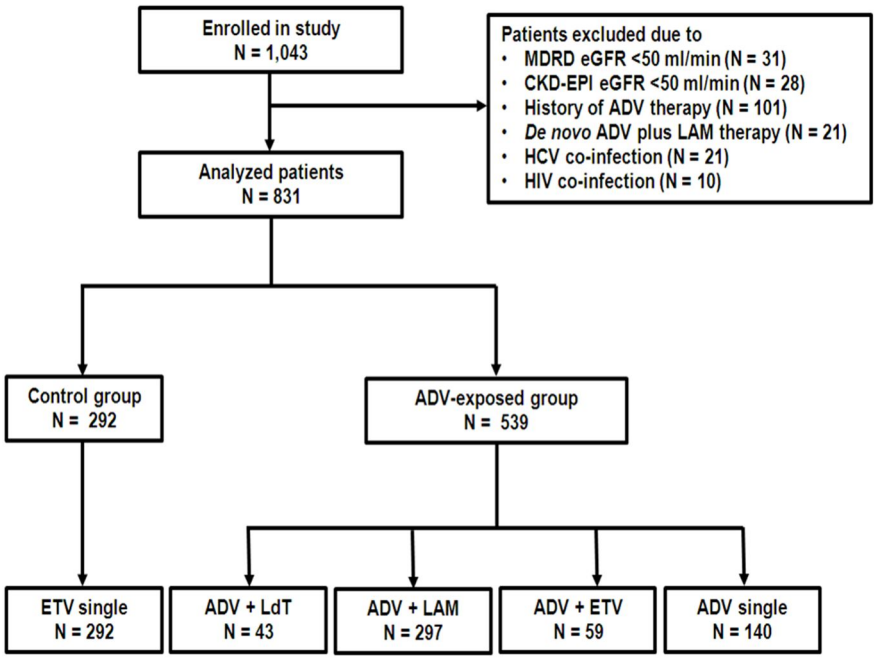


Figure 1. Flow chart of study participants.

Abbreviations: ADV, adefovir; LdT, telbivudine; LAM, lamivudine; ETV, entecavir

Table 1. Baseline patient characteristics

	ADV + LdT	ADV + LAM	ADV + ETV	ADV	ETV	*P* value
Patients (*n*)	43	297	59	140	292	
Ethnicity						
Asian	43 (100%)	297 (100%)	59 (100%)	140 (100%)	292 (100%)	
Age (year)	52.33 ± 11.22	51.40 ± 11.88	53.66 ± 10.43	50.61 ± 10.78	52.76 ± 11.26	0.240†
Sex						0.696*
Female	13 (30.23%)	82 (27.61%)	19 (32.20%)	36 (25.71%)	92 (31.51%)	
Male	30 (69.77%)	215 (72.39%)	40 (67.80%)	104 (74.29%)	200 (68.49%)	
HTN	2 (4.65%)	23 (7.77%)	9 (15.25%)	11 (7.86%)	30 (10.27%)	0.308**
DM	0 (0.00%)	26 (8.75%)	6 (10.17%)	11 (7.86%)	31 (10.62%)	0.161**
CKD-EPI	77.14 ± 15.61	71.93 ± 15.29	80.76 ± 16.49	68.70 ± 13.58	72.14 ± 14.49	<0.001†
Classification						<0.001*
≥ 90 mL/min	11 (25.58%)	42 (14.14%)	23 (38.98%)	12 (8.57%)	39 (13.36%)	
50—90 mL/min	32 (74.42%)	255 (85.86%)	36 (61.02%)	128 (91.43%)	253 (86.64%)	
HBV DNA median, IQR	9140, 220852	70200, 5447045	3370, 488904	11816, 182941	224500, 8599732	<0.001††
log10(HBV DNA) median, IQR	3.96, 3.17	4.85, 3.27	3.53, 3.71	4.07, 3.49	5.35, 4.51	<0.001††
HBeAg-positive rate	21 (52.50%)	103 (53.37%)	30 (65.22%)	59 (48.76%)	96 (40.85%)	0.013*

Values are expressed as mean ± SD or *n* (percentage).

*Chi-square test

**Fisher's exact test

†One-way ANOVA test

††Kruskal-Wallis test

IQR, interquartile range

Abbreviations: ADV, adefovir; DM, diabetes mellitus; ETV, entecavir; HTN, hypertension; LAM, lamivudine; LdT, telbivudine

Changes in renal function during ADV-based combination therapy for CHB

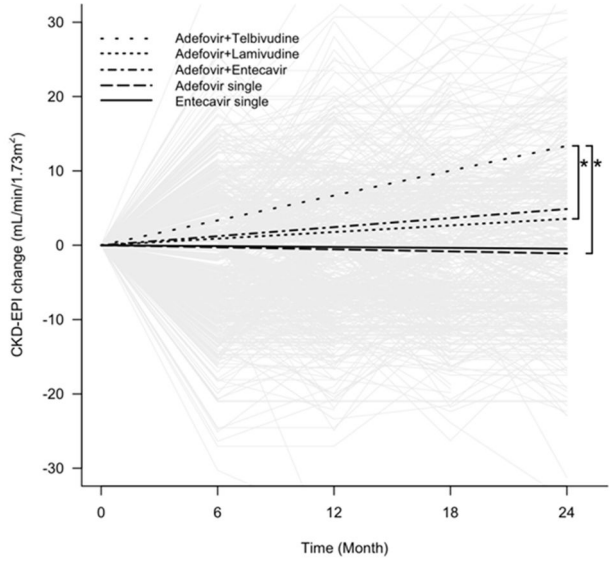
The changes in eGFR over time were evaluated using the linear mixed-effect model and the CKD-EPI formula for eGFR (Figure. 2A). The estimated mean changes in eGFR per month were as follows: +0.557 ml/min for the ADV plus LdT group, +0.148 ml/min for the ADV plus LAM group, +0.203 ml/min for the ADV plus ETV group, −0.046 ml/min for ADV alone and −0.020 ml/min for ETV alone (Figure. 2A). A steady increase in eGFR from baseline was observed in the ADV plus LdT (P<0.001) and ADV plus LAM (P<0.001) groups. However, in the groups treated with ADV plus ETV, ADV alone or ETV alone, eGFR did not change significantly over time. When the groups were compared, eGFR changed more significantly in the ADV plus LdT group than in the ADV plus LAM group, after adjusting for multiple comparisons with the false discovery rate (FDR) correction (P=0.015).

We divided the patients into two groups to investigate the changes in eGFR according to renal function. One patient group had baseline eGFR greater than or equal to 90 ml/min, and the other group had baseline eGFR in

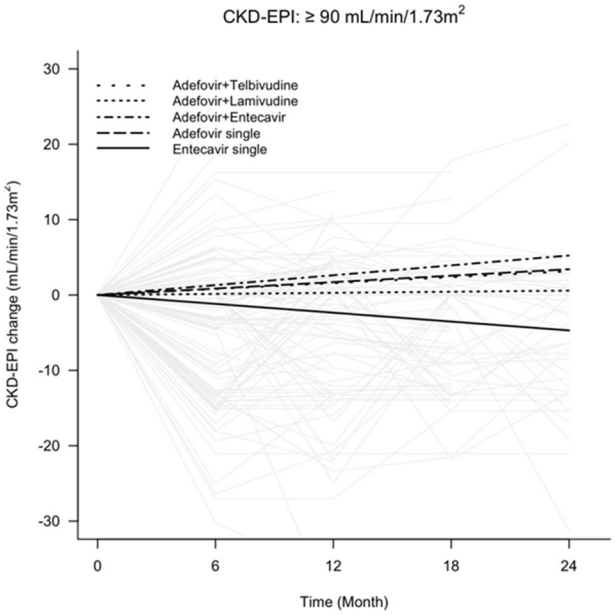
the *Gray Zone* (eGFR ≥50 and lower than 90 ml/min). In patients with baseline eGFR ≥90 ml/min, the estimated mean changes in eGFR over time were not statistically significant for any treatment group (Figure. 2B) (P=0.855).

In patients with baseline eGFR in the *Gray Zone*, eGFR changed significantly over time in the ADV plus LdT and the ADV plus LAM groups. The estimated mean changes in the patients with *Gray Zone* baseline eGFR were as follows: +0.641 ml/min per month in the ADV plus LdT group (P<0.001) and +0.165 ml/min per month in the ADV plus LAM group (P<0.001) (Figure. 2C). Among the three remaining groups, the estimated mean changes in eGFR per month were not statistically significant and were as follows: +0.172 ml/min in the ADV plus ETV group (P= 0.134), –0.065 ml/min in the ADV group (P=0.162), and no change in the ETV alone group (P=0.993). When the eGFR changes were compared between the groups, the ADV plus LdT group showed significantly higher (P<0.001) eGFR changes compared to the other four treatment groups (ADV plus LAM, ADV plus ETV, ADV alone, and ETV alone). The eGFR change was also significantly higher in the ADV plus LAM group compared to the ADV plus ETV, ADV alone, and ETV alone groups (P<0.001). However, the eGFR changes were not significantly different between the ADV plus ETV group and the groups treated with either ADV or ETV alone.

(A)


Adefovir+Telbivudine
Adefovir+Lamivudine
Adefovir+Entecavir
Adefovir single
Entecavir single
CKD-EPI change (mL/min/1.73m²)
Time (Month)
**

(B)


CKD-EPI: ≥ 90 mL/min/1.73m²
Adefovir+Telbivudine
Adefovir+Lamivudine
Adefovir+Entecavir
Adefovir single
Entecavir single
CKD-EPI change (mL/min/1.73m²)
Time (Month)

(C)

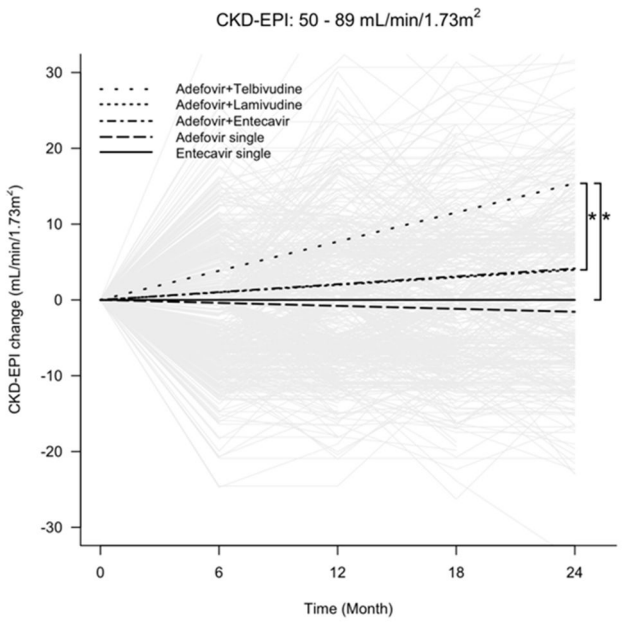


Figure 2. Changes in the renal function of the treatment groups were assessed over the course of 2 years by the following criteria.

(A) Estimated glomerular filtration rate (eGFR) as calculated by the CKD-EPI formula. The patients were divided into two groups according to baseline eGFR for subgroup analysis. **(B)** Changes in eGFR over time using the CKD-EPI formula in patients with baseline eGFR ≥90 ml/min. **(C)** Changes in baseline eGFR between 50 and 90 ml/min. The mean changes in the five treatment groups were estimated using the linear mixed-effects model for repeated measures at each time point. *P* values below 0.05 are indicated by asterisks. The eGFR changes over time in the ADV plus LdT and ADV plus LAM groups were significantly different from each other and from the other groups.

Predictors for significant eGFR change

In the linear mixed-effect model, age, gender, baseline eGFR, and prescribed antiviral therapy were significant predictors for eGFR changes over time (Table 2). Among these variables, the baseline eGFR was most capable of predicting eGFR decreases in CHB patients (estimated value of 0.743, $P<0.001$). Among the five treatment options, the ADV plus LdT therapy caused the greatest improvement in renal function in terms of eGFR over time in the linear mixed-effect model (estimated value of 0.557, $P<0.001$). Treatment with ADV plus LAM was also observed to be a significant predictor for positive eGFR changes, resulting in estimated eGFR values that were 25% lower than those predicted following treatment with ADV plus LdT. In contrast, treatment with ADV alone or ETV alone negatively influenced eGFR over time, although this was not statistically significant (estimated values of –0.046 and –0.020, respectively).

The MDRD equation resulted in higher individual eGFR values in patients with normal renal function when compared to values obtained with the CKD-EPI formula. When eGFR values were calculated by the MDRD rather than the CKD-EPI equation, the slope of the change over time was different, but the general pattern of the results remained the same (data not shown).

Table 2. Predictors of CKD-EPI eGFR decrease from baseline

	Estimate	Standard Error	*P* value*
Age	-0.147	0.024	<0.001
Sex (Female vs. Male)	5.992	0.759	<0.001
Hypertension	-0.156	0.770	0.840
Diabetes mellitus	0.678	0.737	0.358
Baseline CKD-EPI	0.743	0.023	<0.001
ADV plus LdT	0.557	0.133	<0.001
ADV plus LAM	0.148	0.038	<0.001
ADV plus ETV	0.203	0.118	0.086
ADV alone	-0.046	0.045	0.309
ETV alone	-0.020	0.034	0.553

* Results from the linear mixed-effects model for repeated measures

Abbreviations: ADV, adefovir; LdT, telbivudine; LAM, lamivudine; ETV, entecavir

Virologic Response

The median changes in serum concentrations of HBV DNA over 24 months in the five groups are shown in Figure 3. HBV DNA concentrations at baseline were highest in the ETV alone group among five treatment groups. HBV DNA concentrations in the ETV alone group at 24 months were significantly lower than those in the other four groups (P<0.001). The change in serum concentrations of HBV DNA over time were not significantly different between the ADV plus LdT, ADV plus LAM, ADV plus ETV, and ADV alone groups.

We evaluated the possibility that a reduction in HBV DNA levels could be associated with improved eGFR in the ADV plus LdT group. The eGFR change over time was not significantly associated with the changes in serum concentrations of HBV DNA in the ADV plus LdT group when the baseline levels of serum HBV DNA and baseline eGFR were compared with those at each time point (Figure. 4).

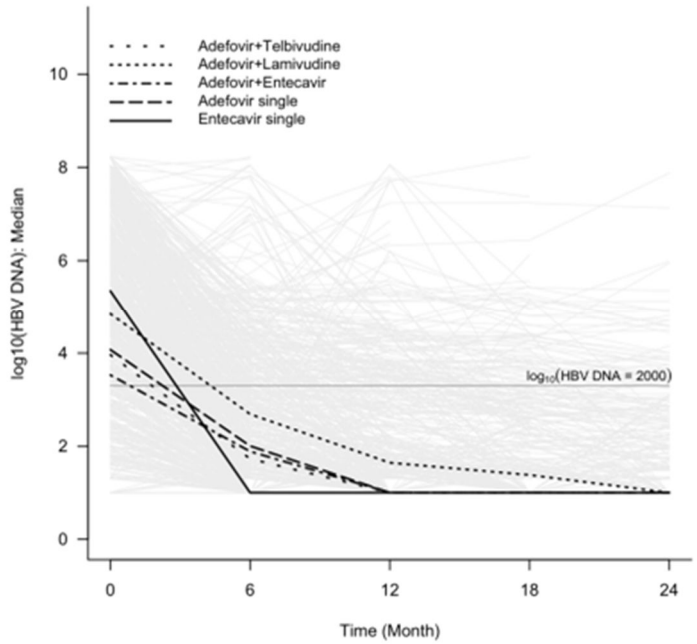


Figure 3. Changes in serum concentrations of HBV DNA over 24 months in five treatment groups with CHB refractory to LAM.

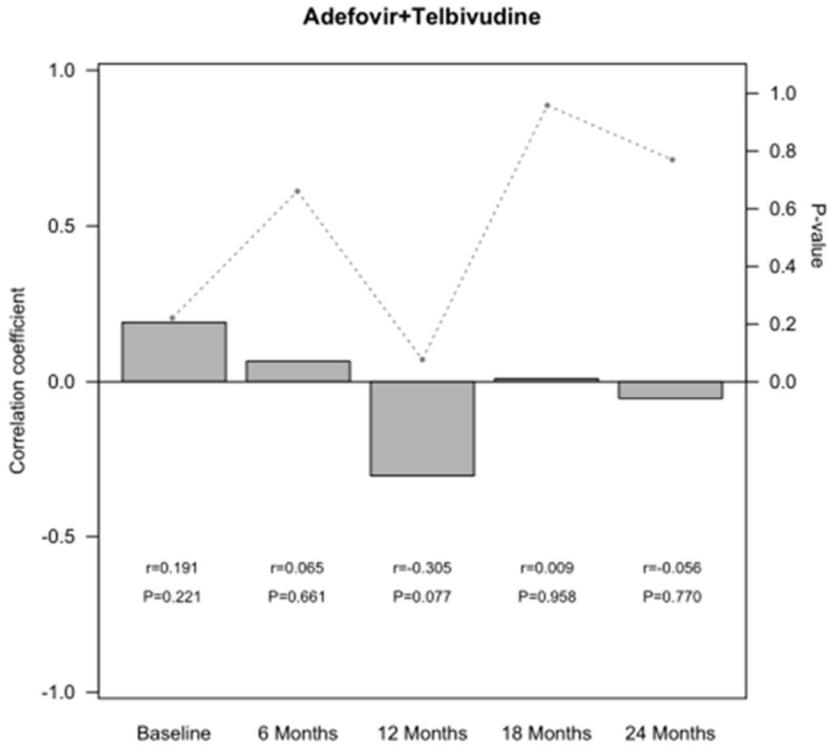


Figure 4. Relationship between changes in the serum HBV DNA levels and eGFR over time in the ADV plus LdT group. The Pearson correlation coefficient was calculated. The serum concentrations of HBV DNA and eGFR of the ADV plus LdT group were compared to baseline values at each time point.

DISCUSSION

Combination therapy is now being regarded as one of therapeutic options for LAM-experienced resistant HBV patients because of higher efficacy than ADV monotherapy by no cross-resistance between these two nucleos(t)ide analogues (34). Considering that the primary goal of antiviral therapy in CHB is to maintain undetectable levels of serum HBV DNA, indefinite anti-viral therapy might be required to achieve the primary goal. Therefore, adverse effect in long-term use of combination therapy became to be one of important issues to make an optimal strategy for CHB treatment in LAM-resistant patients. Nephrotoxicity, one of well-known adverse effects, has been reported as a warning associated with nucleotide analogues such as ADV and tenofovir (35-37). In contrast to this, previous studies reported that LdT significantly improved renal function in CHB patients (6, 19-24). However, there has been lack of evidence reported renal dysfunction in CHB patients with ADV-based combination therapy until now.

A novel, clinically important finding of the present study was that patients treated with ADV plus LdT showed a significant improvement in eGFR over 2 years. While ADV treatment is known to cause nephrotoxicity, LdT treatment showed a strong renoprotective effect, with an estimated protective effect that was 4 times greater than that of LAM (Table 2). However, ETV did not affect renal function when combined with ADV. When LdT, LAM, or ETV was combined with ADV, LdT improved renal function the most effectively.

The eGFR *Gray Zone* has important clinical implications for the safety of CHB patients receiving long-term combination therapies. Renal function is an important safety issue in patients with advanced liver diseases, especially in those with decompensated HBV cirrhosis, as renal dysfunction is associated with high mortality (38). In this study, the baseline eGFR was a better predictive factor of decreasing eGFR than age, sex, or existence of the comorbidities hypertension and diabetes. In other words, patients with low baseline eGFR have a higher tendency to develop renal dysfunction than those with high baseline eGFR. The current study demonstrated that over time, the combination therapy ADV plus LdT improved the renal function of CHB patients with eGFR in the *Gray zone* more than in patients whose eGFR were ≥90 ml/min and this treatment option may stop the vicious cycle between low eGFR and renal dysfunction.

Currently, there are no recommendations for adjusting the dose and/or treatment interval of antiviral agents for CHB patients with *Gray zone* eGFR. In patients with eGFR in the *Gray zone*, LdT in combination treatment could provide an effective treatment option. Naturally, the baseline eGFR is an important predictor of the risk of declining renal function and development of ESRD (39). The renoprotective effects of LdT should be considered, particularly for patients with terminal liver function because the baseline pre-liver transplant eGFR is significantly associated with decreased survival following transplantation and is a prognostic indicator of post-transplant chronic kidney disease (40).

We divided the patients into two groups, including those with eGFR

≥90 and those with eGFR between 50 and 90 ml/min. In this study, we chose an eGFR cut-off value of 50 ml/min, rather than a cut-off of 60 ml/min because the lowest eGFR value requiring adjustments in the dose and/or interval of antiviral drugs is 50 ml/min. In a clinical setting, when antiviral drugs need to be prescribed, the cut-off value of 60 ml/min, which is associated with stage 2 chronic kidney disease, is less clinically meaningful than the 50 ml/min value.

Furthermore, the serum concentrations of HBV DNA did not change significantly among the five groups, except in the ETV alone group. The differences in the HBV DNA concentrations between the ETV alone and other treatment groups could be explained by the fact that the patients in the ETV alone group received ETV as the first-line therapy, in contrast to those treated with the second-line therapy against resistant HBV. Based on the similar antiviral effects among the groups other than the ETV alone group, ADV plus LdT should be considered for patients with eGFR in the *Gray Zone*.

The improvement of renal function in the ADV plus LdT group was not significantly associated with the control of serum HBV DNA levels, indicating that the increase in eGFR was influenced by LdT itself rather than by the control of HBV infection. The eGFR changes observed here following treatment with the ADV/LdT combination therapy suggest that the renoprotective effects of LdT could overcome the nephrotoxicity caused by ADV. As a possible mechanism, Chan et al. suggested that LdT could increase blood flow, thereby improve tubular dysfunction (21). In terms of mechanism of drug excretion, ADV has been shown to cause nephrotoxicity

by inhibiting mitochondrial DNA (mtDNA) replication during the renal excretion (41). In contrast, the main mechanism of LdT excretion is through passive diffusion, not result in mtDNA depletion or toxic effects on function of renal tubule cells (42). Considering the importance of mtDNA in the maintenance of homeostasis in proximal tubule cells, this difference in the excretion mechanism could explain the contrasting effects of these drugs on renal function. However, the specific mechanisms by which LdT exerts its renoprotective effects when used alone or in combination with other drugs are unclear and should be clarified in future studies.

Recently, the CKD-EPI formula was introduced for the calculation of eGFR both in patients with normal kidney function and in those with eGFR in the *Gray Zone*. Previous studies have reported that the CKD-EPI formula was more accurate than the MDRD equation, which was not validated for the evaluation of changes in individuals with eGFR ≥60 ml/min (27). The application of the CKD-EPI formula in the current study demonstrated no significant changes over time in the five groups with eGFR ≥90 ml/min. Neither a renoprotective effect due to treatment with LdT nor nephrotoxicity due to treatment with ADV were found in CHB patients with normal kidney function.

In contrast with some previous studies reporting no significant improvement and/or decrease in eGFR following treatment with ADV plus LAM (43-45), here we found that eGFR in CHB patients with eGFR between 50 and 90 ml/min improved significantly after treatment with ADV plus LAM. However, previous studies evaluated kidney function through serum

creatinine levels and/or eGFR calculated by the MDRD or Cockcroft-Gault equations rather than the CKD-EPI formula, and these studies were therefore limited in their ability to reflect real changes in eGFR in CHB patients in the *Gray zone*. Furthermore, a previous study of Asian CHB patients reported that treatment with ADV plus LAM led to an improvement in serum creatinine levels (46), although the underlying mechanism of this improvement was unclear.

Current guidelines recommend that ETV or tenofovir disoproxil fumarate (TDF) should be used for first-line monotherapy in CHB patients (3). Nephrotoxicity may be a potential concern for HIV patients receiving TDF, although this problem occurs less frequently in CHB patients treated with TDF. Considering the risk of nephrotoxicity following TDF treatment and the renal protective effect of LdT, the efficacy of TDF plus LdT combination therapy in the patients with multidrug resistant HBV on renal function should be clarified in future studies (24).

The rather limited number of enrolled patients and the retrospective design with the short observational period of 2 years may represent limitations of our study. To overcome these limitations, we used a linear mixed-effect model. The clear inclusion and exclusion criteria and the use of the CKD-EPI formula for the assessment of kidney function may counteract the study limitations. The five groups were not well-matched in terms of baseline characteristics. To correct for the baseline differences among the five groups, we treated the baseline patient eGFR as a fixed effect and considered random effects to account for patient variability in the model. In this study, we did not

exclude patients treated with potentially nephrotoxic drugs such as nonsteroidal anti-inflammatory drugs and/or renoprotective drugs such as angiotensin converting enzyme inhibitors. However, only 14% of the patients in this study had hypertension. In addition, we sought to include as many patients as possible, as our primary goal was to identify a general pattern of drug toxicity in the overall population, rather than to observe eGFR changes in specific individuals.

In conclusion, over the course of a 2-year observational period, renal function was significantly improved in CHB patients treated with ADV plus LdT compared to patients treated with ADV alone, ETV alone or other ADV-based combination therapies. Patients with renal insufficiency in the *Gray zone* in particular benefitted from ADV plus LdT combination therapy. The underlying mechanisms of telbivudine's renal protective effects remain to be investigated.

REFERENCES

1. Alberti A. Can serum HBV-DNA be used as a primary end point to assess efficacy of new treatments for chronic hepatitis B? Hepatology. 2003;38(1):18-20. Epub 2003/06/28.

2. Mommeja-Marin H, Mondou E, Blum MR, Rousseau F. Serum HBV DNA as a marker of efficacy during therapy for chronic HBV infection: analysis and review of the literature. Hepatology. 2003;37(6):1309-19. Epub 2003/05/30.

3. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. Journal of hepatology. 2012;57(1):167-85. Epub 2012/03/23.

4. Lok AS, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, et al. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. Gastroenterology. 2003;125(6):1714-22. Epub 2004/01/16.

5. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. Gastroenterology. 2006;131(6):1743-51. Epub 2006/11/08.

6. Wang Y, Thongsawat S, Gane EJ, Liaw YF, Jia J, Hou J, et al. Efficacy and safety of continuous 4-year telbivudine treatment in patients with chronic hepatitis B. Journal of viral hepatitis. 2013;20(4):e37-46. Epub 2013/03/16.

7. Lim YS, Lee JY, Lee D, Shim JH, Lee HC, Lee YS, et al. Randomized trial of the virologic response during up to two years of entecavir-adefovir combination therapy in multiple-drug-refractory chronic hepatitis B virus patients. Antimicrobial agents and chemotherapy. 2013;57(7):3369-74.

8. Seo SY, Kim IH, Sohn JY, Lee S, Kim SH, Kim SW, et al. Long-Term Efficacy of Entecavir Plus Adefovir Combination Therapy versus Entecavir Monotherapy in Adefovir Refractory Chronic Hepatitis B Patients with Prior Lamivudine Resistance. Intervirology. 2013.

9. Ahn SH, Kweon YO, Paik SW, Sohn JH, Lee KS, Kim DJ, et al. Telbivudine in combination with adefovir versus adefovir monotherapy in HBeAg-positive, lamivudine-resistant chronic hepatitis B. Hepatology international. 2011.

10. Inoue J, Ueno Y, Wakui Y, Niitsuma H, Fukushima K, Yamagiwa Y, et al. Four-year study of lamivudine and adefovir combination therapy in lamivudine-resistant hepatitis B patients: influence of hepatitis B virus genotype and resistance mutation pattern. Journal of viral hepatitis. 2011;18(3):206-15.

11. Xu XH, Li GL, Qin Y, Li Q, He FQ, Li JY, et al. Entecavir plus adefovir rescue therapy for chronic hepatitis B patients after multiple treatment failures in real-life practice. Virology journal. 2013;10(1):162.

12. Papatheodoridis GV, Manolakopoulos S, Dusheiko G, Archimandritis AJ. Therapeutic strategies in the management of patients with chronic hepatitis B virus infection. The Lancet infectious diseases. 2008;8(3):167-78. Epub 2007/12/07.

13. Izzedine H, Hulot JS, Launay-Vacher V, Marcellini P, Hadziyannis SJ, Currie G, et al. Renal safety of adefovir dipivoxil in patients with chronic hepatitis B: two double-blind, randomized, placebo-controlled studies. Kidney international. 2004;66(3):1153-8. Epub 2004/08/26.

14. Bayrakci US, Baskin E, Ozcay F, Ozdemir BH, Karakayali H, Haberal M. Renal Fanconi syndrome and myopathy after liver transplantation: drug-related mitochondrial cytopathy? Pediatric transplantation. 2008;12(1):109-12. Epub 2008/01/12.

15. Fabrizi F, Dulai G, Dixit V, Bunnapradist S, Martin P. Lamivudine for the treatment of hepatitis B virus-related liver disease after renal transplantation: meta-analysis of clinical trials. Transplantation. 2004;77(6):859-64. Epub 2004/04/13.

16. Schmilovitz-Weiss H, Melzer E, Tur-Kaspa R, Ben-Ari Z. Excellent outcome of Lamivudine treatment in patients with chronic renal failure and hepatitis B virus infection. Journal of clinical gastroenterology. 2003;37(1):64-7. Epub 2003/06/18.

17. Kamar N, Milioto O, Alric L, El Kahwaji L, Cointault O,

Lavayssiere L, et al. Entecavir therapy for adefovir-resistant hepatitis B virus infection in kidney and liver allograft recipients. Transplantation. 2008;86(4):611-4. Epub 2008/08/30.

18. Matthews SJ. Entecavir for the treatment of chronic hepatitis B virus infection. Clinical therapeutics. 2006;28(2):184-203. Epub 2006/05/09.

19. Gane EJ, Deray G, Liaw YF, Lim SG, Lai CL, Rasenack J, et al. Telbivudine improves renal function in patients with chronic hepatitis B. Gastroenterology. 2014;146(1):138-46 e5. Epub 2013/09/27.

20. Li X, Zhong C, Yang S, Fan R, Peng J, Guo Y, et al. [Influence of adefovir dipivoxil or telbivudine monotherapy on renal function of patients with chronic hepatitis B]. Nan fang yi ke da xue xue bao = Journal of Southern Medical University. 2012;32(6):826-9. Epub 2012/06/16.

21. Chan HL, Chen YC, Gane EJ, Sarin SK, Suh DJ, Piratvisuth T, et al. Randomized clinical trial: efficacy and safety of telbivudine and lamivudine in treatment-naive patients with HBV-related decompensated cirrhosis. Journal of viral hepatitis. 2012;19(10):732-43. Epub 2012/09/13.

22. Hou J, Sun J, Xie Q, Tan D, Ning Q, Niu J, et al. Superiority of ROADMAP Strategy in HBeAg Positive CHB Patients：2 Year Final Result of EFFORT Study APASL Liver Week; Singapore 2013.

23. Piratvisuth T, Komolmit P, Tanwandee T, Sukeepaisarnjaroen W, Chan HL, Pessoa MG, et al. 52-week efficacy and safety of telbivudine with conditional tenofovir intensification at week 24 in HBeAg-positive chronic hepatitis B. PloS one. 2013;8(2):e54279. Epub 2013/02/08.

24. Amarapurkar DN, Patel N. Increased eGFR with telbivudine in combination therapy of chronic hepatitis B infection. Indian journal of gastroenterology : official journal of the Indian Society of Gastroenterology. 2013. Epub 2013/03/21.

25. Sawabe T, Uenotsuchi T, Imafuku S, Kohno H, Arima J, Horiuchi T. Remission of hepatitis B virus-related vasculitis with lamivudine. Annals of internal medicine. 2004;140(8):672-3. Epub 2004/04/21.

26. Tang S, Lai FM, Lui YH, Tang CS, Kung NN, Ho YW, et al. Lamivudine in hepatitis B-associated membranous nephropathy. Kidney international. 2005;68(4):1750-8. Epub 2005/09/17.

27. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Annals of internal medicine. 2009;150(9):604-12. Epub 2009/05/06.

28. Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics. 1982;38(4):963-74. Epub 1982/12/01.

29. Cnaan A, Laird NM, Slasor P. Using the general linear mixed

model to analyse unbalanced repeated measures and longitudinal data. Statistics in medicine. 1997;16(20):2349-80. Epub 1997/11/14.

30. Verbeke G, Molenberghs G. Linear mixed models for longitudinal data. New York, USA: Springer Verlag LLC; 2009.

31. Akaike H. A New Look at the Statistical Model Identification. IEEE Trans Autom Control. 1974(19):716-23.

32. Schwartz G. Estimating the dimension of a model. Annals of Statistics. 1978;6:461-4.

33. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol. 1995;57:-300.

34. Locarnini S, Mason WS. Cellular and virological mechanisms of HBV drug resistance. Journal of hepatology. 2006;44(2):422-31. Epub 2005/12/21.

35. Fontana RJ. Side effects of long-term oral antiviral therapy for hepatitis B. Hepatology. 2009;49(5 Suppl):S185-95. Epub 2009/04/29.

36. Vrouenraets SM, Fux CA, Wit FW, Garcia EF, Furrer H, Brinkman K, et al. Persistent decline in estimated but not measured glomerular filtration rate on tenofovir may reflect tubular rather than glomerular toxicity. AIDS. 2011;25(17):2149-55. Epub 2011/08/23.

37. Ha NB, Garcia RT, Trinh HN, Vu AA, Nguyen HA, Nguyen KK, et al. Renal dysfunction in chronic hepatitis B patients treated with

adefovir dipivoxil. Hepatology. 2009;50(3):727-34. Epub 2009/06/12.

38. de Jongh FE, Janssen HL, de Man RA, Hop WC, Schalm SW, van Blankenstein M. Survival and prognostic indicators in hepatitis B surface antigen-positive cirrhosis of the liver. Gastroenterology. 1992;103(5):1630-5. Epub 1992/11/01.

39. Keith DS, Nichols GA, Gullion CM, Brown JB, Smith DH. Longitudinal follow-up and outcomes among a population with chronic kidney disease in a large managed care organization. Archives of internal medicine. 2004;164(6):659-63. Epub 2004/03/24.

40. Bahirwani R, Campbell MS, Siropaides T, Markmann J, Olthoff K, Shaked A, et al. Transplantation: impact of pretransplant renal insufficiency. Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society. 2008;14(5):665-71. Epub 2008/04/25.

41. Tanji N, Tanji K, Kambham N, Markowitz GS, Bell A, D'Agati V D. Adefovir nephrotoxicity: possible role of mitochondrial DNA depletion. Human pathology. 2001;32(7):734-40. Epub 2001/08/04.

42. McKeage K, Keam SJ. Telbivudine: a review of its use in compensated chronic hepatitis B. Drugs. 2010;70(14):1857-83. Epub 2010/09/15.

43. Lampertico P, Vigano M, Manenti E, Iavarone M, Sablon E,

Colombo M. Low resistance to adefovir combined with lamivudine: a 3-year study of 145 lamivudine-resistant hepatitis B patients. Gastroenterology. 2007;133(5):1445-51. Epub 2007/11/07.

44. Tamori A, Enomoto M, Kobayashi S, Iwai S, Morikawa H, Sakaguchi H, et al. Add-on combination therapy with adefovir dipivoxil induces renal impairment in patients with lamivudine-refractory hepatitis B virus. Journal of viral hepatitis. 2010;17(2):123-9. Epub 2009/08/14.

45. Manolakopoulos S, Striki A, Deutsch M, Mela M, Ketikoglou I, Tzourmakliotis D, et al. Long-term adefovir plus lamivudine therapy does not decrease creatinine clearance in HBeAg-negative chronic hepatitis B patients. Liver international : official journal of the International Association for the Study of the Liver. 2011;31(10):1525-32. Epub 2011/11/19.

46. Fan XH, Geng JZ, Wang LF, Zheng YY, Lu HY, Li J, et al. De novo combination therapy with lamivudine and adefovir dipivoxil in chronic hepatitis B patients. World journal of gastroenterology : WJG. 2011;17(43):4804-9. Epub 2011/12/08.

국문 초록

서론: 만성 B 형 간염환자에서 아데포비어의 투약은 신장기능을 악화시킨다는 점이 여러 문헌을 통해 보고된 바 있다. 이에 반해, 만성 B 형 간염환자에서 텔비부딘의 투약은 신장기능을 향상시킨다는 점이 여러 연구를 통해 밝혀져 있다. 이에 본 연구의 목적은 만성 B 형 간염환자에서 아데포비어를 근간으로 한 병합요법에서 텔비부딘이 신장기능에 미치는 영향을 파악해 보고자 한다.

방법: 만성 B 형 간염 환자에서 아데포비어와 텔비부딘의 병합요법, 아데포비어와 엔테카비어의 병합요법, 아데포비어와 라미뷰딘의 병합요법, 아데포비어 단독 투약, 엔테카비어 단독투약군 총 다섯 투약군에서 신장기능의 변화를 96 주간 관찰하였다. 후향적 관찰 연구로써, 상기 약제를 투약 받은 바 있는 만성 B 형 간염환자 831 명을 대상으로 추정사구체여과율 계산을 통해 신장기능의 변화양상을 분석하였다. 투약군 사이에 추정사구체여과율 변화양상은 선형 혼합모형을 통해 분석되었다.

결과: 다섯 투약군 중에서, 아데포비어와 텔비부딘 및 아데포비어와 라미뷰딘 병용투약군에서 추정사구체여과율의 유의한 상승이 관찰되었다 ($P<0.001$). 특히, 기저 추정사구체여과율이 50 에서 90 ml/min 사이인 환자군에서 아데포비어와 텔비부딘 투약군이 다른 투약군에 비해 가장 유의한 추정사구체여과율의 상승을 보여주었다.

나이, 성별, 기저 신장기능 및 만성 B 형 간염에 대한 약물 투약력이 추정사구체여과율의 변화에 대한 유의한 예측인자로 분석되었다.

결론: 결론적으로 만성 B 형 간염환자에서 아데포비어를 기반으로 한 다른 병합요법과 비교할 때, 아데포비어와 텔비부딘의 병합요법이 신장기능의 향상과 가장 유의한 연관관계를 보이고 있다.

주요어 : 만성 B 형 간염, 신장 기능, 아데포비어 디피복실, 텔비부딘
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