Pretreatment and on-treatment indicators of virologic breakthrough of lamivudine therapy in chronic hepatitis B patients: an Egyptian study

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Summary. – The primary objectives of hepatitis B treatment include improved clinical and histological progression as well as virus eradication. For many years, lamivudine was a first-line therapy for treating hepatitis B virus (HBV) infection. However, its long-term use is associated with high resistance rates (up to 70% after 5 years) due to mutations in the viral YMDD motif. The purpose of this study was to outline factors responsible for the development of viral resistance during long-term lamivudine therapy. Initially, 230 patients receiving lamivudine therapy for chronic hepatitis B (CHB) infection were enrolled in the study. All subjects received follow-ups in the first year to assess viral and biochemical responses. Only 136 and 104 patients received follow-up assessments during the second and third years of treatment, respectively. Viral breakthrough (VBT) occurred in 49 of 230 patients (21.3%). Hepatitis B "e" antigen (HBeAg) status before treatment was significantly associated with VBT in the first 2 years of treatment; however, this effect was not significant in the third year. Pre-treatment HBV DNA levels were predictive of VBT in the HBeAg-positive subgroup after all years of treatment (P = 0.001, P = 0.002, and P = 0.002, respectively). These levels were also predictive in the HBeAg-negative subgroup after 1 year of treatment (P = 0.001). In resource-poor communities, financial concerns drive the selection of antiviral therapy to treat CHB. Lamivudine monotherapy is a relatively cheap and effective treatment to manage CHB. However, consistent follow-ups and treatment modifications appear essential to prevent long-term treatment failure.

Keywords: chronic hepatitis B; lamivudine; predictors of response

Introduction

Chronic hepatitis B virus (HBV) infection is a global health problem: about 2 billions of people have a past or present HBV infection; of these, 350 millions are chronic carriers of HBV. Each year, approximately 1 million people die of diseases associated with HBV infection, including liver failure, cirrhosis, and primary hepatocellular carcinoma (Yi *et al.*, 2012).

Due to inadequate measures to confine the spread of infection, HBV has a high prevalence in many developing countries (Abbas and Siddiqui, 2011). Studies in the Middle East show the prevalence of the hepatitis B surface antigen (HBsAg) to range from 3% to 11% in Egypt (Qirbi *et al.,* 2001).

The primary objectives of hepatitis B treatment include improved clinical and histological progressions and virus eradication (European Association for the Study of the Liver [EASL], 2012). Long-term lamivudine use is associated with a high rate of resistance (up to 70% by the end of 5 years) due to mutations in the viral YMDD motif (Chang *et al.*, 2004).

Viral breakthrough (VBT) is usually followed by biochemical breakthrough, defined by elevated alanine ami-

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Abbreviations: ALT = alanine aminotransferase; CHB = chronic hepatitis B; EASL = European Association for the Study of the Liver; HBeAg = hepatitis B "e" antigen; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; NA = nucleos(t)ide analogue; VBT = viral breakthrough

notransferase (ALT) levels during treatment in patients who had experienced an initial treatment response. Development of viral mutations against antiviral therapies can negate the initial response, and in some cases, lead to hepatitis flares and hepatic decompensation. These mutations can be detected months or even years before biochemical breakthrough occurs (Fung *et al.*, 2006). Thus, early detection and intervention can prevent serious conditions, particularly in patients who are immunosuppressed or with underlying cirrhosis. Another potential consequence of drug-resistant HBV mutants is cross-resistance with other nucleos(t)ide analogues (NAs), thus limiting future treatment options *(*Yim *et al.*, 2006*)*.

Follow-up of patients receiving continued lamivudine treatment has revealed decreased virologic and biochemical responses over time due to viral development of drug-resistance mutations. Several studies reported that patients with sustained viral suppression had lower rates of hepatic decompensation and liver-related mortality (Papatheodoridis *et al.*, 2005).

The purpose of this study was to determine which pre-treatment and on-treatment factors are associated with the development of viral resistance and VBT during long-term lamivudine therapy.

Materials and Methods

Patients. This retrospective study included patients diagnosed with chronic HBV who received lamivudine therapy at the Hepatology Clinic in the National Hepatology & Tropical Medicine Research Institute (NHTMRI) and Tropical Medicine Department in Cairo, Egypt over three years. The study was approved by the Ethics Committee of National Hepatology & Tropical Medicine Research Institute and the Tropical Medicine Department at Ain Shams University Hospitals, Cairo, Egypt, in accordance with local research governance requirements. The trial was registered with the federal clearinghouse for randomized trials: ClinicalTrials.gov (NCT01548820).

Definitions. A biochemical response was defined as normalization of serum ALT levels after the initiation of therapy. A virologic response corresponded to a reduction of HBV DNA to levels undetectable by real-time PCR (HBV PCR assay by real time mx-3000, Qiagen). A partial response was defined as a decrease greater than 1 $\log_{10}$ IU/ml in HBV DNA levels at month 6 of lamivudine therapy, from baseline levels detectable by real-time PCR assay. A primary non-response was defined as a decrease less than 1 $\log_{10}$ IU/ml in HBV DNA levels from baseline at month 3 of lamivudine therapy (EASL, 2012). Viral breakthrough was considered as the first manifestation of antiviral drug resistance and was defined as a 10-fold increase in serum HBV DNA levels from their lowest point during treatment in patients with an initial virologic response (Lok and McMahon, 2009); it was also

defined as the detection of HBV DNA after virologic response (Chae *et al.*, 2007).

Inclusion criteria. Adult Egyptian patients serum-positive for HBsAg for more than 6 months with HBV viraemia above 2,000 IU/ml (10,000 copies/ml) were enrolled in the study. Study participants received lamivudine monotherapy at the recommended dose of 100 mg administered daily. Patients were evaluated every 3 months for laboratory abnormalities.

Exclusion criteria. Patients with positive serology results for IgM antibodies against hepatitis B core antigen (anti-HBc IgM) or hepatitis C virus (HCV) were excluded from the study. Patients diagnosed with hepatocellular carcinoma were also excluded.

Patient assessment. Full medical histories were collected and clinical examinations performed on all study participants. Examinations included laboratory testing to measure complete blood count (CBC), international normalized ratio (INR), liver and renal profiles including ALT assay (Bais *et al.,* 2006), alpha-fetoprotein (AFP) levels, viral markers, and HBV DNA levels by quantitative PCR. Abdominal ultrasound imaging was also performed on study participants.

Patients were monitored for VBT after 1, 2, and 3 years of lamivudine treatment to identify pre-treatment and on-treatment factors associated with breakthrough.

Statistical analysis. IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, USA) was used for data analysis. Quantitative parametric measures were expressed as mean ±SD, quantitative non-parametric measures as median and percentiles, and both numbers and percentages were used to express categorized data. Student's t, Wilcoxon rank-sum, Spearman′s ranked correlation, and Chi square tests were used to analyze data. Probability of error (P) values <0.05 were considered statistically significant.

Results

This retrospective study included 230 patients at the outpatient clinic at the NHTMRI and Tropical Medicine Department in Cairo, Egypt with chronic HBV infections and matched the inclusion criteria; study participants received lamivudine treatment (100 mg once daily) and received follow-ups to assess virologic and biochemical responses to treatment and were tested for VBT. Lamivudine was selected as it was the only oral antiviral CHB therapy available under medical insurance coverage at the beginning of the study period.

Two hundred and thirty patients started the study and were followed up during the first year; however, only 136 and 104 patients received follow-ups during the second and third years. Patients who did not respond to treatment and those that developed VBT and received alternative drug therapies were excluded from analysis. There were 34 women (14.8%) and 196 men (85.2%) in the study population. Their mean age was 34.52±8.12 years (average,

Table 1. Serum ALT and HBV DNA levels in CHB patients at various lamivudune treatment statuses

Treatment status		ALT (IU/l)			
		Mean ± SD	Median	IQR*	P-value
Pre-treatment		56.23±54.62	47.00	32.34–66.15	
Treatment	6 months	36.52±22.33	34.00	22.00–42.00	<0.01
	1 year	35.76±20.61	30.00	22.00–40.00	
	2 years	32.55±19.81	28.00	20.00–35.00	
	3 years	31.34±19.85	29.00	21.00–37.00	
		HBV DNA in HBeAg-positive patiens**			
Pre-treatment		11.33±2.43	10.62	8.70–13.23	
Treatment	6 months	2.77±3.44	0.00	0.00–4.79	<0.01
	1 year	1.42±2.69	0.00	0.00–0.00	
	2 years	1.12±1.32	0.00	0.00–0.00	
	3 years	0.63±1.20	0.00	0.00–0.00	
		HBV DNA in HBeAg-negative patients**			
Pre-treatment		5.33±2.43	4.62	3.70–6.23	
Treatment	6 months	2.77±2.17	0.00	0.00–3.26	<0.01
	1 year	1.42±1.22	0.00	0.00–0.00	
	2 years	0.73±1.21	0.00	0.00–0.00	
	3 years	0.32±1.10	0.00	0.00–0.00	

*IQR; interquartile range; **log number of viral genome IU per ml.

33 years). We have observed a significant drop in mean and median ALT levels after 6 months and up to 3 years of treatment. We also observed a statistically significant drop in mean and median HBV DNA levels in the same time frame in both HBeAg-positive and HBeAg-negative subgroups (Table 1).

Among the studied population (230); primary virologic response was detected in 99 patients (43%), partial response in 100 patients (43.5%) and thirty one patients (13.5%) had primary non response (Table 2).

Pre-treatment factors were analyzed to determine their effect on the development of VBT during treatment. There were no significant associations between age, gender, and pre-treatment ALT levels and VBT rates for all treatment durations (data not shown).

Table 3 shows that the pre-treatment HBeAg status was significantly associated with VBT during the first and second years of treatment; however, this effect was not significant during the third year. Pre-treatment HBV DNA levels were significantly associated with VBT in the HBeAg-positive subgroup during all years of treatment. A significant relationship existed between pre-treatment HBV DNA levels and the development of VBT in the HBeAg-negative subgroup only during the first year of treatment (Table 4).

Only 1 (1.3%) HBeAg-positive patient showed HBsAg seroconversion after 1 year of lamivudine therapy, and no HBeAg-negative patient showed HBsAg seroconversion in the same treatment duration. Among HBeAg-positive patients, 22 (28.2%) showed HBeAg seroconversion after 1 year of drug therapy.

Discussion

A high prevalence of HBeAg-negative variants and inactive HBsAg carrier states exists in Egypt, where HBeAg-negative variants account for more than 80% of CHB cases in older age groups (22–45 years). Inactive HBsAg carriers represent nearly 33% of chronic hepatitis B infections (El-Zayadi, 2007).

Study participants were divided into 2 subgroups based on the pre-treatment HBeAg status to study differences in treatment response and VBT rate and to evaluate the usefulness of pre-treatment HBeAg status and HBV DNA levels as predictors of VBT. The study population was predominantly

Table 2. Response to 6-month-lamivudine therapy in CHB patients

Patients	No. (%)
Total	230 (100)
Virologic response	99 (43.0)
Partial response	100 (43.5)
Primary non-response	31 (13.5)

†Values are given as number (percentage).

Table 3. Influence of the pretreatment HBeAg status on the rate of viral breakthrough in CHB patients treated with lamivudine

Period of LAM therapy	Pretreatment HbeAg-negative patients		Pretreatment HBeAg-positive patients		P-value
	Total	No. (%) of patients showing VBT	Total	No. (%) of patients showing VBT	
1 year	152	20 (13.2%)	78	29 (37.2%)	0.03
2 years	104	21 (20.2%)	32	11 (34.4%)	0.8
3 years	83	23 (27.7%)	21	8 (38.1%)	0.5

VBT = viral breakthrough.

Table 4. Influence of the pre-treatment HBeAg and HBV DNA statuses on the rate of viral breakthrough in CHB patients treated with lamivudine

Period of LAM therapy	HbeAg status	No. (%) of patients without VBT	No. (%) of patients with VBT	P-value
1 year	Negative	102 (68%)	20 (40.8%)	0.001
	Positive	48 (32%)	29 (59.2%)	
2 years	Negative	83 (79.8%)	21 (65.6%)	0.024
	Positive	21 (20.2%)	11 (34.4%)	
3 years	Negative	60 (82.2%)	23 (74.2%)	0.182
	Positive	13 (17.8%)	8 (25.8%)	

	HBV DNA (mean ± SD) **		P-value
	VBT absent	VBT present	
1 year	8.77±4.13	17.14±5.23	0.001*
2 years	8.19±3.76	11.73±3.75	0.001*
3 years	8.75±3.55	13.24±2.30	0.001*

*Statistically significant difference. †Values are given as mean ± SD or number (percentage). **log number of viral genome IU per ml.

HBeAg-negative, with 152 of 230 patients (66.1%) comprising this subgroup.

Only 1 HBeAg-positive patient (1.3%) showed HBsAg seroconversion after 1 year of lamivudine treatment. This result is concordant with reports by Lau *et al.* (2005) and Janssen *et al.* (2005), who found HBsAg seroconversion rates of 1% in HBeAg-positive patients after 1 year of therapy. No HBeAg-negative patient in this study showed seroconversion after 1 year of lamivudine therapy. Marcellin *et al.* (2004) and Lai *et al.* (2007) also reported no seroconversion among HBeAg-positive patients after 1 year of lamivudine therapy.

Among HBeAg-positive patients, 22 (28.2%) showed HBeAg seroconversion after 1 year of lamivudine therapy. Comparable results were reported by other case series studies that reported 32% and 18% HBeAg seroconversion, respectively (Chang *et al.*, 2006; Liaw *et al.*, 2009).

The seroconversion rate reached 93.7% in patients who received follow-ups in the second year of treatment, after exclusion of patients who did not respond to treatment or had developed VBT. The seroconversion rate was 100% by the third year of the study. This finding is similar to a report by Reijnders *et al.* (2010), who found that anti-HBe seroconversion rates increased with continued NA therapy, and were affected by the development of drug resistance.

Lamivudine has been a long-time first-line therapeutic agent for the management of CHB, mainly due to its relatively low cost (Lok and McMahon, 2009), good clinical tolerability, and moderate antiviral efficacy (Wiegand *et al.,* 2010). However, EASL guidelines updated in 2012 instead recommend entecavir and tenofovir as the initial treatments. Because they are potent HBV inhibitors with high barriers to resistance, these drugs can be confidently used as first-line monotherapies. The other 3 NAs are recommended for treating CHB only when the more potent drugs are not available or appropriate. Lamivudine is an inexpensive agent, but engenders very high rates of resistance when used as a long-term monotherapy (Lok *et al.*, 2000; Marcellin *et al.*, 2011).

In this study, virologic response was assessed after 1, 2, and 3 years of lamivudine therapy. After 1 year of lamivudine treatment, 137 of 230 patients (59.6%) showed virologic response. There was no significant difference in the response rate between subgroups after 1 year of therapy. In the HBeAg-positive subgroup, 33 of 78 (42.3%) patients showed virologic response. This is consistent with findings by Chang *et al.* (2006) and Lai *et al.* (2007), who reported virologic response rates of 36% and 43%, respectively, in HBeAg-positive patients after 1 year of lamivudine therapy. The present study observed a virologic response in 104 of 152 (68.4%) patients in the HBeAg-negative subgroup. This finding is consistent with findings by Marcellin *et al.* (2004) and Lai *et al.* (2007) who reported a virologic response rate

of 70% and 73%, respectively, in HBeAg-negative patients after 1 year of lamivudine therapy. Non-response was noted in 44 out of 230 (19.1%) patients.

VBT occurred in 49 of 230 patients (21.3%). In addition, there was a significant difference in the rate of VBT between the 2 subgroups after 1 year of lamivudine therapy.

In our study, long-term treatment with lamivudine resulted in progressively higher VBT rates. After 2 years of therapy, 32 of 136 patients (23.5%) showed VBT; the cumulative rate was for 2 years 35.2% (81 out of 230 patients). After 3 years of therapy, 31 of 104 patients (29.8%) showed VBT, corresponding to a cumulative rate of 48.7% (112 of 230 patients) (Table 3). These findings are in agreement with those of Pawlotsky *et al.* (2008), who reported a cumulative resistance rate in lamivudine therapy after 1, 2, and 3 years of 24%, 38%, and 49%, respectively.

The subgroups showed different VBT rates during the course of treatment. The HBeAg-positive subgroup had VBT rates of 37.2%, 34.4%, and 38.1% after 1, 2, and 3 years, respectively. This observation is concordant with Lau *et al.*(2005), who reported VBT rates of 35%, 37%, and 42% in HBeAg-positive patients after 1, 2, and 3 years of lamivudine therapy, respectively. The HBeAg-negative subgroup had VBT rates of 13.2%, 20.2%, and 27.7% after 1, 2, and 3 years of treatment, respectively, similar to results reported by Marcellin *et al.*(2004)*,* of VBT rates among HBeAg-negative patients of 15%, 24%, and 33% after 1, 2, and 3 years of lamivudine therapy, respectively (Table 3).

We studied the relationship between age, gender, pre-treatment HBeAg status, pre-treatment ALT levels, and pre-treatment HBV DNA levels to determine if these various pre-treatment factors were possible predictors of VBT.

We observed a highly significant relationship between pre-treatment HBeAg status and VBT during the first and second years of treatment. However, this pre-treatment status did not significantly affect the development of VBT in the third year of treatment. These results disagree with those of studies conducted by Yuen (2001), Hadziyannis *et al.* (2007), and Alam *et al.* (2008), who found no significant relationship between the pre-treatment HBeAg status and rates of VBT.

Pre-treatment ALT levels were not significant predictors of VBT for any year. We included in our analysis only those patients with ALT levels twice the normal limit. Our findings agree with those of Alam *et al.* (2008) who also found no significant relationship between pre-treatment ALT levels and development of VBT.

We observed a significant relationship between pre-treatment HBV DNA levels and VBT among HBeAg-positive patients during the first, second, and third years of treatment follow up. This result is similar to the findings by Chae *et al.* (2007) who reported a significant relationship between pre-treatment HBV DNA levels and development of VBT. However, Yuen *et al.* (2007) reported that baseline HBV DNA

levels were not significantly related to VBT occurrence in HBeAg-positive patients on lamivudine therapy. A significant relationship also existed between pre-treatment HBV DNA levels and development of VBT in HBeAg-negative patients during the first year of treatment. However, this relationship was not significant in later years. In contrast, Hadziyannis *et al.* (2007) found no significant relationship between pre-treatment HBV DNA levels and VBT in HBeAg-negative patients, a result also corroborated by a study conducted by Thompson *et al.* (2007).

On-treatment factors, including HBeAg status and ALT and HBV DNA levels, were measured at 6 months to determine if these factors were possible predictors of VBT. We observed a significant relationship between HBeAg status during treatment and development of VBT in the first and second years of treatment. Alam *et al.* (2008) also observed that sustained HBeAg-positive status within 1 year of lamivudine therapy was highly predictive of future VBT.

We found no significant relationship between ALT levels during treatment and VBT. This finding is similar to Chae *et al.* (2007) and Alam *et al.* (2008), who also reported that ALT levels during treatment were not predictive of VBT, presumably because VBT usually precedes biochemical breakthrough.

Several studies have found on-treatment serum HBV DNA levels to be predictive of lamivudine resistance (Lai *et al.*, 2007; Hadziyannis *et al.*, 2007; Thompson *et al.,* 2007). In our study, patients who had not developed a virologic response after 6 months of treatment were more likely to develop VBT later.

A highly significant relationship was found between on-treatment HBV DNA levels and VBT rates after 6 months and 1 year of treatment, respectively (P <0.001). The 6-month HBV DNA levels remained significantly related during the second year of therapy (P <0.005), but the association was not significant during the third year of treatment. Yuen *et al.* (2001) also reported a similar finding in 159 HBeAg-positive CHB patients treated with lamivudine and evaluated for a median period of 30 months: those with HBV DNA levels greater than 103 copies/ml at 6 months had a 63% chance of developing the YMDD mutation associated with VBT, whereas those with HBV DNA levels less than 103 copies/ml had only a 13% chance of developing the mutation.

We observed a significant relationship between HBV DNA levels at 6 months of treatment and occurrence of VBT during the first and second years in the HBeAg-negative subgroup (P <0.005). However, the relationship was not significant in the third year of treatment. This observation is concordant with a study of 139 HBeAg-negative patients treated with lamivudine for 2 years: Chan *et al.* (2007) reported a correlation between 2-year lamivudine resistance rates and serum HBV DNA levels at 6 months, a finding also consistent with Hadziyannis *et al.* (2007), who in-

vestigated the predictive value of serum HBV DNA levels during treatment in a study involving 156 HBeAg-negative patients who received prolonged lamivudine treatment. In their analysis, undetectable HBV DNA levels at 12 and 24 weeks of lamivudine treatment had 93% and 72% predictive values for sustained virologic response at 2 and more than 4 years, respectively.

Our analyses showed a positive correlation between pre- and post-treatment HBV DNA levels and VBT occurrence at 6 months and 1, 2, and 3 years in the HBeAg-positive subgroup. These levels were also positively correlated in the HBeAg-negative subgroup at 6 months and 1 year. However, there was no significant positive correlation at 2 or 3 years of treatment. Alam *et al.*(2008) reported a similar significant positive correlation between baseline HBV DNA levels and VBT in both HBeAg-positive and HBeAg-negative patients in a study of 423 patients followed up for 78 months (P = 0.005).

Multivariate analysis of pre-treatment factors including age, gender, ALT, and HBV DNA levels, and HBeAg status revealed HBV DNA levels to be predictive of VBT in the HBeAg-positive subgroup after 1, 2, and 3 years of treatment. Pre-treatment HBV DNA levels were also predictive of VBT in the HBeAg-negative subgroup after 1 and 2 years of treatment. However, no factors were significantly predictive of VBT after 3 years of treatment. In contrast, Thompson *et al.*(2007) reported resistance rates of 6%, 31%, and 51% at 12, 24, and 48 months, respectively, in a prospective study of 85 patients with CHB who were treated with lamivudine. Multivariate analysis identified the presence of the HBV precore variant, high baseline ALT levels, and persistent viraemia at 24 weeks as independent predictors of early lamivudine resistance. Our findings are also contradictory to those of Alam *et al.* (2008), who found persistent levels of HBV DNA detected by PCR at 6 months of treatment to be an independent predictor of lamivudine resistance by multivariate analysis in a study of 423 chronic HBV HBeAg-positive and HBeAg-negative patients who had not previously received treatment.

In summary, our data show that lamivudine monotherapy is a relatively cheap and effective treatment for management of CHB in carefully selected patients, and that pre-treatment and on-treatment level of HBV DNA and rates of HBeAg seroconversion are helpful predictors of treatment response as well as markers to inform continued treatment strategies.

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