Role of HBV genotype in predicting response to lamivudine therapy in patients with chronic hepatitis B

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Background: Predictors of response of chronic hepatitis B (CHB) to lamivudine therapy need better definition. Whether hepatitis B virus (HBV) genotypes could serve as such a predictor has not been well studied. Aim: To study the association of HBV genotypes with the outcome of lamivudine treatment in patients with CHB. Methods: Seventy-six patients with CHB (45 HBeAg +ve) received lamivudine 100 mg/day, orally for 12 mo. Infecting HBV genotypes were determined in pre-treatment specimens using restriction fragment length polymorphism. End-of-treatment response (ETR) and sustained viral response (SVR) were defined as undetectable HBV DNA (<0.5 pg/mL) at 12 and 18 months, respectively. Results: ETR was observed in 26 (34%) and SVR in 11 (14%) patients receiving lamivudine. The pre-treatment characteristics of the responders and non-responders were comparable. Genotypes A and D were observed in 28 (37%) and 48 (63%) patients, respectively. The frequency of genotypes A and D was comparable between responders (28.6% vs. 37.5%) and non-responders (71.4% vs. 62.5%), respectively (p=ns). Of the 26 responders, SVR could be evaluated in 20 subjects; 9 (45%) relapsed and 11 achieved SVR. Patients with genotype D achieved higher SVR rate than genotype A (10 of 48, 28.8% vs. 1 of 28, 3.5% p =0.0359). Conclusions: Forty-five percent of Indian patients with CHB who achieve ETR relapse, and SVR to lamivudine therapy is achieved in 14%. Patients with genotype D achieve higher SVR rate than with genotype A. [Indian J Gastroenterol 2005;24:12-15]

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The search for predictors of response to antiviral therapy in patients with chronic hepatitis B (CHB) continues. Younger age, female gender, shorter duration of infection, elevated ALT levels, HBeAg positivity, low HBV DNA levels, and mild chronic hepatitis have been reported to predict better therapeutic response.1-5

Among patients with chronic hepatitis C, genotype of the infecting virus determines the response rate; thus, patients with genotype 1b HCV infection respond to therapy less frequently than those with genotypes 2 and 3.6,7,8

The heterogeneity in disease manifestation and, more importantly, response to antiviral treatment in HBV infection could also be related to HBV genotype. Based on inter-group divergence of 8% or more in the complete genome sequence or 4.1% or more divergence of the surface gene, eight genotypes of HBV have been described.9,12 Whereas genotype A is universally present, genotypes B and C are predominant in South East Asia, China and Japan.12,13 The predominant HBV genotypes in India are genotypes A and D.14,15

HBV genotypes are known to influence response to interferon therapy. In Germany, HBeAg seroconversion rate with interferon treatment was higher among patients with genotype A than D (37% vs. 6%).16 In a study from Asia, HBeAg loss was higher in patients with genotype B (41%) than in those with genotype C (15%).17 A study of 35 HBeAg-negative patients found a better response to interferon in patients who had HBV genotype A (70%) than those with genotype D/E (40%).18

There are limited data on the association of HBV genotypes and response to lamivudine therapy.19,20 There is no large prospective study on this issue, particularly from Asia. Such information would help in better patient selection for lamivudine therapy.

Methods

Patients with CHB were included in this prospective study if they fulfilled the following criteria: (i) HBSAg positive for >6 mo, (ii) HBV DNA levels >0.5 pg/mL, with or without HBeAg positivity, (iii) histological evidence of chronic hepatitis or cirrhosis, (iv) serum ALT levels >2 times upper limit of normal. Exclusion criteria included: (i) age below 12 years, (ii) co-existent HCV, HDV or HIV infection, (iii) presence of autoimmune liver disease (positive ANA or ASMA in 1:80 dilution), Wilson’s disease (decreased serum ceruloplasmin and presence of K-F ring), (iv) Child class C liver disease, (v) alcohol intake >80 g/d for more than 5 years, (vi) advanced systemic disease, including cardiac, renal, neurological or thyroid disease, (vii) pregnancy, (viii) hepatocellular carcinoma (alpha fetoprotein >400 ng/mL, with CECT evidence of space-occupying liver lesion or evidence of tumor on needle aspiration cytology), and (ix) lack of consent. The study protocol was approved by the institutional ethics committee.

Lamivudine was given orally in a dose of 100 mg/day for 12 months. Patients were followed up clinically till 6 months after completion of lamivudine therapy.
Liver and renal function tests every month. HBsAg, HBeAg and anti-HBe antibody were determined before the start of therapy and at three-monthly intervals thereafter, using commercially available ELISA kits (Organon Teknika, Netherlands). HBV DNA was measured at baseline and then every three months using a commercially available hybrid capture assay (Digene, Maryland, USA) with a sensitivity of 0.5 pg/mL. Liver histological activity index (HAI; grade) and degree of fibrosis (stage) were evaluated using the modified Knodell’s criteria in a biopsy obtained during a three-month period preceding the start of treatment, by a pathologist who was unaware of clinical or biochemical findings.

HBV genotype was determined using restriction fragment length polymorphism. In brief, using DNA extracted from serum using the standard phenol-chloroform method, a 485-bp DNA segment from the pre-S region was amplified and digested with restriction enzymes Ava II and Dpn II (New England Biolabs, Beverly, MA) in two separate reactions. The digested products were resolved on 3% agarose gel electrophoresis, and genotypes were assigned based on the band patterns.

Assessment of response
The definitions used for assessing response to treatment were as follows:

**End-of-treatment response (ETR).** Loss of HBeAg (in HBeAg-positive subjects) and quantifiable HBV DNA (in both HBeAg-positive and HBeAg-negative subjects) at the end of 12-month therapy.

**Sustained viral response (SVR).** Persistent loss of HBeAg and/or quantifiable HBV DNA, at six months after cessation of therapy.

**Relapse.** Reappearance of HBeAg or quantifiable DNA after stopping therapy, within the 6-months follow-up period.

**Statistical analysis**
Inter-group comparisons were done using chi-squared test with Yates’ correction and Fisher’s exact test for categorical variables, and two-sample Student’s t test and Mann-Whitney U test for continuous variables. Multivariate analysis was done using logistic regression model and SPSS 10.05 software.

**Results**
Of the 76 patients with CHB included in the study, 45 (59%) were HBeAg positive. Of the HBeAg-positive patients, 27 (60%) and 18 (40%) had genotypes A and D, respectively. Of the HBeAg-negative, anti-HBe positive patients 30 had genotype D and one had genotype A.

**ETR**
None of the 76 patients lost HBsAg during therapy. Twelve of 45 (26%) HBeAg-positive and 14 of 31 (45%) HBeAg-negative patients had ETR (p=ns); these included 8 of 28 (29%) patients with genotype A and 18 of 48 (38%) with genotype D (p=ns).

At the end of therapy, mean serum ALT levels became normal (38 [3.3] IU/L) in subjects who had ETR, but remained high (62 [3] IU/L) in those without ETR. The pre-treatment demographic characteristics, including age, gender, ALT and HBV DNA levels, were comparable in those with and without ETR (Table). The mean HAI and fibrosis score were significantly lower in responders than in non-responders.

**SVR and relapse**
At the end of 6-month follow up, no additional patient showed response. Of the 26 patients with ETR, 4 were lost to follow up and 2 died; of these, 4 patients had genotype D and 2 had genotype A, and 4 were HBeAg positive. Of the remaining 20 responders, 9 (45%) had relapse and 11 (55%) had SVR. Thus, overall SVR was 11 of 76 (14%). Eight of these 20 patients were HBeAg positive and 12 were HBeAg negative at baseline. The number of relapsers was higher in HBeAg-positive than in HBeAg-negative patients (7/8 vs. 2/12; p<0.05).

Of the patients who had ETR and were followed up for 6 months post-treatment, SVR was achieved in 10 of 14 (71.4%) patients with genotype D and 1 of 6 (16.6%) with genotype A (p=0.0379). Overall SVR rate among all those treated was also higher among patients with genotype D (10/48 [29%]) than those with genotype A (1/28 [4%]; p=0.04).

The 11 patients who achieved SVR were comparable in age with non-responders. There was a male predominance in both groups. The pre-treatment serum ALT levels and viral load were also comparable in non-responders and subjects who achieved SVR (ALT 87 [10.4] vs. 104 [10.2] IU/L, p=ns; HBV DNA median 115 [range, 0.5-16000] pg/mL vs. 137 [0.5-10800] pg/mL, p=ns).

**Predictors of sustained response**
A multivariate analysis that included age, sex, serum ALT, HBV DNA, HAI and HBeAg status revealed that
the combination of HBeAg-negative phenotype and genotype D has 4-fold higher SVR than that of HBeAg-positive phenotype and genotype A (9 of 14 [64.2%] vs. 1 of 6 [16.6%]). HBeAg-negative phenotype was associated with genotype D; 30 of 31 HBeAg-negative patients had genotype D. The other factors did not predict response to lamivudine treatment.

Discussion

Prevalence of various HBV genotypes varies geographically; therefore response to antiviral therapy is likely to differ in various parts of the world. In the present study, 34% of 76 patients with CHB treated with lamivudine had ETR. Of these 11 of 20 who could be evaluated achieved SVR.

In recent years, viral genotypes are being evaluated as determinants of lamivudine response and emergence of drug-resistant mutants. In our study population, only genotypes A (32%) and D (68%) were found. The percentage of subjects infected with these genotypes among responders and non-responders was not different. Our observation is similar to that of Buti et al,20 Eastern Asia and the Far East have predominantly genotypes B and C.6 In a series of 31 consecutive HBeAg-positive patients treated with lamivudine, a better virological response was observed with genotype B than with C.19 In a study from Germany, HBV subtype ayw responded significantly better than subtype ayw.25 Genotypes are generally subtype-specific; thus correlation of HBV subtype and lamivudine response can be extrapolated to HBV genotype and response associations.

In the present study, genotype D was associated with SVR more often than genotype A (71.4% vs. 16.6%). Our observations are in conformity with the results of Zollner et al,23 revealing higher rates of SVR in genotype D/subtype ayw.

The durability of lamivudine-induced response in HBeAg seroconversion is controversial. It is reported to be durable in Caucasian patients,24 whereas groups from Korea25,26 and Taiwan27 report a high relapse rate. It has been shown that the relapse rate was higher in patients older than 25 years27 and in patients whose HBV DNA remained more than 4.7 x 10^3 genome per mL during treatment.25 The duration of post-therapeutic therapy plays an important role in preventing relapse. In Korea, the cumulative relapse rate in seroconverters was 63% after discontinuation of lamivudine treatment.26 Another Korean study revealed that the durability of response was 90% after the first and second year in patients who received more than 6 months’ additional lamivudine after seroconversion.28

Factors like age and gender did not determine response to lamivudine therapy. In an Asian study24 involving 345 patients with HBeAg-positive CHB, pretreatment ALT level exceeding 5 times normal was the strongest determinant for HBeAg seroconversion in response to lamivudine, whereas HBV DNA level and presence of cirrhosis did not have an effect. In a similar US study29 of 805 patients, elevated baseline ALT levels and HAI score were important predictors of HBeAg loss. We also observed similar results. The percentage of subjects with chronic hepatitis and cirrhosis was comparable between the responders and non-responders. However, pre-treatment histological features including HAI and fibrosis score were significantly lower in responders. Although baseline serum ALT levels did not differ significantly between the two groups, they were marginally higher in the responders. Pre-treatment HBV DNA levels were not different in the two groups.

In summary, our results demonstrate that with 12 months’ lamivudine therapy, 45% of Indian patients with chronic hepatitis B achieved ETR and 14% achieved SVR. Patients with genotype D HBV infection achieved SVR more often than those with genotype A infection. Larger studies are needed to further assess the role of HBV genotypes in selection of patients with CHB for lamivudine or interferon therapy.

References


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