ORIGINAL ARTICLE

Tenofovir therapy in chronic hepatitis B infection: 48-week results from Izmir Province, Turkey

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ABSTRACT

Objectives: The goal of therapy in chronic hepatitis B infection (CHB) is to impede liver injury by suppressing viral replication. The study was aimed to determine the efficacy of tenofovir (TDF) in CHB infection for 48 weeks.

Materials and methods: We retrospectively analyzed the data of 45 CHB patients treated by tenofovir. The patients were divided into two groups based on their hepatitis B e antigen status (HBeAg). Those who were eligible to therapy received TDF 300 mg once daily for 48 weeks. Serum alanine aminotransferase levels (ALT), hepatitis B virus DNA (HBV DNA), and viral serological markers were checked at three-month intervals. Liver biopsy scores were determined in all patients.

Results: The mean age ± standard deviation (SD) was 35.8 ± 17.0 years, 26 (57.8 %) were male, and seven patients (15.5%) were treatment-experienced by a nucleos(t)ide analogue before TDF. HBeAg was positive in 17 (37.8%) patients. At week 48 among HBeAg positive (HBeAg +) patients' biochemical and virological response rates at month-3, -6 and -12 were 64.7%, and 100%, 70.6%, and 94.1%, and 88.2%, and 64.7%, respectively. The serological response in HBeAg + patients was 29.4%. For HBeAg negative (HBeAg -) patients; biochemical, and virological response rates were 64.3%, and 96.4% at month 3; 82.1%, and 96.4% at month 6; and 100%, and 85.7% at month 12, respectively. At week 48 both groups had significant virological response (p<0.001).

Conclusion: Treatment in CHB with TDF leads to HBV DNA suppression without evident resistance for 48-week, and is well tolerated. *J Microbiol Infect Dis 2012; 2(3): 87-92*

Key words: Hepatitis B, chronic, tenofovir disoproxil

Kronik hepatit B infeksiyonunda tenofovir tedavisi: İzmir bölgesinden 48 haftalık sonuçlar

ÖZET

Amaç: Kronik hepatit B (KHB) infeksiyonunda hedef viral replikasyonu baskılayarak karaciğer hasarının engellenmesidir. Bu çalışmada KHB infeksiyonunda 48 hafta süreyle tenofovir (TDF) tedavisinin etkinliğini belirlemek amaçlanmıştır.

Gereç ve yöntem: Tenofovir ile tedavi edilen 45 KHB hastasının verileri retrospektif olarak incelendi. HBeAg durumlarına göre hastalar iki gruba ayrıldı. Tedaviye uygun olan hastalara 48 hafta süreyle 300 mg/gün TDF verildi. 3 ay aralarla serum alanin aminotransferaz (ALT), hepatit B viral DNA (HBV DNA) ve viral serolojik belirteçleri istendi. Karaciğer biyopsi skorları belirlendi.

Bulgular: Hastaların ortalama yaşı ± SD 35,8 ± 17 yıl idi ve 26'sı (%57,8) erkekti. Yedi hasta daha önce bir nükleos(t)id analoğu ile tedavi almıştı. 17 hastada (%37,8) HBeAg pozitifti. 48. haftada HBeAg pozitif (HBeAg +) olgularda 3, 6 ve 12. aylarda biyokimyasal ve virolojik yanıt oranları sırasıyla %64,7 ve %100, %70,6 ve %94,1 ile %88,2 ve %64,7 idi. Serolojik yanıt oranı ise %29,4 idi. HBeAg negatif (HBeAg -) olgular için 3. ayda biyokimyasal ve virolojik yanıt oranları %64,3 ve %96,4, 6. ayda %82,1 ve %96,4 ve 12. ayda %100 ve %85,7 idi. 48. haftada her iki grupta anlamlı virolojik yanıt saptandı (p<0,001).

Sonuç: KHB infeksiyonunda TDF tedavisi 48 hafta içinde direnç gözlenmeden HBV DNA baskılanması sağlayabilmekte ve iyi tolere edilmektedir.

Anahtar kelimeler: Hepatit B, kronik, tenofovir disoproxil

INTRODUCTION

Chronic hepatitis B virus (CHB) infection is a serious global public health problem associated with cirrhosis, liver failure, and hepatocellular carcinoma (HCC). The goal of CHB therapy is to decrease the risk of complications such as cirrhosis and hepatocellular carcinoma by potent and durable suppression of viral replication. Several major advances in the treatment of CHB have been made over the last 15 years.[1] The patients with medium to advanced liver disease and with HBV DNA >2000 IU/ml should be treated. Cirrhotic patients with detectable HBV DNA can be treated. All patients with treatment indications can be treated by oral antivirals.[2] Major determinants of long-term therapy necessitate that a chosen-drug should have acceptable tolerability, minimal toxicity, potent activity, a compliant dosing regimen, and minimal selection for resistance.[3]

Recent studies have suggested that the HBV DNA level is directly correlated with the risk of disease progression independently of the HBeAg status and serum ALT level. The HBV DNA cutoff value for discriminating patients with HBeAg - CHB from inactive carriers has recently been recommended to be 2000 IU/mL. However, the recent study has suggested that the patient should not be classified as "inactive" based on a single reading of <2000 IU/mL, since HBV DNA levels can fluctuate to lower than 2000 IU/mL in patients with HBeAg - CHB. It has been shown that with a cutoff value of 200 IU/mL HBV reactivation within one year can be predicted with a sensitivity of 85.4%, and a specificity of 69.5%.[4-6]

Drugs recommended for treatment of patients with CHB can be divided into two main groups based on their mechanism of action, namely immunomodulatory drugs like alpha interferons, and antiviral drugs including lamivudine (LAM), telbivudine (LdT), entecavir (ETV), adefovir (ADV), and tenofovir.[2,7,8]

Tenofovir disoproxil fumarate (TDF) is a prodrug of tenofovir that has been approved for treatment of HIV-1 infection since 2001 and was approved for treatment of chronic HBV infection in 2008. The prodrug is hydrolyzed to tenofovir, and then phosphorylated by cellular enzymes to form tenofovir diphosphate, which competitively inhibits HIV-1 and HBV polymerases, and after incorporation into DNA, acts as a chain terminator. Oral antiviral therapies in long-term eventu-

ally leads to some level of resistance: up to 70% after 4 years with LAM; up to 29% in HBeAg - patients after 5 years with ADV; 25.1% and 10.8% in HBeAg + and HBeAg - patients, respectively, after 2 years with LdT; and 1.2% after 5 years with ETV. TDF has been shown to be effective in patients with a LAM-resistant virus, and no resistant mutations after 48 weeks of therapy.[7] The purpose of this study was to investigate the response rates during 48 weeks of therapy with TDF in CHB infection.

MATERIALS AND METHODS

This study was conducted in concordance with the ethical guidelines of the 1975 Helsinki Declaration, and was approved by Tepecik Research and Training Hospital Ethical Committee.

The study enrolled patients mono-infected with HBV who had HBeAg - or HBeAg + CHB with compensated liver disease and pretreatment Knodell histological activity index (HAI) of 5 or more (on a scale of 0 to 18, with higher scores indicating more severe chronic hepatitis). The treatment-eligible patients, i.e. the patients with evidence of viral replication, either HBeAg + with a nonamplified HBV DNA values >104 copies/mL, or HBeAg - with HBV DNA > 2000 IU/mL (~345 copies/mL) and serum ALT levels that were more than two times the upper limit of normal (ULN) or with evidence of moderate-to-severe activity on liver biopsy, received TDF (Viread, Gilead Sciences, USA) 300 mg once daily for 48 weeks. The data of the study patients were retrospectively analyzed.

Key exclusion criteria were co-infection with human immunodeficiency virus (HIV-1), hepatitis C or D virus, evidence of hepatocellular carcinoma, liver decompensation, prior liver transplantation, and concomitant renal failure.

Serum ALT levels, viral serological markers including hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs), HBeAg and antibodies to HBeAg (anti-HBe) were assessed at three-month intervals. HBV DNA levels were studied by real-time polymerase chain reaction (PCR). Patients underwent liver biopsies within one month of biochemical and serological test results. The statuses of CHB of the patients were blinded to both the laboratory team and pathologists. All specimens were assessed and scored

twice with interval of 2 weeks according to the Knodell HAI by a single experienced pathologist who was blinded to all clinical information and virological results.

Three of seven pre-treated patients with any of nucleos(t)ide analogues received LAM only, and remaining four received both LAM and ADV. None of the patients used any form of interferon (INF) before enrolling to the study. The resistance analysis to LAM and ADV was assessed using the multiplex PCR and reverse hybridization tools (INNO-LIPA HBV DR v2, Innogenetics, Belgium).

According to the criteria of the Turkish Association for the Study of the Liver we defined normalization of serum aminotransferases as biochemical response, and loss of HBeAg with or without anti-HBe as serologic response. Virological response was defined as more than 2 log10 IU/mL reduction in HBV DNA levels at the end of treatment.[13]

Data handling and analysis were performed with SPSS software for Windows, version 15.0 (SPSS Inc., Chicago, IL). The comparison of gender ratios between groups was determined by using Chi-square (χ^2) test, and p value <0.05 was considered significant. The comparisons of variables between groups and of percent changes of repeated measurements (DNA and ALT levels) against baseline values (expressed as median with minimum and maximum values) were analyzed by nonparametric Mann-Whitney test, and p value <0.05 was considered significant. Within groups the dependent variables (viral DNA loads, and ALT levels) measured with time intervals were compared by using nonparametric Wilcoxon Signed Ranks Test and p<0.05 was considered significant.

The measures made two times by the same pathologist were categorized according to given scores (i.e. ≤9 and >9). McNemar test was used to measure the consistency between two readings. The degree of agreement was determined by kappa statistic.

RESULTS

There were 26 males (57.8%) and mean age ± SD was 35.8 ± 17 years. Overall characteristics of the patients and the comparisons of variables between groups are given in Table 1. 38 patients (84.5%) did not receive any nucleos(t)ide therapy before (naive). HBeAg was positive in 17 patients (37.8%). Basal DNA loads did not significantly differ between groups (p=0.082), but both basal ALT levels and Knodel scores were significantly low in HBeAg - group than HBeAg + group, p values of less than 0.001.

Figure 1 and 2 shows the decrement of viral DNA burden and ALT levels at 3-month intervals, respectively. The change in the level of HBV DNA was characterized by a precipitous, statistically significant decrease at month 3 in both groups (p=0.001). Decrements of ALT levels were significantly significant compared to baseline value in both groups at month 3, and kept the significance throughout the follow-up period.

Knodell scores performed by the same pathologist was not significantly different (McNemar test, p=0.25), and two readings were significantly consistent (kappa=0.869, p<0.001). Five of seven patients treated previously with any nucleos(t)ide analogue other than TDF showed virological response at 12 month. We achieved virological response at 48 week in one patient with mutation of LAM-resistance. No major clinical side effects were reported during treatment with TDF.

Table 1. Characteristics and the comparison of basal parameters between groups

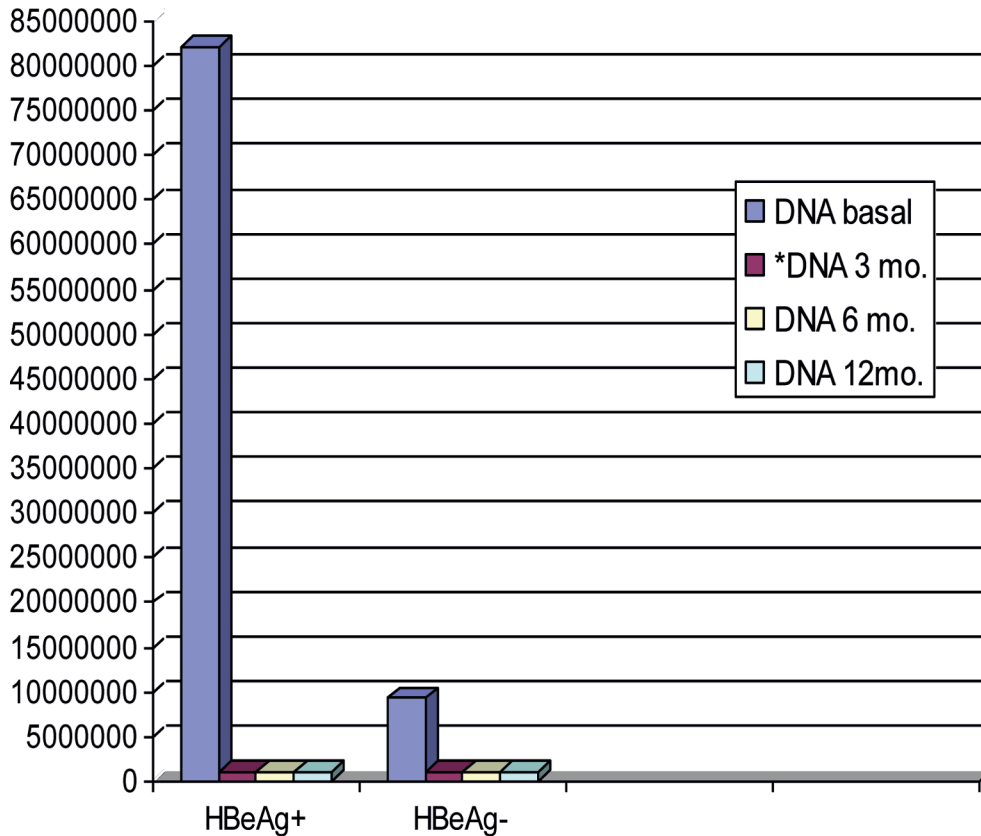| Variables	HBeAg +	HBeAg -	P value
Number of patients (%)	17 (37.8)	28 (62.2)	-
Mean age	36.4 ± 2	35.1 ± 1	0.082
Male (%)	8 (47.1)	18 (64.3)	0.043
No.of naive (%)	13 (76.5)	25 (89.3)	0.032
Mean ALT level (IU/mL)	137.2 ± 2	95.2 ± 2	0.001*
Median HBV DNA (copies/mL)	8.2×10^7	9.3×10^6	0.082
Median Knodell HAI	10	9	0.001*
No. of patients with resistance to LAM or ADV (%)	1 (5.9)	1 (3.6)	-

*Statistically significant.

Figure 1. Virological responses of both groups on consecutive 3-month intervals

*Statistically significant decrease was achieved at 3-month. There were no significant changes between intervals after that period.
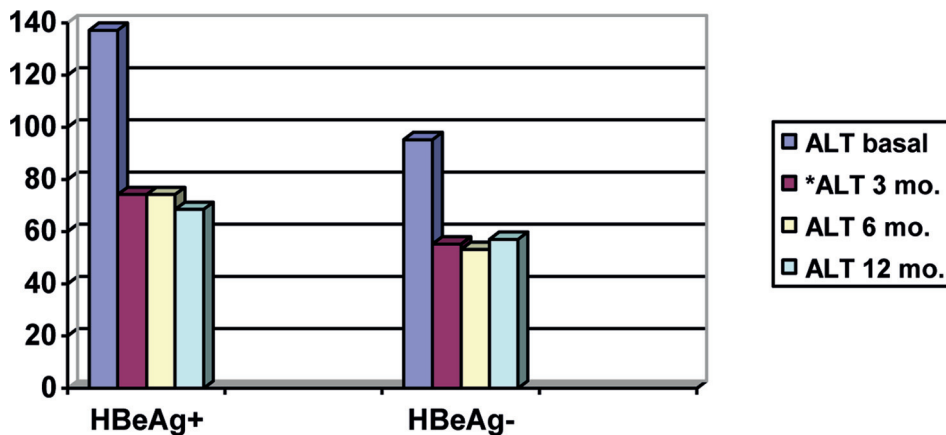

Figure 2. ALT decrease after TDF treatment in both groups

*ALT levels were significantly decreased at month-3 in both groups, and kept significance thereafter.

DISCUSSION

In this study TDF was shown to be effective as early as the third month of the beginning of treatment in CHB patients. Hepatitis B virus (HBV) infection is a global public health problem with estimated nearly 400 million HBV carriers in the world, of whom approximately one million die annually from related liver disease The prevalence of CHB estimated from blood donor studies in our country has decreased from 5.2% to 2.97%.[1,2] The clinical spectrum varies between

individuals depending on viral factors and immunological and genetic factors of the host. In the study conducted in our country by Kandemir et al, individuals with HLA-DRB1*14-*15 and HLA-DQB1*01 genotypes were more likely to develop CHB infection.[3] The prevalence of CHB infection in Western Europe in cross-sectional studies has been shown to be 0.3-1.5%, whereas in some regions of Asia and Africa it reaches 9-12% of the population.[4]

Recent studies have suggested that the HBV DNA level is directly correlated with the risk of disease progression independently of the HBeAg status and serum ALT level Akhan et al in their study determined the association between serum levels of biochemical markers and histological findings of CHB patients. They grouped the patients according to the scoring system for chronic hepatitis modified from Scheuer. The higher HBV DNA levels were associated with the higher degree of fibrosis (group 2) in CHB patients. The ALT levels were higher in both groups, and the level was significantly higher in the group.[2,5,6] Our study, in accordance with the forementioned study, showed that the mean ALT level was significantly higher in HBeAg + patients, and correlated with significantly higher histological score as well. Although HBV DNA level was also higher in HBeAg + group, the difference was not found to be significant (p=0.082).

The HBV DNA cutoff value for discriminating patients with HBeAg - CHB from inactive carriers has recently been recommended to be 2000 IU/mL.[5,7] It has been suggested in the study by Kim et al that with a cutoff value of 200 IU/mL HBV reactivation within one year can be predicted with a sensitivity of 85.4%, and a specificity of 69.5%.[8] Multiple reports have shown that maintenance of viral suppression is a key determinant of therapeutic outcomes for patients with CHB, and that a sustained HBV DNA response was correlated with serologic, histologic, or biochemical responses.[7,9]

We need prolonged viral suppression with long-term treatment as covalently closed circular DNA of the virus presumably persist lifetime within infected hepatocytes.[10] Treatment strategies for CHB include interferon, LAM, ADF, telbivudine, ETV, and TDF. TDF, a new nucleoside analogue licensed in 2008 for the treatment of HBV infections in Europe and the United States,

has been shown to be both efficacious and safe.[11] It may be used as first-line therapy in treatment-naïve patients, and has more potent antiviral activity than ADF and also suppresses wild-type as well as LAM-resistant HBV.[12]

In our study, in patients with compensated CHB monoinfected with HBV, TDF was effective in both HBeAg + and HBeAg - groups at week 48. We did not observe significant loss of HBsAg or seroconversion at week 48. This might be explained by development of resistance and relatively short duration of treatment. Therefore, long-term treatments with oral therapies to maintain viral suppression, and also potent therapies that offer barrier against development of resistance are desirable, since antiviral resistance and poor adherence are main risk factors for treatment failure and subsequent reversal of improvement.

The development of resistant strains of HBV is another major issue related to efficacy of drug. The clinical relevance of certain mutations conferring decreased susceptibility to TDF is dubious, although evidence for cross-resistance with adefovir-resistant mutations has been observed.[13,14] Currently, there have been no reports of virological resistance to TDF among HBV- monoinfected patients, and even resistance to TDF is rare after up to four years of treatment.[4] It has been recommended as an additional drug after failure of previous nucleos(t)ide analogues.[15]

In our study TDF resistance was not detected, and no significant side-effects were encountered. Major limitation of this study was the lack of post-treatment liver biopsies to follow up the level of histological response.

In concludion, this 48 week study revealed that TDF was effective for the treatment of both HBeAg - and HBeAg + CHB patients, and for the patients who had previously treated with LAM. It had favorable long-term safety without significant side effect profile.

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