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The efficacy and safety of tenofovir in the prevention of Hepatitis B virus recurrence following liver transplantation

LIVER

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Original Article

ABSTRACT

Background/Aims: In this study, a tenofovir disoproxil fumarate (TDF) + hepatitis B immunoglobulin (HBIG) regimen was compared with lamivudine (LAM) + HBIG to determine the efficacy and safety of TDF in the prevention of hepatitis B virus (HBV) recurrence following liver transplantation (LT).

Materials and Methods: Thirty-six patients, 18 treated with TDF+HBIG (TDF group) and 18 with LAM+HBIG (LAM group), were evaluated retrospectively over a median 36-month follow-up in the Liver Transplantation Outpatient Unit of Dokuz Eylül University after having an LT. In the TDF group, TDF treatment was initiated in six patients due to resistance to LAM, in one patient due to relapse, in three patients to prevent relapse, and in eight patients due to *de novo* hepatitis. In the LAM group, LAM therapy was initiated in two patients due to de novo hepatitis and in 16 patients to prevent relapse.

Results: In the TDF group, an increase of greater than 0.5 mg/dL in creatinine values was observed in two patients. In the LAM group, creatinine values did not increase to greater than 0.5 mg/dL. No cases of acute renal failure associated with TDF or LAM, mild or serious adverse events, or HBV recurrence were observed among the patients. Glomerular filtration rates (GFRs) of these patients were calculated with a modification of renal disease (MDRD) formulation. There was no significant difference ($p<0.05$) in the GFRs between the two groups.

Conclusion: The results of this study, after a 36-month follow-up period, were encouraging and demonstrated that TDF therapy is safe and efficacious in treating HBV-positive organ transplant patients. However, patients should be monitored carefully in terms of renal function. Given the limited experience with TDR in LT, this study is of importance due to its long follow-up period.

Keywords: Tenofovir, lamivudine, liver transplantation, efficacy, safety

INTRODUCTION

Significant improvements in the survival of grafts and patients who received hepatitis B virus (HBV) liver transplantation (LTs) have been made in the past 20 years. In the early 1990s, the use of hepatitis B immunoglobulin (HBIG) significantly reduced recurrence rates (1). In the late 1990s, the use of lamivudine (LAM) contributed to improved outcomes for transplant patients with HBV (2). However, prolonged LAM use has been associated with the highest resistance rates: the HBV resistance rate for a period of one year is nearly 10% and for a period of three years is as high as 50% (3). In recent years, combined use of HBIG and antivirals (LAM or adefovir) has decreased the rates of recurrence after LT. Although the efficacy of these combinations has been shown to be superior to the efficacy of HBIG or nucleotide analogues (NUCs) alone, there is still controversy about the optimal protocol (4,5).

Therefore, a more effective nucleotide analogue with a lower resistance rate is required to prevent the recurrence of HBV after LT.

The aim of this study was to evaluate the efficacy and safety of prophylaxis with TDF and HBIG in preventing the recurrence of HBV after living donor LT.

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MATERIALS AND METHODS

Patients

The medical records of 132 patients who underwent orthotopic LT (OLT) for HBV-related end-stage liver disease were retrospectively analyzed from January 1997 to May 2012. The study protocol was approved by the Ethics Committee of the Medical Faculty of Dokuz Eylül University. All patients provided informed consent for this study. Out of these 132 patients, there were only 18 patients who had used TDF. Patients who had similar demographics with the TDF group were chosen for the LAM group. Patients were divided into two groups: a) patients who used LAM monotherapy before LT for initial anti-HBV treatment and continued to use only LAM as the anti-viral regimen after transplantation (LAM group), and b) patients whose initial and post-operative therapy was TDF (tenofovir group). In the TDF group, TDF treatment was initiated in 6 patients due to resistance to LAM, in 1 patient due to relapse, in 3 patients to prevent relapse, and in 8 patients due to *de novo* hepatitis. In the LAM group, LAM therapy was initiated in two patients due to *de novo* hepatitis, and in 16 patients to prevent relapse. The groups were demographically similar. The patients were followed up for 36 months after beginning treatment.

Prophylaxis with TDF and LAM

Preoperative LAM was given at a dose of 100 mg/day from January 1997 to May 2012 to the first group of recipients with HBV-related end stage liver disease. During the same period, TDF was given at the dose of 245 mg/day to the second group. Preoperative LAM or TDF prophylaxis was followed by combined usage with HBIG after transplantation. The first application of HBIG at a dose of 4,000 IU was administered intramuscularly (IM) during the operation in the anhepatic phase of LT; and 2,000 IU [10 mL] after reperfusion, as well as 2,000 IU IM HBIG daily thereafter, until the HBsAb titer was greater than 200 IU/mL and the HBsAg was seronegative. This protocol was followed by 1,200 to 2,000 IU HBIG intramuscularly, when the HBsAb titer fell below 100 IU/mL.

Immunosuppression

All patients received cyclosporine or tacrolimus, azathioprine or mycophenolate mofetil (MMF) and steroids. Methylprednisolone was initiated as a 1-gram intravenous bolus immediately after reperfusion of the hepatic graft and then progressively decreased from 100 mg/d to 15 mg/d before discharge. Corticosteroids and MMF (500 mg twice daily) were withdrawn at 3 months to 6 months after OLT. The dosage of cyclosporine/tacrolimus was adjusted according to blood levels and renal function. Immunosuppressive dosing was adjusted according to therapeutic drug levels and renal function.

Diagnosis of HBV recurrence and follow-up

Hepatitis B virus DNA and serum HBV markers (including hepatitis B surface antigen [HBsAg], antibodies to HBsAg [HBsAb], hepatitis B e antigen [HBeAg], antibodies to HBeAg [HBeAb], and hepatitis B core antibody [HBcAb]) were assessed before initiating antiviral therapy and LT. Activation of HBV was diagnosed when HBsAg and/or HBVDNA became positive in the serum of the recipients. HBsAg, HBsAb and HBV DNA were measured at three-month intervals after LT.

Hepatitis B virus DNA was detected in serum with a hybridization assay (Digene Hybrid Capture, Gaithersburg, USA with a sensitivity of 5 pg/mL until March 2002, and then was replaced by Bayer Versant, Berkeley, USA with a sensitivity of 2.5 pg/mL). After February 2003, a polymerase chain reaction (PCR)-based method was added for serum HBV DNA detection (COBAS Amplicor HBV Monitor test, Roche Diagnostics, Branchburg, USA). We next employed the RealArt HBV RG PCR Kit (Artus, Hilden, Germany) with a sensitivity of 100 copies/mL.

Serum serologic markers of HBV were measured by chemiluminescent enzyme immunoassay. Liver and kidney functions were monitored postoperatively on a daily basis for 15 days after LT and twice a week thereafter, as well as 30 days after surgery as monthly follow-up. When the transplanted liver showed abnormal liver function, a percutaneous fine-needle aspiration biopsy was performed. In the event of disruption of renal function, drug toxicities were re-evaluated.

Statistical analysis

Statistical package for the social sciences (SPSS) statistical software (Version 15.0; SPSS Inc. Chicago, IL, USA) was used. Descriptive tests were performed on the demographic data of the patient groups. A Wilcoxon test was utilized to compare non-numeric and numeric values. $P<0.05$ was considered statistically significant.

RESULTS

Demographics

During the period between January 1997 and May 2012, 132 HBsAg-positive patients received anti-viral therapy to prevent post-transplant HBV recurrence. Of these 132 patients, two groups of 18 patients with similar demographic recipients were chosen. The LAM group was comprised of 12 men and 6 women. The TDF group included 17 men and 1 woman. The baseline characteristics of these patients are listed in Table 1. The two groups of patients did not differ significantly by age, primary disease or serological markers of HBV prior to OLT. The average donor age was 45.72±10.36 years in the LAM group and 50.83±6.63 in the TDF group. The median follow-up period was 36 months.

Hepatitis B recurrence

No HBV recurrence was detected over a median 36 month follow-up in either group.

Patient' survival

One patient from the lamivudine group died due to recurrence of HCC. There is no exitus reported in the TDF group.

Table 1. Baseline characteristics of 36 patients

	LAM group (n) 18	TNF group (n) 18
Age (years)	45.72±10.36	50.83±6.63
Gender (males/females)	12/6	17/1
Primary disease		
Chronic hepatitis B (n)	18	18
Liver cirrhosis, HCC - (n)	14	11
Liver cirrhosis, HCC + (n)	4	7
HBV Genotype D (n)	18	18
HBV markers before OLT		
HBsAG+ (n)	18	17
HBsAG- *(n)	0	1*
HBeAg (+) (n)	4	10
HBV DNA (+)before OLT (n)	15	11
Follow-up period (months)	38	40
Average Meld score	16	13
Transplant type		
Cadaveric (n)	9	10
Live (n)	9	8
Exitus (n)	1	0
Cause of exitus	Recurrence of HCC	

**De novo* hepatitis B after LT
HCC: hepatocellular carcinoma; HBV: hepatitis B; OLT: orthotopic liver transplantation; HBsAG: hepatitis B surface antigen; HBeAg: hepatitis B e antigen; n: number

Table 2. Mean creatinine values with standard deviations in both groups during the follow-up period

	Creatinine values of the LAM group (mean±SD; mg/dL)	Creatinine values of the TDF group (mean±SD; mg/dL)
Before LT	0.84±0.44	1.00±0.25
After LT		
0 months	0.91±0.30	1.04±0.24
3 months	0.95±0.20	1.14±0.26
6 months	0.96±0.29	1.10±0.25
12 months	0.96±0.26	1.10±0.24
24 months	0.93±0.21	1.20±0.27
36 months	0.83±0.10	1.49

LT: liver transplantation; LAM: lamivudine; TDF: tenofovir disoproxil fumarate; SD: standard deviation

Safety of prophylaxis with TDF+HBIG

Serum creatinine and serum electrolyte levels were recorded at the beginning of tenofovir and lamivudine therapy, 1 month and 3 months later, and at subsequent three-month intervals. The follow-up values of creatinine for the LAM and TDF groups are given in Table 2 and Figure 1. During the follow-up period, there was no statistically significant ($p<0.05$) change in serum creatinine levels after treatment between the LAM and TDF groups.

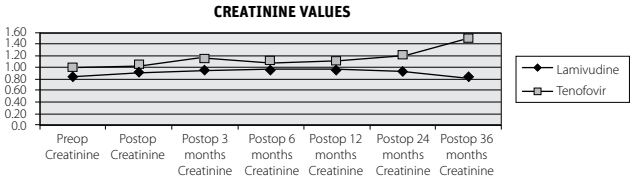

Figure 1. Mean creatinine values for both groups during the follow-up period.

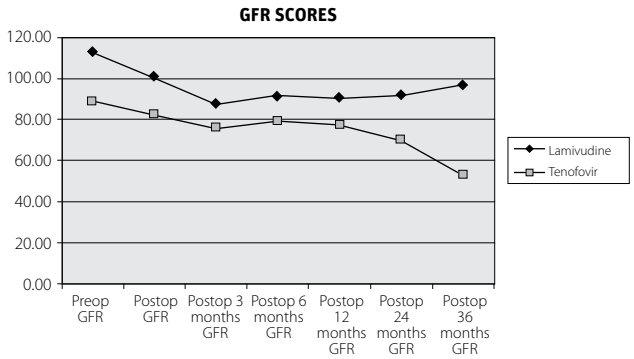

Figure 2. GFR scores during the follow-up period.

Table 3. Mean GFR values during the follow-up period

	GFR values of the LAM group (mean±SD)	GFR values of the TDF group (mean±SD)
Before LT	112.82±42.48	88.61±24.03
After LT		
0 months	100.94±53.76	82.76±20.35
3 months	87.96±23.81	76.25±19.48
6 months	91.01±32.01	79.01±21.65
12 months	90.73±35.96	77.42±17.94
24 months	91.80±30.39	70.24±18.66
36 months	96.58±4.71	53.28

LT: liver transplantation; LAM: lamivudine; TDF: tenofovir disoproxil fumarate; GFR: glomerular filtration rates; SD: standard deviation

Glomerular filtration rates (GFRs) of these patients were calculated with a modification of renal disease (MDRD) formulation. The follow-up values of GFRs for the LAM and TDF groups are given in Table 3 and Figure 2. There was no significant difference ($p<0.05$) in the GFRs of these two groups.

When changes (differences) in creatinine values were assessed, it was found that the average and standard deviation of differences between the initial and after transplantation values of the LAM group were 0.07 and 0.42, respectively, and the average and standard deviation of differences between the initial and after transplantation values of the TDF group were 0.11 and 0.19, respectively. The difference between these two

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Table 4. Changes in comparison with the initial creatinine values during the follow-up period, and the comparison between the two groups

	Δ creatinine values of the LAM group		Δ creatinine values of the TDF group		
	n	(mean±SD)	n	(mean±SD)	p
After LT					
0 months	18	0.07±0.42	14	0.11±0.19	0.849
3 months	18	0.11±0.38	13	0.16±0.23	0.984
6 months	17	0.11±0.42	15	0.09±0.23	0.427
12 months	17	0.13±0.47	16	0.09±0.27	0.304
24 months	15	0.11±0.45	12	0.16±0.22	0.807
36 months	3	-0.06±0.25	1	0.74	0.180

Δ: changes in comparison with the initial value.
LT: liver transplantation; LAM: lamivudine; TDF: tenofovir disoproxil fumarate; SD: standard deviation

Table 5. Changes in comparison with the initial GFR values during the follow-up period, and comparison between the two groups

	Δ GFR values of the LAM group		Δ GFR values of the TDF group		
	n	(mean±SD)	n	(mean±SD)	p
After LT					
0 months	16	-3.88±44.46	12	4.53±15.89	0.676
3 months	9	-2.58±51.89	5	5.67±18.79	0.549
6 months	16	2.43±40.07	15	5.23±22.55	0.813
12 months	17	9.21±44.35	16	8.70±23.41	0.235
24 months	15	11.86±46.80	11	17.30±21.77	0.979
36 months	3	4.68±26.36	1	69.21	0.180

Δ: changes in comparison with the initial values.
LT: liver transplantation; LAM: lamivudine; TDF: tenofovir disoproxil fumarate; GFR: glomerular filtration rates; SD: standard deviation

values was not statistically meaningful (p=0.849). Other follow-up values are shown in Table 4. No significant difference was found between these values.

Additionally, GFR changes were assessed and compared between the two groups. Although the 36th month GFR value was lower in the TDF group, the difference between the two groups was not significant. The changes in comparison with the initial values are shown in Table 5.

None of the patients in either the LAM or TDF group had mild or serious adverse effects such as acute renal failure or HBV recurrence.

DISCUSSION

Effective anti-viral therapy after LT is critical in preventing the recurrence of HBV infection; long-term antiviral therapy is needed after LT. Therefore, the use of antiviral drugs with a high genetic barrier to resistance is especially important in the outcome of post-transplant patients. HBIG was the first agent to prevent HBV recurrence but its high cost, low availability, HBIG failures, and the emergence of escaped mutant HBV strains sparked a search for alternative agents. The introduction of LAM provided an effective suppressor of HBV after LT. However, long-term use of LAM is associated with an increased risk for the development of YMDD mutations, which results in LAM resistance. The combined use of LAM and/or adefovir with HBIG has reduced the risk of re-infection of the graft, but the resistance rates observed in clinical practice may limit long-term use. Therefore, the choice of a new nucleotide analogue with a high genetic barrier to resistance is especially crucial. However, the efficacy and safety of the new and more potent antivirals (entecavir and tenofovir) in transplant patients still remains to be determined.

In this study, we demonstrated that TDF with HBIG provides safe and effective prophylaxis against recurrence of HBV infection after LT. The baseline characteristics of patients in the TDF and LAM groups were statistically identical.

Our results showed that all patients who received TDF had good responses and had no serious adverse effects.

Although the mean creatinine values in the beginning were observed to be mildly higher, and GFR values lower in the TDF group, there was no statistically significant difference between the LAM and TDF groups during the follow-up period. It is known that nephrotoxic immunosuppressive drugs such as cyclosporine and tacrolimus, which must be taken after LT, have decisive effects on GFR and creatinine values. This may cause a high risk of nephrotoxicity in both groups. Nephrotoxic effects of tenofovir can only be assessed objectively if other nephrotoxic immunosuppressive drugs are not used, which is not possible in LT patients. Such factors as hypertension, diabetes mellitus and being elderly, which have not been taken into consideration in this study, may increase the risk of nephrotoxicity. These are the weaknesses of our study.

In our previous study with 40 patients and 16 months of follow-up, no recurrence was observed with HBIG and LAM (6). In other studies, this percentages varied between 0% and 20% (7,8). The fact that the ratio of HBV resistance to LAM over a 5-year period was greater than 60% emphasized the importance of drugs without any HBV resistance, such as TDF, in preventing relapse in recent LT patients. It is possible to presume that, with HBIG and TDF, the HBV recurrence rate was low because no resistance to these drugs was reported. At the same time, extensive prospective studies are needed on the use of TDF without HBIG or with the combination of short-term HBIG treatment. This is important in terms of reducing the cost of HBIG therapy.

Limited studies have been published on the use of tenofovir in liver transplant patients. W.G. Neff et al. (9) performed a ret-

rospective review of the medical records of LT patients for HBV from June 1994 to May 2003. A breakthrough of HBV infection was noted in 14 patients after LT. Of these 14 patients, 5 patients were switched from lamivudine to tenofovir therapy. No adverse reactions were noted in the patients treated with tenofovir.

M. Jimenez-Perez et al. (10) assessed the efficacy and safety of entecavir and/or tenofovir for prophylaxis and treatment of hepatitis B recurrence post-LT. Two patients received entecavir and tenofovir, while two other patients received only tenofovir. The median duration of follow-up was 9.5 months. There were no significant adverse effects from these drugs and no alteration of renal function during the follow-up period.

M. Daude et al. (11) initiated tenofovir therapy in seven patients (three kidney transplant recipients, three liver transplant recipients, and one cardiac transplant recipient) with chronic HBV infection and a partial response to adefovir (n=7), lamivudine (n=5), or entecavir (n=5) therapy. HBV DNA clearance was not achieved in all patients. The results of this pilot study are encouraging and demonstrate that tenofovir therapy is safe and efficacious in treating HBV-positive organ transplant patients.

In another study, 21 LT recipients receiving HBIG without evidence of HBV recurrence with or without NUC were enrolled. Patients received their last injection of HBIG at initiation of tenofovir/emtricitabine and were followed up for 31.1±9.0 months. Substitution of tenofovir/emtricitabine for HBIG prevented recurrence of HBVDNA in 100% of the patients. However, three patients developed reversible acute renal failure; on renal biopsy, one had possible tenofovir/emtricitabine induced acute tubular necrosis (12).

Finally, Cholongitas et al. (13) found no significant differences in renal function (evaluated by GFRs using Cr-EDTA) between the group of patients under nucleotide analogues (i.e., adefovir or TDF [with or without LAM]) compared with under nucleoside analogues (i.e., entecavir monoprophylaxis) during the follow-up period.

In conclusion, the results of this study over the median 36-month follow-up period are encouraging and demonstrate that tenofovir therapy is safe and efficacious in treating HBV-positive LT patients. Given the limited experience with tenofovir in LT settings, this study has significance for long follow-up periods.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Dokuz Eylül University.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - M.A., G.D.H.; Design - G.D.H., M.A.; Supervision - G.D.H., M.A.; Resource - M.A., S.K., T.Ü.; Materials - G.D.H.,T.U.; Data Collection&/or Processing - G.D.H.; Analysis&/or Interpretation - M.A.; Literature Search - G.D.H., M.A.; Writing - G.D.H., M.A.; Critical Reviews - M.A., S.K., İ.A.

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

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