DOI: 10.4274/vhd.galenos.2026.2025-10-3
Viral Hepat J 2026;32(2):51-59

Longitudinal Renal Function Changes and Pilot Mitochondrial Epigenetic Analysis in Chronic Hepatitis B Patients Treated with Nucleos(t)ide Analogues

Nükleoz(t)id Analogları ile Tedavi Edilen Kronik Hepatit B Hastalarında Böbrek Fonksiyonlarındaki Uzun Dönemli Değişimler ve Pilot Mitokondriyal Epigenetik Analiz

Güliz Uyar Güleç[1], Sevin Kırdar[2], Ceylan Ak[3], Mehtap Kılıç Eren[4], Hüseyin Örün[5]

[1]*Aydın Adnan Menderes University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Aydın, Türkiye*
[2]*Aydın Adnan Menderes University Faculty of Medicine, Department of Medical Microbiology, Aydın, Türkiye*
[3]*Aydın Adnan Menderes University Institute of Health Sciences, Department of Medical Biology, Aydın, Türkiye*
[4]*Aydın Adnan Menderes University Faculty of Medicine, Department of Medical Biology, Aydın, Türkiye*
[5]*Republic of Türkiye Ministry of Health, Central Community Health Directorate, Şırnak, Türkiye*

ABSTRACT

Objectives: Long-term use of nucleos(t)ide analogues (NAs) has been associated with mitochondrial dysfunction and potential adverse effects. This study aimed to evaluate nephrotoxicity in treated and treatment-naïve chronic hepatitis B (CHB) patients and to explore DNA methyltransferase 1 (DNMT1) expression as a potential epigenetic mechanism.

Materials and Methods: Adult CHB patients receiving tenofovir disoproxil fumarate (TDF) or entecavir (ETV) for ≥12 months comprised the "under treatment" group, while untreated patients formed the "treatment-naïve" group. Renal parameters at baseline and months 3, 6, and 12 were analyzed retrospectively. Longitudinal changes in glomerular filtration rate (GFR) were assessed using linear mixed-effects modeling. DNMT1 expression in peripheral blood mononuclear cells was evaluated as a pilot analysis.

Results: A total of 158 patients were analyzed; 91 were treated and 67 were treatment-naïve. Median GFR values declined significantly from 102.3 at the baseline to 102.1 at the 12th month in the treated group (p=0.012). In longitudinal analysis, each additional time point was associated with a mean GFR decline of 1.65 mL/min/1.73 m^2 (95% confidence interval: -3.13 to -0.16; p=0.030). Age was independently associated with a reduction in GFR (β=-0.75 per year; p<0.001). No significant differences in renal outcomes were observed between TDF and ETV. In the pilot epigenetic analysis (n=25), DNMT1 expression was comparable between treated and treatment-naïve patients (median 1.1789 vs. 0.7565; p=0.466).

ÖZ

Amaç: Nükleos(t)id analoglarının (NA) uzun süreli kullanımı, mitokondriyal işlev bozukluğu ve olası yan etkilerle ilişkilendirilmiştir. Bu çalışmada, tedavi altındaki ve tedavi-naiv kronik hepatit B (KHB) hastalarında nefrotoksisitenin değerlendirilmesi ve potansiyel bir epigenetik mekanizma olarak DNA metiltransferaz 1 (DNMT1) ekspresyonunun araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Tenofovir disoproksil fumarat (TDF) veya entekavir (ETV) tedavisini ≥12 ay boyunca alan yetişkin KHB hastaları "tedavi altındaki" grubu oluştururken, tedavi görmemiş hastalar ise "tedavi-naiv" grubu oluşturdu. Başlangıçta ve 3., 6. ve 12. aylarda ölçülen böbrek parametreleri retrospektif olarak analiz edildi. Glomerüler filtrasyon hızındaki (GFR) uzunlamasına değişiklikler, doğrusal karışık etki modellemesi kullanılarak değerlendirildi. Pilot çalışma olarak, periferik kan mononükleer hücrelerinde DNMT1 ekspresyonu değerlendirildi.

Bulgular: Toplam 158 hasta analiz edildi; 91'i tedavi altında, 67'si tedavi-naiv hastaydı. Tedavi altındaki grupta medyan GFR değerleri, başlangıçtaki 102,3'ten 12. ayda 102,1'e istatistiksel olarak anlamlı bir düşüş gösterdi (p=0,012). Boylamsal analizde, her bir ek zaman noktası ortalama 1,65 mL/dk/1,73 m^2'lik bir GFR düşüşü ile ilişkilendirildi (%95 güven aralığı: -3,13 ila -0,16; p=0,030). Yaş, GFR azalmasıyla bağımsız olarak ilişkilendirildi (yılda -0,75 β; p<0,001). TDF ve ETV arasında böbrek fonksiyon sonuçlarında anlamlı bir fark gözlenmedi. Pilot epigenetik analizde (n=25), DNMT1 ekspresyonu tedavi görmüş ve tedavi görmemiş hastalar arasında benzerdi (medyan 1,1789'a karşı 0,7565; p=0,466).

Address for Correspondence: Güliz Uyar Güleç, MD, Aydın Adnan Menderes University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Aydın, Türkiye
E-mail: guliz.uyar@adu.edu.tr **ORCID ID:** orcid.org/000-0002-8565-1042

Received: 13.10.2025 **Accepted:** 02.07.2026 **Epub:** 21.07.2026 **Publication Date:** 01.09.2026

Cite this article as: Uyar Güleç G, Kırdar S, Ak C, Kılıç Eren M, Örün H. Longitudinal renal function changes and pilot mitochondrial epigenetic analysis in chronic hepatitis B patients treated with nucleos(t)ide analogues. Viral Hepat J. 2026;32(2):51-59

Conclusion: Long-term NA therapy is associated with a modest decline in renal function. Although DNMT1 expression was not significantly altered, potential mitochondrial effects warrant further investigation in larger prospective studies.
Keywords: DNA methyltransferase 1, epigenetics, hepatitis B virus, nephrotoxicity, nucleos(t)ide analogues

Sonuç: Uzun süreli NA tedavisi, böbrek fonksiyonunda hafif bir düşüşle ilişkili bulunmuştur. DNMT1 ekspresyonunda istatistiksel olarak anlamlı bir değişiklik gözlenmemiş olsa da, olası mitokondriyal etkiler nedeniyle daha geniş kapsamlı prospektif çalışmalarla bu konunun daha ayrıntılı olarak araştırılması gerekmektedir.
Anahtar Kelimeler: DNA metiltransferaz 1, epigenetik, hepatit B virüsü, nefrotoksisite, nükleos(t)id analogları

Introduction

Although its prevalence varies by country, chronic hepatitis B (CHB) remains a global problem. According to World Health Organization data for 2022, the number of people with CHB is 254 million; the number of new cases is 1.2 million per year; and the number of deaths from cirrhosis and hepatocellular carcinoma (HCC) attributable to hepatitis B virus (HBV) infection is 1.1 million (1).

The main objective in treating CHB is to enhance patients' life expectancy, to improve their quality of life, and to prevent complications, such as HCC or liver failure, caused by CHB. Prevention of HBV reactivations and transmission from mother to infant are secondary targets of treatment (2).

Nucleos(t)ide analogues (NA) and pegylated interferon alpha (PegIFNa) are two main treatment options for CHB. NAs approved for HBV treatment include lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine (LdT), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF). While ETV, TDF, and TAF are associated with a high barrier against HBV resistance, LAM, ADV, and LdT are associated with a low barrier to HBV resistance (3).

Due to the risk of virological relapse and hepatic decompensation after discontinuation of treatment, patients must continue NA treatment for life, exposing them to several side effects of long-term treatment (4).

Both HBV infection and long-term NA use may adversely affect renal function. All NAs are excreted by the kidneys in unchanged form and some of them cause nephrotoxicity through dose-dependent proximal tubular damage. Nephrotoxicity is manifested by elevated serum creatinine, proteinuria, nephrogenic diabetes insipidus, hypophosphatemia or the more severe form of Fanconi syndrome (5).

The prevalence and course of extrahepatic side effects which are most frequently reported during NA treatment are not known exactly and may be associated with mitochondrial dysfunction (6). Since NA, which exerts its effects by inhibiting the viral DNA polymerase, can also inhibit the DNA polymerase involved in mitochondrial DNA (mtDNA) replication, mitochondrial side effects may occur. Furthermore, it has been shown that mtDNA, like nuclear DNA, contains 5-methylcytosine (5mC) at CpG dinucleotides and thus epigenetic modification may play a potential role in the regulation of transcription of mtDNA (7,8). So far, very few experimental data are available regarding the epigenetic regulation of mtDNA in physiological and various pathological conditions.

Different types of epigenetic processes have been described. However, methylation of CpG islands is an important mechanism in higher-order eukaryotes. Vertebrate nuclear DNA contains three DNA methyltransferases (DNMTs): DNMT1, DNMT3A, and DNMT3B. Under the action of these enzymes, a methyl group is added to the 5′ position of the base cytosine to form 5mC, and they can methylate DNA *de novo*. Mitochondria have been reported to contain a specific DNMT isoform (mtDNMT1), suggesting a potential role for DNA methylation in mitochondrial gene regulation. DNMT3A has been reported to associate with mitochondria under certain conditions. Emerging evidence indicates that alterations in mtDNA methylation may be linked to DNMT1 expression in patients receiving NA therapy, supporting the exploratory evaluation of DNMT1 as an epigenetic marker in the present study (9,10). To the best of our knowledge, no research in our country has examined the epigenetic effects of NA treatment in patients with CHB.

This study investigated the effect of NA treatment on the development of nephrotoxicity in patients with CHB and the potential modulation of DNMT1 as an epigenetic mechanism.

Materials and Methods

Patient Selection and Nephrotoxicity Analyses

This study included adult patients with CHB who were followed and treated at the Infectious Diseases and Clinical Microbiology Outpatient Clinic of Aydın Adnan Menderes University Hospital from January 2010 to December 2024. Ethical approval was obtained from the Aydın Adnan Menderes University Clinical Research Ethics Committee (approval no: 2019/07, date: 11/06/2020).

According to the health practice communiqué in our country, patients who meet the treatment criteria may initiate therapy with TDF or ETV. The decision is left to the clinician. Patients who were older than 18 years and had complete data were included in the study. Patients co-infected with other hepatitis viruses and human immunodeficiency virus (HIV), pregnant women, patients with known kidney disease, and patients using nephrotoxic drugs were excluded from the study. Patients with CHB who initiated NA treatment (TDF or ETV) for the first time and continued it for at least 12 months were included in the "under treatment" group. Those with CHB who were followed without treatment were classified as "treatment-naïve".

Nephrotoxicity was assessed by retrospective review of patient data from the hospital information system. Age, gender, comorbidities (diabetes mellitus and hypertension), medication

and its duration, baseline HBV-DNA level, HBeAg and anti-HBe, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, glomerular filtration rate (GFR) (mL/min/1.73 m^2), and phosphorus levels were recorded. GFR was calculated using the short modification of diet in renal disease formula, which includes sex, age, race, and serum creatinine values. To assess the development of nephrotoxicity, urea, creatinine, GFR, and phosphorus values at 3 months (T1), 6 months (T2), and 12 months (T3) after the start of treatment were recorded. Renal endpoints were defined as creatinine clearance <50 mL/min, serum creatinine ≥0.5 mg/dL increase from baseline and serum phosphate <2 mg/dL as defined in Buti et al. (11).

mRNA Isolation and qRT-PCR Analysis

Epigenetic analyses were designed as pilot analyses to generate preliminary data on DNMT1 expression. Blood samples were collected into ethylenediaminetetraacetic acid tubes from patients diagnosed with CHB who attended the outpatient clinic for their routine check-ups and provided informed consent between June 2022 and January 2023. The mRNA was isolated from peripheral blood mononuclear cells (PBMCs) of the patients included in the study using a total RNA isolation kit (Thermo Fisher Scientific, Massachusetts, USA). After RNA isolation, DNA contamination was removed by treatment with DNase I (Thermo Fisher Scientific, Massachusetts, USA). Synthesis of cDNA from the obtained RNA was performed using one µg of RNA, random hexamer primers, and reverse transcriptase (Thermo Fisher Scientific, Massachusetts, USA). Gene expression analysis was performed with SYBR Green PCR Master Mix (ABM, Richmond, Canada) on a StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), using mtDNMT1 gene-specific forward (5′-TCCCTGGGCATGGCCGGCTC-3′) and reverse (5′-CTCTTTCCAAATCTTTGAGCCGCC-3′) primers, and forward (5′-TCCGAGATGCCGGCGCGTACC-3′) and reverse (5′-CTCTTTTCCAAATCTTTGAGCCGCC-3′) primers for total DNMT1. The *GAPDH* gene was used as an internal control, and forward (5′-GAAGGTGAAGGTCGGAGT-3′) and reverse (5′-CATGGGTGGAATCATATATTGGA-3′) primers were used to amplify it. Real-time PCR was performed after denaturation at 95 °C for 3 minutes, followed by 40 cycles of 15 seconds at 95 °C, 30 seconds at 58 °C, and 30 seconds at 72 °C. For quantification of mRNA levels, each sample was run in duplicate, and the average of the duplicate measurements was used to calculate the gene's mRNA expression. Relative mRNA levels were calculated according to the 2 -(ΔCt) approach.

Statistical Analysis

Descriptive statistics are provided as frequencies and percentages for categorical variables, and as mean ± standard deviation or median (min-max) for continuous variables. The normality of distributions was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. For normally distributed continuous variables, comparisons between two groups were made using the Student's t-test, while the Mann-Whitney U test was used for non-normally distributed variables. Dependent groups were analyzed using the Friedman test because the data were not normally distributed.

GFR measurements in treated patients at baseline (T0), 3 months (T1), 6 months (T2), and 12 months (T3) were analyzed longitudinally using a linear mixed-effects model with a random intercept for each patient. Time was entered as a categorical fixed effect with T0 as the reference level. The final model included time, antiviral agent (TDF vs. ETV), age, sex, prior diabetes mellitus, and prior hypertension as fixed effects. To explore potential drug-specific renal trajectories, a time-by-drug interaction was initially tested; however, because this term did not improve model fit, the simpler model without the interaction was retained based on fit indices, including Akaike's information criterion. Regression coefficients, standard errors, p-values, and 95% confidence intervals (CIs) were reported.

The distribution of DNMT1 values was tested for normality using the Shapiro-Wilk test because the sample size was small. Since the data did not conform to a normal distribution, the difference between the two groups was analyzed using the Mann-Whitney U test.

All analyses were performed in R version 4.5.0. Data import and management were conducted using the readxl, dplyr, and tidyr packages; standard statistical tests were performed using base R/stats; linear mixed-effects models were fitted using lme4 and lmerTest; adjusted marginal means and contrasts were obtained using emmeans; and figures were generated using ggplot2 and patchwork. A two-sided p-value of <0.05 was considered statistically significant.

Results

Data from 158 patients were analyzed for nephrotoxicity. The mean age of the patients was 49.25±12.9 years. Of these, 71 (45%) were female. The "under treatment" group included 91 (57.6%) patients, while the "treatment-naïve" group included 67 (42.4%) patients. Patient characteristics by treatment status are presented in Table 1. The duration of treatment varied between 12 and 164 months. Age, HBV-DNA, basal ALT, and AST levels were significantly higher in the treatment group. Basal phosphorus level was lower in the treatment group than in the treatment-naïve group (p=0.004). No significant differences were observed between the two groups in basal urea, creatinine, and GFR. HBeAg was positive in 16.5% of the treatment group and 1.5% of the treatment-naïve group.

In the treatment group, renal parameters were analyzed longitudinally by comparing baseline (T0) values with follow-up measurements (T1, T2, and T3). Phosphorus, urea, and creatinine levels remained stable over time, whereas GFR demonstrated a statistically significant decline (p=0.012) (Figure 1). Longitudinal changes in renal function parameters across follow-up visits are summarized in Table 2.

When the treatment group was analyzed according to the therapeutic agent, 74 (81.3%) were receiving TDF and 17 (18.7%) were receiving ETV. No significant difference was found when baseline and control values of renal parameters were compared by treatment type. However, basal urea values were higher in the ETV group than in the TDF group (p=0.039 and p=0.014); this difference disappeared over time (Table 3).

Table 1. Characteristics of the study group by the under treatment or not

Variables	Treatment-naïve (n=67)	Under the treatment (n=91)	p-value	X^2	z	t
Age (years)	46.0 (19-68)	53.0 (24-76)	**0.003**		-2.958	
Sex			**0.011**	6.524		
Female	38 (56.7)	33 (36.3)				
Male	29 (43.3)	58 (63.7)				
Pre-existing* diabetes	9 (42.9)	12 (57.1)	0.964	0.002		
Pre-existing* primary hypertension	20 (66.7)	10 (33.3)	**0.003**	8.925		
Duration of the treatment (months)	NA	76.1±40.6 (12-164)	NA			
HBV-DNA	958 (10-170000000)	590000 (0-224766065)	**<0.001**		-6.850	
HbeAg			**0.005**	7.953		
Negative	66 (98.5)	76 (83.5)				
Positive	1 (1.5)	15 (16.5)				
Anti-HBe			**0.005**	7.953		
Negative	1 (1.5)	15 (16.5)				
Positive	66 (98.5)	76 (83.5)				
ALT	20.0 (12-166)	31.0 (10-646)	**<0.001**		-4.674	
AST	21.0 (9-105)	29.0 (13-656)	**<0.001**		-4.405	
P at the baseline	3.4±0.6	3.1±0.6	**0.004**			2.909
Urea at the baseline	25 (12-64)	27 (11-53)	0.092		-1.683	
Creatinine at the baseline	0.75 (0.52-1.73)	0.77 (0.53-1.07)	0.058		-1.899	
GFR at the baseline	104.7 (30.7-130.9)	102.3 (71.0-136.7)	0.329		-0.976	

*: Prior to treatment initiation for NA treated patients or prior to hepatitis B diagnosis for treatment-naïve group. HBV: Hepatitis B virus, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, P: Phosphorus, GFR: Glomerular filtration rate, NA: Nucleos(t)ide analogues

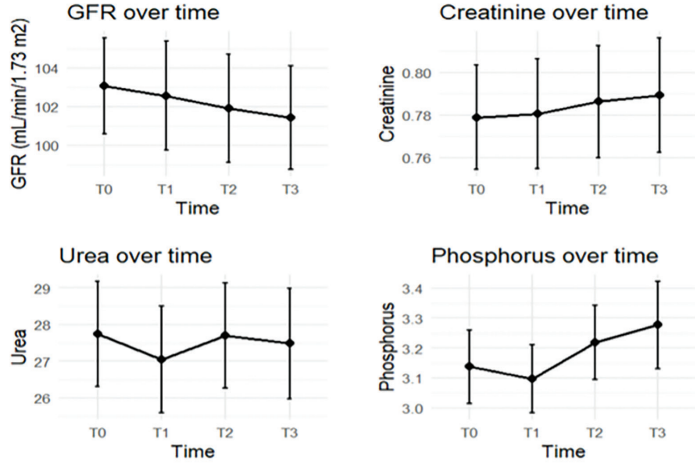

Figure 1. Descriptive trends in renal function parameters over time in nucleos(t)ide analogues-treated patients. Mean values with 95% confidence intervals are shown for glomerular filtration rate (GFR), creatinine, urea, and phosphorus at T0, T1, T2, and T3

Table 2. Longitudinal changes in renal function parameters across follow-up visits in treated patients (n=91)

Parameters	Baseline	T1	T2	T3	p-value	X^2
Phosphorus	3.1 (1.9-4.7)	3.1 (1.9-4.4)	3.2 (1.6-4.8)	3.2 (1.3-5.6)	0.076	6.877
Urea	27 (11-53)	26 (5-51)	27 (11-50)	27 (14-49)	0.909	0.544
Creatinine	0.77 (0.53-1.07)	0.79 (0.46)	0.79 (0.53-1.16)	0.79 (0.56-1.19)	0.551	2.103
GFR	102.3 (71.0-136.7)	102.0 (65.0-139.6)	102.8 (64.4-134.0)	102.1 (62.0-127.6)	**0.012***	10.907

*: The difference due to the third time point (12th month, T3), for all post-hoc analysis with Wilcoxon signed-rank p>0.05 except for between the baseline (T0) and T3 with p=0.012. GFR: Glomerular filtration rate

Table 3. Laboratory parameters by therapeutic agents

Parameters	TDF (n=74)	ETV (n=17)	p-value	t	z
P at baseline	3.16±0.63	3.06±0.40	0.430	0.798	
P at T1	3.1 (1.9-4.4)	3.1 (2.2-4.1)	0.580		-0.553
P at T2	3.2 (1.6-4.8)	3.1 (2.3-3.7)	0.520		-0.643
P at T3	3.27±0.74	3.31±0.47	0.648	-0.456	
Urea at baseline	27 (11-53)	34 (18-42)	**0.039**		-2.065
Urea at T1	26 (5-44)	29 (23-51)	**0.014**		-2.452
Urea at T2	27 (11-50)	30 (17-43)	0.130		-1.515
Urea at T3	27.0±7.5	29.4±6.0	0.239	-1.185	
Creatinine at baseline	0.77±0.11	0.82±0.14	0.116	-1.587	
Creatinine at T1	0.77±0.13	0.81±0.13	0.286	-1.074	
Creatinine at T2	0.79±0.13	0.78±0.15	0.858	0.180	
Creatinine at T3	0.78 (0.56-1.19)	0.80 (0.56-1.03)	0.558		-0.586
GFR at baseline	103.8±12.2	100.2±11.9	0.273	1.103	
GFR at T1	103.1±13.8	100.4±13.6	0.478	0.712	
GFR at T2	101.8±13.7	102.2±14.4	0.931	-0.087	
GFR at T3	101.6±12.8	100.8±14.9	0.830	0.216	

P: Phosphorus, GFR: Glomerular filtration rate, TDF: Tenofovir disoproxil fumarate, ETV: Entecavir

In the adjusted linear mixed-effects model that included time, antiviral agent, age, sex, prior diabetes mellitus, and prior hypertension, GFR at 12 months was significantly lower than at baseline (β=-1.65 mL/min/1.73 m^2, 95% CI: -3.13 to -0.16; p=0.030). The differences at 3 months (β=-0.52, p=0.495) and 6 months (β=-1.18, p=0.121) were not statistically significant. Increasing age was independently associated with lower GFR (β=-0.75 per year, 95% CI: -0.87 to -0.62; p<0.001). No statistically significant associations were observed for antiviral agent, sex, prior diabetes, or prior hypertension (Table 4).

Renal end points defined by Buti et al. (11) were found in three patients. When these patients were analyzed in detail, the first case was a 70-year-old woman who had been receiving TDF for 102 months and had been diagnosed with hypertension and osteoporosis. Increased creatinine and decreased GFR were detected. Case 2 was a 59-year-old male patient who developed increased creatinine and proteinuria in the 107th month of TDF treatment. Hypophosphatemia was detected in the test results of the 3rd patient, a 62-year-old male with diabetes, hypertension, and coronary artery disease. He was followed up by the nephrology service and was diagnosed with chronic kidney disease. He had been receiving TDF for 42 months. Treatment was changed to TAF in these three patients.

DNMT1 gene expression in RNA samples extracted from the PBMCs of the eight CHB patients treated with NA, compared with the 17 treatment-naïve patients, was analyzed by real-time PCR assay (Figure 2). Ten (40%) of the patients were female, 15 (60%) were male, and the mean age was 51+14.37 years. In epigenetic analysis, median DNMT1 levels in the under treatment group were 1.1789 [interquartile range (IQR) 0.3202-1.5584)] and in the treatment-naïve group were 0.7565 (IQR 0.2740-1.3293). DNMT1 levels were similar between the two groups (p=0.466; z=-0.728) (Figure 3).

Table 4. Adjusted linear mixed-effects model for GFR during the first 12 months of NA therapy

Variable	β estimate	95% CI	p-value
Time: T1 (3 months) vs. T0	-0.52	-2.00 to 0.96	0.495
Time: T2 (6 months) vs. T0	-1.18	-2.66 to 0.31	0.121
Time: T3 (9 months) vs. T0	-1.65	-3.13 to -0.16	0.030
Drug: ETV vs. TDF	-0.42	-4.18 to 3.34	0.828
Age (per year)	-0.75	-0.87 to -0.62	<0.001
Sex: female vs. male	0.06	-3.01 to 3.12	0.972
Prior diabetes: yes vs. no	-1.49	-6.18 to 3.19	0.534
Prior hypertension: yes vs. no	-1.88	-7.30 to 3.53	0.497

Linear mixed-effects model with random intercept for patient ID. Reference categories: T0, TDF, male sex, no prior diabetes, and no prior hypertension. GFR: Glomerular filtration rate, TDF: Tenofovir disoproxil fumarate, ETV: Entecavir, CI: Confidence interval, NA: Nucleos(t)ide analogues

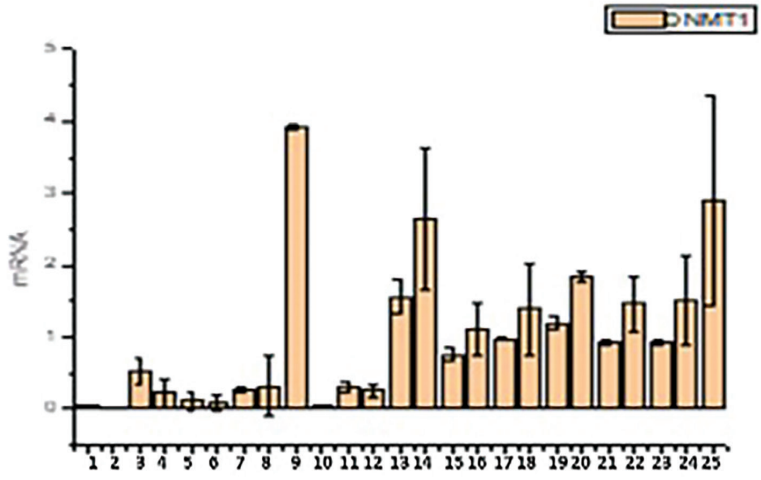

Figure 2. Schematic representation of DNMT1 values in samples subjected to epigenetic analysis
DNMT1: DNA methyltransferase 1

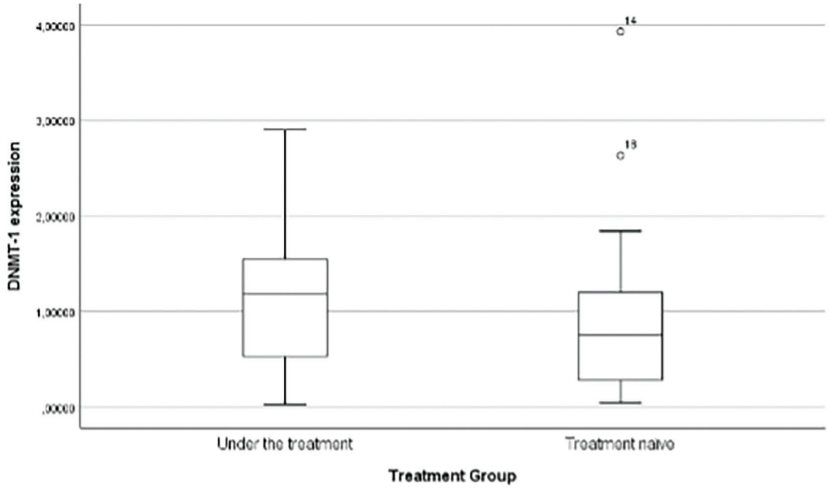

Figure 3. Comparison of DNMT1 values according to treatment status
DNMT1: DNA methyltransferase 1

Discussion

Of the two currently approved treatment classes for CHB—peg-IFN alpha and NAs—the NAs represent the cornerstone of therapy. They effectively suppress viral replication, improve liver inflammation, and reduce the risk of progression to cirrhosis and HCC. However, functional cure (sustained loss of HBsAg with undetectable HBV-DNA) is achieved in ≤1% of patients, necessitating long-term treatment. This is largely due to the persistence of covalently closed circular DNA and integrated HBV-DNA, which are not targeted by NAs. Although NAs have an excellent safety profile, long-term use may be associated with increased healthcare costs, drug non-adherence, and risk of renal and bone toxicity. Consequently, there exists a clear demand for innovative, brief therapeutic interventions that can result in a significant rate of functional enhancement (12,13). While oral administration offers advantages, NAs have limited immunomodulatory effects and do not adequately induce cell-mediated immunity (14).

In this study, we investigated whether nephrotoxicity, one of the possible side effects, developed in patients treated with NA. One hundred and fifty-eight patients with CHB whose follow-up values were recorded at 3-6 month intervals were analyzed. Data from 91 patients who received treatment with NA and from 67 treatment-naïve patients were compared. Our findings suggest a modest decline in renal function during the first 12 months of NA therapy. Although the follow-up period was relatively short, the magnitude of decline was small. Age emerged as the strongest independent predictor of lower GFR. Although no significant difference was detected between TDF and ETV, the comparison should be interpreted cautiously given the marked imbalance in sample sizes between treatment groups (TDF: 74 vs. ETV: 17).

Although deterioration in renal function after NA use was reported in similar studies, its potential effect on renal failure remains uncertain.

In a study involving 110 patients receiving TDF for 48-171 weeks, creatinine levels increased significantly at weeks 12, 24, 48, 72, and 96, and eGFR levels decreased significantly at these five time points. They found that being over 60 years of age and basal bilirubin values were independent risk factors for renal failure (15). Age-related decline in renal function is a well-recognized physiological process that may, in part, explain the observed longitudinal reduction in GFR. In our mixed-effects model, age was independently associated with GFR decline, suggesting that treatment-related effects should be interpreted in the context of baseline renal aging.

In a prospective study involving 105 patients, including 33 with cirrhosis and 72 without, GFR significantly decreased in the non-cirrhotic group during the first year of treatment with TDF ($p<0.001$). Fortunately, GFR partially recovered by week 96 without the need for interruption of treatment (16).

Among patients treated with NA, baseline and follow-up renal values did not differ significantly between the TDF and ETV groups. In a retrospective cohort study of 1917 patients receiving ETV and 1509 patients receiving TDF, TDF treatment was associated with a higher risk of renal dysfunction (17).

A retrospective study of 10,642 patients with CHB found that NA treatment—particularly TDF—was associated with a higher risk of chronic kidney disease progression compared with untreated patients. In contrast, ETV and TAF showed similar risks to untreated patients, with no difference between the two (18).

In a meta-analysis of randomized controlled trials of HBV patients treated with ETV, TDF and TAF, increases in creatinine and decreases in GFR were compared with respect to renal side effects. ETV and TAF had less effect on GFR than TDF, while ETV had less effect on an increase in creatinine than TAF and TDF. As a result, it was concluded that TDF negatively affected renal tissue compared to ETV and TAF (19).

In a retrospective study of 1061 untreated CHB patients and 366 patients receiving TAF, 190 besifovir dipivoxil maleate (BSV) and 2029 ETV, renal function decline for ≥3 consecutive months was assessed as the primary outcome. Compared with each matched untreated group, changes in estimated GFR over time were higher in the ETV group ($p=0.010$), although similar in the TAF ($p=0.073$) and BSV groups ($p=0.926$) (20). Consistent with these findings, TDF therapy was switched to TAF in three patients in our cohort who developed renal involvement during follow-up.

Real-world cohort and meta-analysis data have shown that there is no clinically significant difference in renal dysfunction between commonly used agents such as tenofovir and ETV, and that they have similar renal safety profiles. Differences in study design, patient populations, baseline renal risk, and follow-up duration must be taken into account when evaluating nephrotoxicity associated with NA.

Drugs can impair mitochondrial function through multiple mechanisms. Several antibiotics and antiviral agents have been shown to inhibit mitochondrial protein synthesis or interfere with mtDNA replication. Consequently, many drugs currently in clinical use may cause organ toxicity, at least in part, through mitochondrial disruption (21). In particular, nucleoside reverse transcriptase inhibitors used in HIV treatment have been associated with mitochondrial toxicity due to inhibition of mtDNA polymerase-γ, leading to impaired mtDNA replication and mitochondrial dysfunction (22). Although tenofovir and ETV appear to have relatively low mitochondrial toxicity compared with earlier agents, experimental and clinical studies suggest that mitochondrial alterations may still occur, particularly in renal proximal tubular cells (9,23-25).

DNMT1 has been implicated in HBV-related hepatocarcinogenesis, and increased DNMT1 expression has been associated with HCC in previous studies (26,27). However, epigenetic alterations potentially related to NA therapy in patients with CHB have not been extensively investigated.

The median DNMT1 level was 1.1789 (IQR: 0.3202-1.5584) in the treatment group and 0.7565 (IQR: 0.2740-1.3293) in the treatment-naïve group; there was no statistically significant difference between

the groups. In contrast, Madeddu et al. (9) reported significantly higher DNMT1 expression in NA-treated patients compared with untreated patients [HBV-naïve: 0.61 (IQR 0.34-0.82); NA-treated: 4.09 (IQR 3.52-5.15); p<0.00001], and DNMT1 expression was also associated with treatment duration.

The absence of a significant difference in DNMT1 expression between treated and treatment-naïve patients in our study may have several biological explanations. MtDNA methylation is a dynamic and context-dependent process that may be influenced by mitochondrial stress responses rather than direct drug exposure alone. Compensatory mechanisms may therefore maintain mitochondrial epigenetic homeostasis despite prolonged NA therapy. Furthermore, DNMT1 expression measured in PBMCs may not fully reflect mitochondrial epigenetic alterations occurring in target tissues such as renal tubular cells or hepatocytes.

Another possible explanation is treatment heterogeneity. TDF and ETV differ in their effects on mtDNA polymerase-γ and mitochondrial function. Due to the limited number of ETV-treated patients in our cohort, drug-specific effects could not be evaluated separately, and pooling the treatments may have attenuated detectable epigenetic differences. In addition, the epigenetic component of this study was designed as a pilot exploratory analysis with a small sample size, which limited statistical power and increased the risk of type II error.

Taken together, these findings should be interpreted cautiously and primarily as hypothesis-generating. Larger prospective studies incorporating tissue-specific analyses and drug-stratified designs are needed to clarify the potential relationship between long-term antiviral therapy and mitochondrial epigenetic regulation. Nevertheless, these preliminary findings contribute to the limited body of clinical data exploring mitochondrial epigenetic modulation in patients with CHB receiving long-term NA therapy.

Study Limitations

This study has several limitations. The relatively small sample size may have reduced statistical power and limited the generalizability of the findings. Furthermore, the retrospective design restricted the availability of detailed clinical and laboratory data.

The epigenetic analysis was conducted in a limited subgroup of patients and restricted to DNMT1 expression due to practical limitations. The inability to assess additional mitochondrial epigenetic regulators limits mechanistic interpretation. Nevertheless, this study provides preliminary insights into the potential epigenetic effects of NA therapy and may serve as a foundation for future studies.

Conclusion

Long-term NA therapy in patients with CHB was associated with modest longitudinal changes in renal function. While these findings are consistent with previous reports suggesting potential nephrotoxic effects of antiviral therapy, the observed decline in renal function may also partially reflect age-related physiological changes. Therefore, careful assessment of baseline renal function and regular monitoring during therapy remain important in clinical practice. The exploratory evaluation of DNMT1 expression did not reveal significant differences between treated and untreated patients, and therefore does provide direct evidence for mitochondrial epigenetic alterations associated with therapy. Nevertheless, these findings contribute to the limited clinical data exploring mitochondrial epigenetic regulation in the context of long-term antiviral treatment. Larger prospective studies are needed to clarify the potential relationship between antiviral therapy, mitochondrial function, and renal outcomes.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Aydın Adnan Menderes University Clinical Research Ethics Committee (approval no: 2019/07, date: 11/06/2020).

Informed Consent: Informed consent was obtained from the patients included in the pilot epigenetic part of the study.

Footnotes

Authorship Contributions

Concept: G.U.G., S.K., M.K.E., Design: G.U.G., S.K., M.K.E., H.Ö., Data Collection or Processing: G.U.G., C.A., M.K.E., Analysis or Interpretation: G.U.G., S.K., H.Ö., Literature Search: G.U.G., S.K., C.A., Writing: G.U.G., S.K., H.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This research was supported by Aydın Adnan Menderes University Research Fund (project number: TPF-19038).

References

1. World Health Organization. Global hepatitis report 2024: action for access in low- and middle-income countries [Internet]. Geneva: World Health Organization; 2024. Available from: https://www.who.int/publications/i/item/9789240091672
2. Demirtürk N, Köse A, Ural O, Asan A, Barut Ş, Sümer Ş, Şimşek F, Türker N. Management of chronic hepatitis B infection: a consensus report of the Study Group for Viral Hepatitis of the Turkish Society of Clinical Microbiology and Infectious Diseases-2023 update. Klimik Derg. 2023;36(Suppl 1):1-22.
3. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol. 2017;67:370-398.
4. Di Dato F, Iorio R. Expanding indications for chronic hepatitis B treatment: is it really desirable to treat everyone? World J Gastroenterol. 2024;30:2294-2297.
5. Kayaaslan B, Guner R. Adverse effects of oral antiviral therapy in chronic hepatitis B. World J Hepatol. 2017;9:227-241.
6. Fung J, Seto WK, Lai CL, Yuen MF. Extrahepatic effects of nucleoside and nucleotide analogues in chronic hepatitis B treatment. J Gastroenterol Hepatol. 2014;29:428-434.
7. Ghosh S, Sengupta S, Scaria V. Comparative analysis of human mitochondrial methylomes shows distinct patterns of epigenetic regulation in mitochondria. Mitochondrion. 2014;18:58-62.
8. Shock LS, Thakkar PV, Peterson EJ, Moran RG, Taylor SM. DNA methyltransferase 1, cytosine methylation, and cytosine hydroxymethylation in mammalian mitochondria. Proc Natl Acad Sci U S A. 2011;108:3630-5.
9. Madeddu G, Ortu S, Garrucciu G, Maida I, Melis M, Muredda AA, Mura MS, Babudieri S. DNMT1 modulation in chronic hepatitis B patients and hypothetic influence on mitochondrial DNA methylation status during long-term nucleo(t)side analogs therapy. J Med Virol. 2017;89:1208-1214.

10. Mohan J, Ghazi T, Chuturgoon AA. A critical review of the biochemical mechanisms and epigenetic modifications in HIV- and antiretroviral-induced metabolic syndrome. Int J Mol Sci. 2021;22:12020.
11. Buti M, Tsai N, Petersen J, Flisiak R, Gurel S, Krastev Z, Aguilar Schall R, Flaherty JF, Martins EB, Charuworn P, Kitrinos KM, Subramanian GM, Gane E, Marcellin P. Seven-year efficacy and safety of treatment with tenofovir disoproxil fumarate for chronic hepatitis B virus infection. Dig Dis Sci. 2015;60:1457-1464.
12. Gopalakrishna H, Ghany MG. Perspective on emerging therapies to achieve functional cure of chronic hepatitis B. Curr Hepatol Rep. 2024;23:241-252.
13. Kim SW, Yoon JS, Lee M, Cho Y. Toward a complete cure for chronic hepatitis B: novel therapeutic targets for hepatitis B virus. Clin Mol Hepatol. 2022;28:17-30.
14. Akbar SMF, Al Mahtab M, Yoshida O, Aguilar J, Gerardo GN, Hiasa Y. Development of therapy based on the exploration of biological events underlying the pathogenetic mechanisms of chronic hepatitis B infection. Biomedicines. 2023;11:1944.
15. Jung WJ, Jang JY, Park WY, Jeong SW, Lee HJ, Park SJ, Lee SH, Kim SG, Cha SW, Kim YS, Cho YD, Kim HS, Kim BS, Park S, Baymbajav B. Effect of tenofovir on renal function in patients with chronic hepatitis B. Medicine (Baltimore). 2018;97:e9756.
16. Zheng S, Liu L, Lu J, Zhang X, Shen H, Zhang H, Xue Y, Lin L. Efficacy and safety of tenofovir disoproxil fumarate in Chinese patients with chronic hepatitis B virus infection: a 2-year prospective study. Medicine (Baltimore). 2019;98:e17590.
17. Jung CY, Kim HW, Ahn SH, Kim SU, Kim BS. Tenofovir is associated with higher risk of kidney function decline than entecavir in patients with chronic hepatitis B. Clin Gastroenterol Hepatol. 2022;20:956-958.e2.
18. Hong H, Cho M, Lim C, Choi WM, Lee D, Shim JH, Kim KM, Lim YS, Lee HC, Choi J. Longitudinal changes in renal function in patients with chronic hepatitis B on antiviral treatment. Aliment Pharmacol Ther. 2024;59:515-525.
19. Liu Z, Zhao Z, Ma X, Liu S, Xin Y. Renal and bone side effects of long-term use of entecavir, tenofovir disoproxil fumarate, and tenofovir alafenamide fumarate in patients with hepatitis B: a network meta-analysis. BMC Gastroenterol. 2023;23:384.
20. Lee JS, Jung CY, Lee JI, Ahn SH, Kim BS, Kim SU. Comparison of decline in renal function between patients with chronic hepatitis B with or without antiviral therapy. Aliment Pharmacol Ther. 2023;58:99-109.
21. Will Y, Dykens J. Mitochondrial toxicity assessment in industry--a decade of technology development and insight. Expert Opin Drug Metab Toxicol. 2014;10:1061-1067.
22. Fromenty B. Alteration of mitochondrial DNA homeostasis in drug-induced liver injury. Food Chem Toxicol. 2020;135:110916.
23. Birkus G, Hitchcock MJ, Cihlar T. Assessment of mitochondrial toxicity in human cells treated with tenofovir: comparison with other nucleoside reverse transcriptase inhibitors. Antimicrob Agents Chemother. 2002;46:716-723.
24. Venhoff N, Setzer B, Melkaoui K, Walker UA. Mitochondrial toxicity of tenofovir, emtricitabine and abacavir alone and in combination with additional nucleoside reverse transcriptase inhibitors. Antivir Ther. 2007;12:1075-1085.
25. Lebrecht D, Venhoff AC, Kirschner J, Wiech T, Venhoff N, Walker UA. Mitochondrial tubulopathy in tenofovir disoproxil fumarate-treated rats. J Acquir Immune Defic Syndr. 2009;51:258-263.
26. Stella L, Santopaolo F, Gasbarrini A, Pompili M, Ponziani FR. Viral hepatitis and hepatocellular carcinoma: from molecular pathways to the role of clinical surveillance and antiviral treatment. World J Gastroenterol. 2022;28:2251-2281.
27. Zeisel MB, Guerrieri F, Levrero M. Host epigenetic alterations and hepatitis B virus-associated hepatocellular carcinoma. J Clin Med. 2021;10:1715.