Original Article

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Prevalence of Flares after Stopping Lamivudine in Lamivudine Treated Chronic Hepatitis B at Siriraj Hospital

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ABSTRACT

Lamivudine is the first of oral agent approved for the treatment of chronic hepatitis B and has demonstrated efficacy for both hepatitis B e positive and negative chronic hepatitis B patients. One major problem of lamivudine treatment is the high proportion of post-treatment hepatitis flare that makes withdrawal of the drug to be very difficult. Another problem is the development of YMDD mutation in C domain during treatment. We retrospectively analyzed 114 Thai patients with chronic HBV infection after cessation of lamivudine treatment at Siriraj hospital. All patients were administered 7 with 5-150 mg of lamivudine per day for >6 months. Treatment was withdrawn in patients who had either HBe seroconversion or undetectable hepatitis B virus (HBV) DNA level together with normal aminotransferases. For chronic hepatitis HBeAg positive patients, 35 patients (50.7%) relapsed after stopping lamivudine treatment at 8.4+5.7 months (range 1.8-24.8 months). Threety-four (49.3%) patients who sustained HBeAg seroconversion after stopping lamivudine were followed for 18.5 ± 14.3 (mean ± SD) months (range 1.4-56.3 months). We found the cumulative relapse rate in patients after post-treatment at 1 and 2 years were 35.7% and 59%, respectively. For HBeAg negative patients, 20 (45%) patients relapsed at a mean duration of 10.2 ± 11.2 months (range 1.6-44.3 months) after stopping treatment. Those 24 (55%) patients who sustained responded were followed at a mean of 19.0 ± 15.1 months (range 1.9 ± 48.5 months). We found the cumulative relapse rate in patients after post-treatment at 1 and 2 years were 38% and 47%, respectively. In conclusion, these results demonstrated that responses in both e positive and e negative chronic hepatitis B by lamivudine were not durable in this endemic area.

Key words : lamivudine, chronic hepatitis B, flares

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BACKGROUND

Chronic hepatitis B virus infection affected more than 300 million people worldwide and more than 75% of those are of Asian origin[1]. This leads to an increased risk of developing cirrhosis, hepatic decompensation and hepatocellular carcinoma. In Thailand, the prevalence of HBsAg carrier was estimated to be at 9% and 5% in the year 1973 and 2000 respectively.[2-4] Previous studies have showed that there are approximately 10% of spontaneous seroconversions in HBeAg and 1% of HBsAg loss during long term follow up.

Interferon is the first drug for chronic hepatitis B treatment. In patients with HBeAg positive chronic hepatitis B, clearance of HBeAg is achieved in about 30-35% after treatment with interferon α for 4 to 6 months.[5] In HBeAg negative patients however, treatment with interferon α for at least 12 months results in a sustained biochemical response in only 15-25% of patients.[6,7]. Beside this limited efficacy, interferon α therapy is associated with numerous side effects as well as limited action in patients with advanced cirrhosis and transplanted liver. Asian people trend to respond less than caucasian probably due to the different in hepatitis B genotype. Genotype D and C had a response rate lower than genotype A and C. In Thailand, the prevalence of genotype C and B were 70% and 30% respectively[4,8].

Lamivudine, a 2',3' didesoxynucleoside, is the first oral agent approved for treatment of chronic hepatitis B. This drug inhibits the activity of viral polymerase and thereby hault HBV replication and is very cost effective. The seroconversion rates of HBeAg positive patients to anti HBe can be increased with the longer duration of treatment. (2 years; 27% to 3 years; 40%, 4 years; 47% and 5 years; 50%).[9] HBeAg negative patients with chronic hepatitis B who taking lamivudine 100 to 150 mg daily had virological remission 77% at year 1, 52% at year 2, and 42% at year 3.[10] One major problem of lamivudine treatment is the high proportion of post-treatment hepatitis flare that makes withdrawal of drug becomes difficult. Another problem is the development of YMDD mutation in C domain during treatment. The YMDD mutant rates of HBeAg positive patients given lamivudine has been reported at 1 year = 24-32%, 2 year = 38-42%, 3 year = 49-53%, 4 year = 66-67% and 5 year = 69-70%.[11,12] The YMDD mutant rates of HBeAg negative patients given lamivudine has been reported in 1 year = 10% and 2 year = 56%.[13] Long term treatment of HBeAg positive and negative patients with lamivudine is associated with increasing rates of viral resistance. Ninety percent of responders in HBeAg negative patients relapse after1 year withdrawal of the treatment[14].

At present, treatment guidelines suggest that lamivudine should be continued for about 3-6 months after HBeAg seroconversion.[15,16] However, for HBeAg negative patients, there is no strict guideline. Probably, continuation of treatment until undetectable HBV DNA and normalized ALT level is the most popular practice.[16]

METHOD

We retrospectively analyzed 115 Thai patients (88 men and 27 women) with chronic HBV infection after cessation of lamivudine treatment at Siriraj hospital between July 1993 and October 2005. All patients were administered 75-150 mg of lamivudine per day for ≥6 months. In chronic hepatitis HBeAg positive patients, lamivudine was continued until HBeAg seroconversion and ALT level <40 IU/L. For chronic hepatitis HBeAg negative patients, lamivudine was continued until undetectable serum HBV DNA level was achieved with normal ALT. After stopping lamivudine, they were followed at 1-4 month intervals without further treatment. Follow up period from time when lamivudine was stopped to the time of relapse or last follow up. Patients with other causes of liver diseases (co-infected with hepatitis C, immune deficiency virus and alcoholic) were excluded. Pregnant and lactating women were also excluded. The endpoint for the analysis was a relapse. All enrolling patients gave written informed consent.

Clinical Monitoring

Evaluations at each visit included medical histories, physical examination, hepatitis B serologic marker and routine serum chemistries. (AST, ALT) If serum ALT ≥2 UNL was identified, serum HBV DNA level, serum HBeAg and anti-HBe was studied. HBV DNA viral load was done by using Roche HBV Amplicor version 1.0. (Branchburg, USA) (lowest detectable HBV DNA level are <200 copies/ml). Serum HBeAg and AntiHBe were measured by using the routine commercially available immunoassays Imx (Abbott, Chicago, Illinois, USA).

Definition of relapse

A: Relapse was defined as an increased in serum ALT ≥2 UNL and reappearance of serum HBV DNA ≥100,000 copies/ml measured by Roche Amplicor HBV monitor or a positive serum HBe Ag.

B: Virological relapse was defined as an increased in serum ALT <2 UNL and appearance of HBV DNA ≥100,000 copies/ml measured by Roche Amplicor HBV monitor positive or a positive serology for HBe Ag.

Statistical Analysis

Data was expressed as mean ± SD or median (range). The cumulative relapse rate was calculated by Kaplan-Meier method and the difference was determined by the log rank test. Cox's proportional hazard model was used in multivariate analysis. A p value of <0.05 was considered to be statistically significant. The analysis software used was the Statistical Package for Social Science (SPSS Inc., Chicago III., USA), version 11.5.

RESULTS

There were 115 patients with chronic hepatitis B both (HBeAg positive and negative were included). One HBeAg positive patient was excluded due to lost to follow up so there were 114 patients available for analysis. Table 1 shows baseline data for age, gender, baseline AST levels and ALT levels, incidence of cirrhosis and previous treatment. Higher serum HBV DNA levels were observed at baseline in HBeAg positive patients (4.7×10^8 copies/ml[range : 700-9×10^9 copies/ml]) compared to HBeAg negative patients (2.8×10^7 copies/ml [range : 200-2 $\times 10^8$ copies/ml]) After stopping lamivudine, the patients were followed regularly at the mean interval of 3.4-3.9 months in both groups. Thirty-five patients with chronic hepatitis HBeAg positive relapsed after stopping lamivudine treatment [8.4 ± 5.7 (mean ± SD) months (range 1.8-24.8 months)], 8/35 (22.9%) of whom were previously treated with interferon alfa. The characteristic of lamivudine relapsers and non relapsers in chronic hepatitis HBeAg positive patients were compared as shown in Table 2. By Univariate and multivariate analysis; age, sex, pretreatment serum AST/ALT levels, dosage of lamivudine, total duration of lamivudine treatment and duration of additional lamivudine treatment after HBeAg seroconversion are not different between the two groups. Threety-four patients who sustained HBeAg seroconversion after stopped taking lamivudine were followed for 18.5 ± 14.3 months [(mean ± SD) (range 1.4-56.3 months)].

During the follow up period, the cumulative relapse rate in patients with HBeAg seroconversion after post-treatment at 1 and 2 years were 35.7% and 59%, respectively. (Figure 1)

For HBeAg negative patients, 44 patients had negative HBV DNA and lamivudine treatment was discontinued. During the period of follow up, 20 patients relapsed at a mean duration of 10.2 ± 11.2 months (mean ± SD; range 1.6-44.3 months). Those 24 patients who sustained their responses were followed at a mean of 19.0 ± 15.1 months (mean ± SD) (range 1.9 ± 48.5 months). Three of relapsers (15%) were previously treated with IFN-α after stopped taking

Table 1 Baseline characteristic of patients

	HBe Ag positive (N = 69)	Hbe Ag negative (N = 44)
Age (mean ± SD) (yrs)	41.5 ± 12.6	48.4 ± 10.3
Sex (male : female)	53/16	35/9
HBV Viral load (previous treatment) (mean ± SD) (copies/ml)	$4.7 \times 10^8 \pm 1.7 \times 10^9$	$2.8 \times 10^7 \pm 6.0 \times 10^8$
Lab		
AST (mean ± SD) U/l	143.9 ± 107.6	167.1 ± 136.8
ALT (mean ± SD) U/l	245.6 ± 174.9	289.1 ± 263.6
Albumin (mean ± SD) g/dl	4.2 ± 0.5(N = 41)	4.3 ± 0.4(N = 32)
Globulin (mean ± SD) g/dl	3.9 ± 1.0 (N = 40)	3.7 ± 0.7 (N = 32)
History of cirrhosis	4 (5.8%)	9 (20%)
Previous treatment	17 (24.6%)	6 (13.6%)

Table 2 Comparison of chronic hepatitis HBeAg positive patients with and without clinical relapse after treatment withdrawal

	Relapse (N = 35)	No relapse (N = 34)	p value
Age (mean ± SD) (yrs)	43 ± 13.7	39 ± 11.3	0.63
Sex (male : female)	28/05	23/11	1
HBV Viral load (previous treatment) (mean ± SD) (copies/ml)	$8.5 \times 10^8 \pm 2.2 \times 10^8$ (N = 15)	$5.2 \times 10^7 \pm 9.6 \times 10^8$ (N=14)	N/A
Lab			
TB (mean ± SD) mg/dl	1.14 ± 1.2 (N = 16)	0.84 ± 0.4 (N = 18)	N/A
AST (mean ± SD) U/l	151.97 ± 131.4	135.5 ± 76.8	0.53
ALT (mean ± SD) U/l	258.23 ± 205.2	232.65 ± 138.8	0.55
Albumin (mean ± SD) g/dl	4.20 ± 0.6 (N = 22)	4.29 ± 0.4 (N = 19)	N/A
Globulin (mean + SD) g/dl	3.97 ± 1.2 (N = 21)	3.78 ± 0.7 (N = 19)	N/A
History cirrhosis	3 (8.6%)	1 (2.9%)	0.61
History of previous treatment	11 (31.4%)	7 (14.7%)	N/A
Interferon treatment-	8 (22.8%)	5 (11.7%)	N/A
Lamivudine treatment	3 (8.6%)	2 (5.9%)	N/A
Previous alcohol use	21 (60%)	15 (34.1%)	0.25
Duration of treatment (mean+SD)months	24.74 ± 13.7	23.46 ± 8.7	0.65
Duration of seroconversion until stopping lamivudine (months)	11.33 ± 8.4	12.85 ± 9.2	0.48
Dose of lamivudine(mg/day)	107.86 ± 19.9	112.5 ± 23.2	N/A
Lamivudine user (75:100:150 mg/d)	1:28:06	1:24:09	0.37
Interval of F/U(months)	3.4 ± 0.88	3.97 ± 0.8	0.1
Duration of follow up (months)	8.47 ± 5.7	19.53 ± 14.3	0

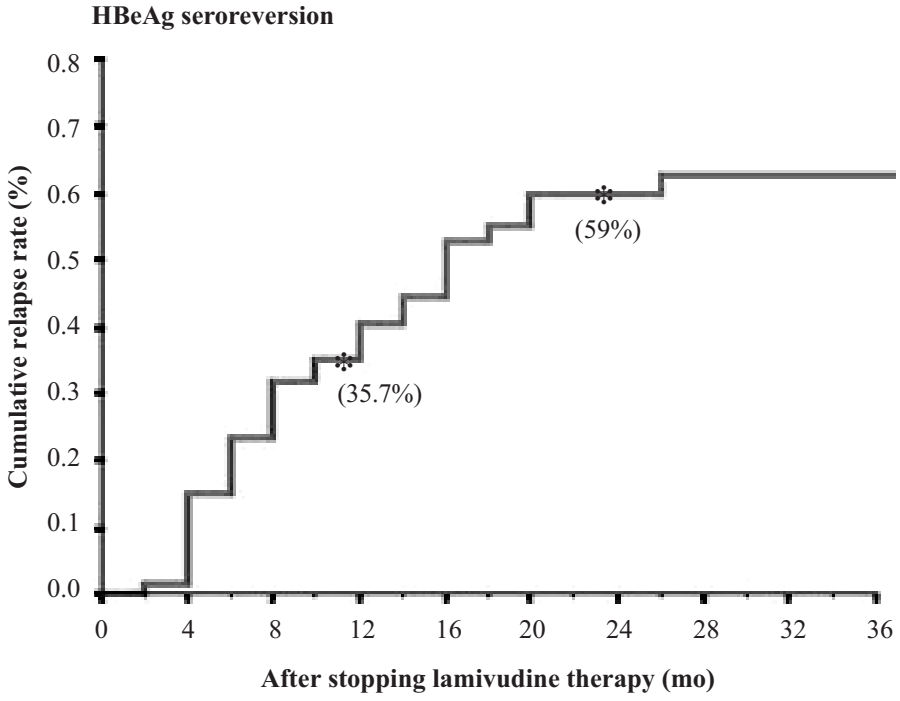

Figure 1 The cumulative relapse rates of HBeAg positive patients after lamivudine cessation. Those at 1 and 2 years were found to be 35.7 and 59%, respectively.

Table 3 Comparison of chronic hepatitis HBeAg negative patients with and without clinical relapse after treatment withdrawal

	Relapse (N = 20)	No relapse (N = 24)	p value
Age (mean ± SD) (yrs)	49.2 ± 9.4	47.7 ± 11.1	0.19
Sex (male:female)	16/04	19/05	0.37
HBV Viral load (previous treatment) (mean + SD) (copies/ml)	$4 \times 10^7 \pm 7.4 \times 10^7$	$1.76 \times 10^7 \pm 4.2 \times 10$	0.23
Lab			
TB (mean ± SD) mg/dl	0.91 ± 0.7 (N = 8)	3.57 ± 9.4 (N = 16)	N/A
AST (mean ± SD) U/l	151.5 ± 139.5	180.1 ± 136.3	0.5
ALT (mean ± SD) U/l	254.15 ± 280.8	318.13 ± 250.6	0.43
Albumin (mean ± SD) g/dl	4.24 ± 0.4(N = 14)	4.27 ± 0.5(N = 18)	N/A
Globulin (mean ± SD) g/dl	3.70 ± 0.8 (N = 14)	3.67 ± 0.6 (N = 18)	N/A
History cirrhosis	4 (20%)	5 (20.8%)	1.0
History of previous treatment	3 (15%)	3 (12.5%)	N/A
Interferon treatment	3 (15%)	2 (8.4%)	N/A
Lamivudine treatment	0	1 (4.2%)	N/A
Previous alcohol use	10 (50%)	15 (62.5%)	0.54
Duration of treatment (mean ± SD) months	27.95 ± 10.1	27.30 ± 8.2	0.81
Duration undetectable HBV DNA until stopping lamivudine (mean + SD) (months)	9.10 ± 7.3	10.58 ± 7.5	0.51
Dose lamivudine (mg/day)	100	104.17 ± 14.1	N/A
Lamivudine users (100:150 mg/d)	20/0	22/2	0.2
Interval of F/U(months)	3.1 ± 1.2	3.29 ± 1.0	0.6
Duration of follow up (months)	10.29 ± 11.2	18.99 ± 15.11	035

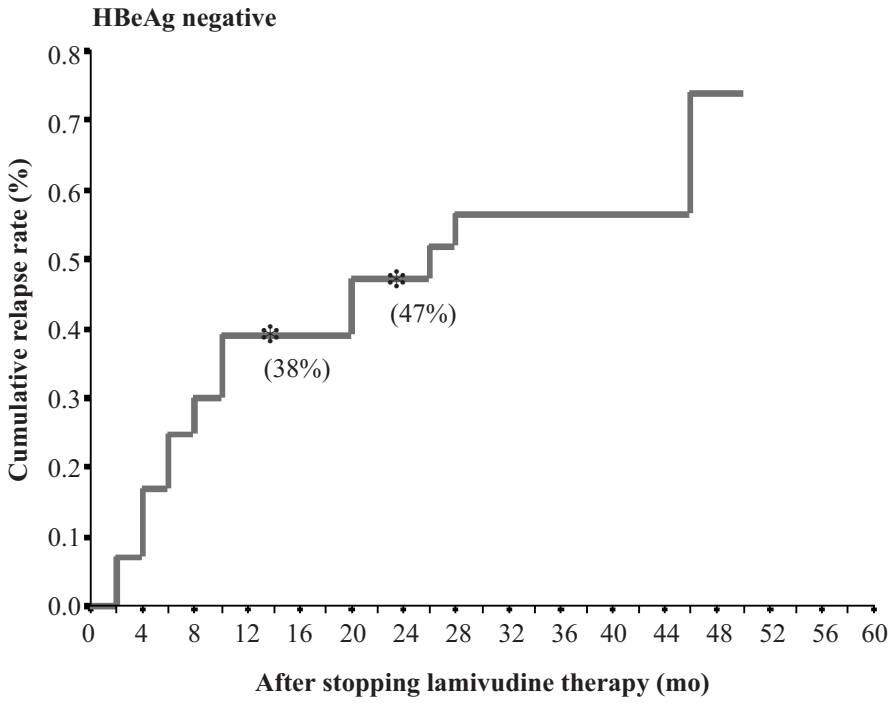

Figure 2 The cumulative relapse rates of HBeAg negative patients after lamivudine cessation. Those at 1 and 2 years were found to be 38 and 47%, respectively.

lamivudine. These were followed regularly at a mean interval of 3.1-3.2 months. Characteristic of both group were compared as shown in Table 3. By in univariate and multivariate analysis; age, sex, pretreatment serum AST and ALT levels, serum HBV DNA level, dose of lamivudine, total duration of lamivudine treatment and duration of additional lamivudine treatment after undetectable serum HBV DNA are not different between the two groups.

The cumulative relapse rate in HBeAg negative after post-treatment at 1 and 2 years were 38% and 47%, respectively. (Figure 2)

DISCUSSION

In this study, 35/69 (51%) of HBeAg positive patients relapsed after HBeAg seroconversion. Most of relapses occurred within 17 months after cessation of lamivudine treatment and usually accompanied by ALT flares and reappearance of HBeAg. In our retrospective study, we do not know the data on isolated virological relapse because we monitored only AST/ALT and HBeAg/Anti-HBeAg and measured HBV DNA only if transaminitis developed. The time from stopping treatment to relapse was 8.4 ± 5.7 months with culmulative relapse rate at 1 and 2 year of 36% and 60%, respectively. This finding was similar to the Korean study[17], where they found cumulative relapse rates at 1 and 2 years post-treatment to be 37% and 49%, respectively. Byun, *et al.*[18] found that the cumulative relapse rates at 6 months and 1 year post-treatment were 58% and 66%, respectively. The cause of the high relapse rate after HBeAg seroconversion is not clear. It may be caused by immune tolerance, which is found commonly in childhood infection such as in our country.[19,20] It has been suggested that lamivudine therapy can restore immune response to HBV with reduction of viral load.[21] However, long standing infections in vertically transmitted patients may make this immune response incomplete.[19,20] Another possibility is the difference of HBV genotypes, for example in Korea, almost all chronic hepatitis B patients were genotype C (96.3%), and this is similar to Japan, Taiwan, Thailand and China where genotype B and C can coexist. These are different from Western countries where genotype A and D are predominant. Chien *et al.*[22], demonstrated that the relapse rate was significantly higher for patients with genotype C than those with genotype B.

In fact, previous studies of the durability of interferon induced HBeAg responses focused on a group of those with treatment-associated responses measured at ≥6 months after discontinuation of therapy.[23-27] Korenman, *et al.*[25], assessed the durability of HBeAg responses after interferon therapy in 23 patients. Only 20 patients had post-treatment durable responses lasting ≥6 months. (excluding 3 relapsers) Similarly, Lok, *et al.*[26] and Lau *et al.*[24], reported a poor result of long term follow up studies involving interferon-treated patients in whom HBeAg had cleared within 1 year of starting a several-month course of therapy.

The durability of HBeAg seroconversion after lamivudine therapy was reported to be 80-90% in the first studies performed in Western countries.[28,29] Song, *et al.*[17], confirmed that patients treated with 100 mg/d of lamivudine for approximately 13 months[30], the 6 months durability of HBeAg seroconcersion measured from cessation of therapy was only 43%, and post-treatment responses were sustained primarily in patients with HBV DNA suppression to $<4.7 \times 10^3$ genomes/ml.

The present study shows no different in age, sex, pretreatment ALT levels, pretreatment HBV DNA levels, total duration of lamivudine treatment, and duration of additional lamivudine treatment after seroconversion between both groups. Similarly, Lee, *et al.*[31] and Byun KS, *et al.*[18], in the multivariate analysis reported that the significant risk factors of relapse included; older age, a higher serum bilirubin level, and a shorter duration of additional lamivudine therapy. Duration of additional lamivudine therapy showed a mean of 6 ± 5 months in relapsers. A significant reduction in cumulative relapse rate was in a group with continued treatment for at least 10 months.[18] The relapsers in our study had a mean duration at 11.33 ± 8.4 months before seroconversion. Ryu SH, *et al.*[32] reported that, in multivariate analysis, older age and the presence of precore mutation before the initiation of therapy appears to be two independent predictive factors for post-treatment viral relapse.

HBeAg negative patients after stopping lamivudine treatment in our study had a mean age at 48.4 ± 10 years. Twenty of 44 patients relapsed after followed up, like HBeAg positive patients. Our study showed time to follow up who had relapsed to be 10.29 ± 11 months. Fung SK, *et al.*[33], showed that 7 of 37 HBeAg negative patients who had undetectable HBV DNA levels and normal ALT at least 3 occasions dur-

ing the second year of therapy had clinical relapse. The median time to clinical relapse was 12 months (range 3-27 months), most patients had genotype C. Previous studies in European patients showed a high relapse rate when treatment was withdrawn after 12 months of therapy.[34] Huang YH, *et al.*(35), reported that pretreatment HBV DNA level was the only determinant for early biochemical relapse after lamivudine withdrawal. HBV DNA ≥12 Meq/ml were nine times more likely to relapse within 12 months as HBV DNA <12 Meq/ml. Huang explained the appearance that HBV might be suppressed by lamivudine at a lower level and it would take a longer time to recover after lamivudine withdrawal. Duration of undetectable HBV DNA until stopping drug in relapsers was (9.10 ± 7.3 months (range 0.6-24.5 months).

Our study had some limitations 1) it was a retrospective analysis 2) no previous histology was available for evaluation.

In conclusion, our study in HBeAg positive patients, thirty-five (51%) patients relapsed after stopped taking lamivudine treatment at 8.4 ± 5.7 months (range 1.8-24.8 months). For HBeAg negative patients, during the period of follow up, there were 20/44 (45%) patients relapsed at mean duration of 10.2 ± 11.2 months (range 1.6-44.3 months) after stopping the medication. The relapse rate after withdrawal of lamivudine treatment following HBeAg loss or undetectable HBV DNA level was high. HBeAg positive patients had an earlier relapse time than HBeAg negative patients. No significant risk factor for relapse was identified in both groups. Many guidelines are recommended 3-6 months additional lamivudine therapy after HBe seroconversion or undetectable HBV DNA level but this is not a guarantee for a durable response. The optimal length of additional lamividine therapy that minimizes relapse and drug resistance should be determined by a future study.

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