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ORIGINAL ARTICLE

Pretreatment and on-treatment predictors of viral breakthrough in lamivudine therapy for chronic hepatitis B

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Abstract
Purpose There are remarkable advances in the treatment of chronic hepatitis B (CHB) in the last few years. Unfortunately, prolonged antiviral treatment is associated with increasing risk of drug resistance/viral breakthrough (VBT), which may lead to flare-up and rapid decompensation. We have designed this study to predict the pretreatment and on-treatment factors responsible for development of VBT.
Methods This study was conducted during the period of February 2000 to November 2007. We have included 423 patients who received lamivudine (LAM) therapy for at least 1 year and at least 2 follow-ups at 6 months' interval. Follow-up period was 12–78 months. Chi-square test, student's *t* test, and logistic regression analysis were performed to prove the validity.
Results Of the 423 study cases, 367 (86.8%) were of male patients and 261 (61.7%) patients were HBeAg positive; the age of the patients was 30.8 ± 12.9 years. Development of VBT was 4.4, 22.8, 45.3, and 74% at 1, 2, 3, and 4 or more years, respectively. Pretreatment high HBV DNA ($P = 0.005$) and female sex ($P = 0.01$) were associated with VBT and pretherapy ALT ($P = 0.698$), HBeAg status ($P = 0.273$), and age ($P = 0.059$) were not associated. Duration of treatment, failure to lose HBeAg at 1 year, and HBV DNA nonresponder at 6 months were significantly ($P = 0.001$) associated with development of VBT.
Conclusion Persistence of HBeAg at 1 year and HBV DNA nonresponder at 6 months are good predictors of development of VBT.

Keywords Viral breakthrough · Lamivudine · Chronic hepatitis B · Viral resistance · HBV DNA

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Introduction

There are remarkable advances in the treatment of chronic hepatitis B (CHB) in the last few years. One of the nucleoside analogue lamivudine (LAM) has been shown to be highly effective in inhibiting hepatitis B virus (HBV) replication [1–3]. LAM therapy for CHB patients with advanced fibrosis significantly reduced the incidence of hepatic decompensation and hepatocellular carcinoma [4]. It is becoming increasingly clear that CHB management requires long-term therapy. Unfortunately prolonged LAM treatment is associated with increasing risk of drug

resistance, which may be cross-reactive. Emergence of antiviral resistance may lead to viral and biochemical breakthrough and sometimes hepatitis flare-up and rapid decompensation [5, 6]. A high incidence of viral breakthrough (VBT) that results from viral resistance is a major disadvantage of prolonged LAM therapy for CHB [7]. Selective amplification of resistant mutants is the main concern of long-term LAM therapy [8, 9]. During continuation of LAM treatment, exacerbation of CHB was reported in 40.6% of patients carrying resistant mutants [10]. In liver transplanted cases, LAM resistance is associated with recurrent HBV infection that leads to advanced hepatic fibrosis and severe necroinflammatory changes [11]. Furthermore, hepatic decompensation and death can occur, particularly in patients with cirrhosis [10, 12–15], and the risk of hepatocellular carcinoma may also be increased in patients with LAM resistance [16]. Prediction of factors responsible for VBT may prevent these complications in future. We have designed this study to predict the pretreatment and on-treatment factors responsible for the development of viral resistance/VBT in prolonged LAM therapy.

Materials and methods

Patients

We have included 423 cases of patients who were treated with 100 mg of LAM daily orally during the period of February 2000 to November 2007 in the Department of Hepatology of Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. Duration of treatment was 12–78 months. We have continued the drug till 6 months after HBeAg seroconversion or up to clearance of HBsAg in HBeAg-negative patients. Of these, 317 were naive and 106 were interferon failure cases. The protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the departmental ethical review committee.

Inclusion and exclusion criteria

The inclusion criteria consisted of the following conditions: (i) HBsAg positive for at least 6 months; (ii) HBeAg positive or negative; (iii) serum ALT levels 2 times upper limit of normal; (iv) HBV DNA detectable by hybridization assay/$\geq 10^5$ copies/ml in HBeAg-positive cases and $\geq 10^4$ copies/ml in HBeAg-negative and interferon failure cases; (v) patients who had completed LAM therapy for at least 12 months and at least 2 follow-up at 6 months' interval; and (vi) patients older than 18 years. Patients were excluded if they had previously received antiviral therapy except interferon or had other causes of hepatitis. We have followed the patients with HBV DNA, ALT, HBeAb, and HBeAg whenever appropriate in every 6 months.

Methods

HBV serological markers were detected using enzyme-linked immunoabsorbent assays (Abbott Laboratories, North Chicago, IL). Serum HBV DNA was determined by a solution hybridization assay based on hybrid capture (Digen Hybrid-CaptureII Standard Test, Digene Corporation, Gaithersburg, MD) between 2000 and 2004 with the detection range of 1.42×10^5 to 1.7×10^9 copies/ml, and thereafter a target-amplification assay based on competitive polymerase chain reaction (Amplicor HBV Monitor™, Roche Molecular Systems, Pleasanton, CA) with the usual detection range of 300 to 10^6 copies/ml, which increased with dilution. Age, sex, HBeAg, log HBV DNA, log ALT before starting LAM and HBeAg, ALT, and DNA response at 6 months as well as duration of LAM therapy were analyzed as predictors of development of VBT.

Definitions

Initial HBV DNA response was defined as HBV DNA level $\leq 10^5$ copies/ml or 2 $\log_{10}$ reduction from baseline HBV DNA level at 6 months [17, 18]. *VBT* was defined as >1 $\log_{10}$ copies increase in HBV DNA from nadir after an initial virologic response or HBV DNA could be detected again after the previous report of under the detection limit [7].

Statistical analysis

Statistical testing was performed using SPSS, version 11.5 (SPSS Inc., Chicago, IL). Results were expressed as mean ± SD. HBV DNA and serum ALT levels were logarithmically transformed for analysis. Continuous variables were compared using student's t test and categorical data were compared by chi-square test. Factors associated with VBT were analyzed by logistic regression analysis, and $P < 0.05$ was considered statistically significant.

Results

Demographic characteristics

During this study period, we have treated 1,925 CHB patients with LAM, but only 423 patients fulfilled the inclusion criteria: age ranging from 18 to 78 years; male-to-female ratio was 6.6:1; 162 (38.3%) were HBeAg negative; 395 were CHB patients and 28 patients were cirrhotic and treatment duration was 12–78 months; and before starting LAM therapy, ALT levels were

Table 1 Demographic characteristics of the patients

Characteristic	Total patients ($n = 423$)	VBT ($n = 123$)	Non VBT ($n = 300$)	P value[a]
Age (years)	30.8 ± 12.9	28.7 ± 11.6	31.6 ± 13.4	0.059
Male, *n* (%)	367 (86.8)	99 (80.5)	268 (89.3)	0.018
HBeAg positive, *n* (%)	261 (61.7)	81 (65.9)	180 (60)	0.273
Log ALT, U/l	2.0 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	0.698
Log HBV DNA, copies/ml	7.8 ± 1.4	8.1 ± 1.4	7.1 ± 1.4	0.005
Mean duration of treatment, months	24.8 ± 13.7	35.9 ± 13.6	20.3 ± 10.9	0.001
Cirrhosis, *n* (%)	28 (6.6)	6 (4.9)	22 (7.3)	0.399

Note: VBT, viral breakthrough was defined as >1 $\log_{10}$ copies increase in HBV DNA from nadir after an initial virologic response or HBV DNA could be detected again after the previous report of under the detection limit [7]

[a] P values were determined by student's t test and chi-square test

134.7 ± 199.5 U/l, AST levels were 106.5 ± 231.1 U/l, and log HBV DNA levels were 7.8 ± 1.4 (Table 1).

Response to LAM therapy

In this series, 137 patients were treated for 1 year, 145 for 2 years, 64 for 3 years, 54 for 4 years, and 23 for 5 years or more with LAM. Overall, HBV DNA responses were 53.7%, ALT normalization 52.6%, HBeAg loss 35.9%, and seroconversion 26%. Development of VBT was 4.4, 22.8, 45.3, and 74% at 1, 2, 3, and 4 or more years, respectively. VBT was 29% in treatment-naive LAM patients and 29.2% in interferon failure LAM patients.

Predictors of VBT

Pretreatment age ($P = 0.059$) and ALT ($P = 0.698$), AST ($P = 0.392$), and HBeAg ($P = 0.273$) levels had no significant effect, but female sex ($P = 0.01$) and HBV DNA level ($P = 0.005$) had significant association with the development of VBT. Cirrhosis and CHB had equal chance ($P = 0.399$) for the appearance of VBT. On-treatment HBV DNA response ($P = 0.007$) at 6 months, HBeAg positivity ($P = 0.001$) at 12 months, and duration of LAM therapy ($P = 0.001$) had significant predictive value on the appearance of VBT during continuation of LAM therapy. HBV DNA nonresponders at 12 months were also highly susceptible to VBT during continuation of LAM therapy. With logistic regression analysis, duration of LAM therapy, failure to lose HBeAg at 12 months, and HBV DNA nonresponder at 6 months had significant association with VBT. ALT normalization at 6 months or 1 year had no significant association with VBT.

Discussion

We have included 423 cases from treatment-naive and interferon failure groups for the duration of 12–78 months. This is the largest series ever reported on VBT/resistance with LAM therapy for a long duration treatment. Baseline predictors of VBT were HBV DNA level and female sex. Duration of LAM therapy, HBeAg positivity at 1 year, and HBV DNA nonresponder at 6 months had high prediction of development for VBT with logistic regression analysis. Baseline HBV DNA level is significant predictor in other study [7]. But in comparison to on-treatment predictors, baseline HBV DNA is not significant in our study. This comparison was not done in the study of Chae and Hann. Females are more prone to VBT in our series, which contradicts this report. This is because females continued the treatment for a longer duration than males ($P = 0.02$) in our study. Study from Taiwan reported that HBeAg, HBV DNA level, and ALT level and treatment duration were the major determinant of resistance for LAM therapy [19]. In our study, pretreatment ALT and HBeAg had no significant association with VBT. We have included only those patients who had ALT levels twice the normal levels, so we could not compare ALT levels lower than these and normal levels. On the other hand, Chae and Hann studied the genotypic resistance, whereas we observed VBT only.

On-treatment predictors are better than pretreatment predictors of VBT in our study; this is in agreement with Japanese study [20]. They observed that persistence of HBV DNA at 3 months was the strongest predictor, but it was 6 months in our study. We have followed up the patients every 6 months and determined HBV DNA with different method in the earlier part of the study. Follow-up of 6 months' interval may cause possible delay in the detection of VBT. Study from Australia reported that high baseline ALT and persistent viremia at 6 months are independent predictors of development of viral resistance [21]. On-treatment HBeAg status was not studied as predictor of VBT in previous studies. We have observed that failure to lose the HBeAg within 1 year of LAM therapy was highly predictive of VBT in future. It is easy and will save the cost of treatment. It is very important for Asian poor patients who cannot afford such treatment.

In our study, a VBT of 4.4, 22.8, 45.3, and 74% at 1, 2, 3, and 4 or more years, respectively, was reported. It was observed in other studies that genotypic resistance to LAM increases from 15 to 30% after 1 year of treatment to 70% after 5 years of treatment [2, 22–24]. It is almost similar for longer duration but was less for shorter duration because we have studied VBT, whereas others had studied genotypic resistance. We have obtained the limitations that we could not analyze effect of genotype on VBT and genotypic resistance in this large series.

We conclude that persistence of HBeAg at 1 year and HBV DNA nonresponder at 6 months are good predictors of development of VBT continuation of LAM therapy. On-treatment predictors are better than the baseline predictors. We recommend that HBV DNA nonresponder at 6 months or persistence of HBeAg at 1 year is the candidate for additional antiviral therapy. Further genotypic study may help in future.

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