CONCISE COMMUNICATION

Quantitative Hepatitis B Virus DNA Testing for the Early Prediction of the Maintenance of Response during Lamivudine Therapy in Patients with Chronic Hepatitis B

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To determine whether a dramatic decrease in hepatitis B virus (HBV) DNA levels within the first months of lamivudine therapy can predict the emergence of YMDD variants in patients with chronic hepatitis B, quantitative testing was done every 3 months on serum samples from 35 patients who were treated with lamivudine for >1 year. The decline in HBV DNA levels from baseline to month 3 was higher in 22 responders than in 13 nonresponders (mean ± SD, 4.16 ± 1.06 vs. 2.88 ± 1.77 log10 copies; P = .002), whereas no differences were observed in patients with and without YMDD variants at 1 year of therapy. At 3 months, HBV DNA was undetectable in 77% of the responders, whereas, after 1 year, it was undetectable in 23% of nonresponders, 40% of patients with YMDD variants, and 74% of those without variants. Therefore, quantitative HBV DNA testing is very useful in deciding whether to continue therapy, because of the low likelihood of response in patients who remain HBV DNA positive at month 3 of treatment.

Lamivudine, the first specific oral antiviral therapy for chronic hepatitis B, has been proven safe and efficacious in controlled studies, but the appropriate duration of lamivudine therapy has not been defined, especially in anti–hepatitis B e (HBe)–positive patients [1–4]. In HBe antigen (HBeAg)–positive chronic hepatitis, an established parameter for discontinuing therapy is HBeAg clearance and seroconversion to anti-HBe, because it indicates a sustained response. Studies using lamivudine for 1 year demonstrated a 20% HBeAg negativity rate, and prolonging therapy to 3 years increased HBeAg loss to 40% (and even higher in patients with elevated alanine aminotransferase [ALT] levels) [2, 4–6]. Prolonging lamivudine therapy is associated with the emergence of variants in the highly conserved YMDD motif of the hepatitis B virus (HBV) polymerase [7, 8]. The emergence of these variants increased from 14% with 1 year of lamivudine therapy to 49% with 3 years of therapy [9]. These variants have been associated with a lower sensitivity to lamivudine, as indicated by abnormal ALT levels and serum HBV DNA positivity.

More than 90% of patients receiving lamivudine therapy achieve a rapid and significant inhibition of viremia [1–3]. Reports of HBV kinetics during lamivudine therapy are scarce, and the role of late clearance of HBV DNA in response has not been extensively evaluated [10, 11]. This could be an important issue in identifying patients for whom prolonged therapy might be associated with an increase in sustained HBV DNA suppression.

The aim of this study was to determine whether measuring quantitative HBV DNA early (i.e., at month 3) during lamivudine therapy in patients with chronic hepatitis B is useful in predicting maintenance of response to therapy and the emergence of YMDD variant HBV species.

Patients and Methods

Patients. Thirty-five patients (25 men and 10 women; mean age, 46.1 years; range, 24–62 years) with HBV DNA–positive chronic hepatitis B (15 HBeAg positive and 20 anti-HBe positive) and elevated ALT levels (mean, 133 U/L; range, 49–364 U/L) were treated orally with 100 mg of lamivudine (Zeffix; Glaxo Wellcome) once daily for >1 year (range, 12–24 months) in an open-label trial. A pretreatment liver biopsy that was performed in 31 patients revealed chronic active hepatitis in 22 and cirrhosis in 9. Patients were evaluated every 3 months during lamivudine therapy, and a blood sample was obtained at each visit. Patient responses to lamivudine therapy were classified as "initial," "maintained," or "non" responses. Initial response was defined as the clearance of serum HBV DNA in 2 consecutive hybridization assay (branching DNA [bDNA]) determinations. Maintained response was defined as undetectable serum HBV DNA throughout the therapy period (≤1 year). Nonresponse was defined as the persistence of serum HBV DNA or reappearance after an initial clearance.
Serologic markers. HBsAg was assayed by using a commercial immunoassay (Abbott-Auszyme Mc; Abbott Laboratories). HBsAg and anti-HBe were detected by using RIA (DiaSorin).

Quantitation of serum HBV DNA level. Two methods were used to determine the serum HBV DNA level. The first method, a conventional chemiluminescent bDNA oligonucleotide assay (Quantiplex; Chiron) has a lower limit of detection of $7.0 \times 10^3$ genome/mL. HBV DNA was tested by using this technique on entry and later, to assess the response to therapy.

The second method used to determine the HBV DNA level was a real-time polymerase chain reaction (PCR) assay, in which HBV DNA was extracted from serum by using spin columns (QiAamp DNA mini columns; Qiagen), and their concentrations were determined by using a programmable DNA high-speed Thermal Cycler (LightCycler; Roche Diagnostics). The oligonucleotide sequences of primers corresponding to the HBV core gene were as follows: HBVE, 5'-GACCACCAAAAGCCCGCTAT-3' (2299–2316), and HBVR, 5'-GGAGATTGAGATCTTCTGCGAC-3' (2442–2421). Hybridization was done by using 2 different short oligonucleotides that hybridize to 2 adjacent internal sequences of the amplified PCR fragment. One probe, HBVLC, 5'-CGAGATTGAGATCTTCTGCGAC(AG/ C)AG(A/G)TCTC-3' (2386–2411), is labeled at the 5' end and is phosphorylated at the 3' end; sequencing reactions were analyzed in a DNA sequencer (ABI PRISM 310; Perkin-Elmer Cetus) and with a red fluorophore (LightCycler 640; Roche Diagnostics). The other probe, HBVF1, 5'-GA(G/C)CGAGATTGAGATCTTCTGCGAC-3' (2361–2384), was labeled with fluorescein at the 3' end. The nucleotide sequences have been numbered according to genotype C (GenBank accession no. V00867). The PCR conditions were 4 mM MgCl$_2$, 3 pmol of each hybridization probe, 20 pmol of 2 PCR primers, 2 mL of hybridization probe mix (LightCycler Fast Start DNA Master probe mix; Roche Diagnostics), and 10 mL of HBV DNA samples, in a total volume of 20 mL.

As a standard for quantification, we used HBV DNA plasma standard containing $\sim 4.4 \times 10^4$ HBV DNA genome copies/mL (HBV DNA Quantiplex; Chiron). From the plasma standard, we prepared 6 serial dilutions in negative plasma to obtain concentrations between $10^3$ and $10^6$ HBV DNA genome copies/mL. The detection limit of this assay was $\sim 1 \times 10^3$ genomes/mL. This method was used to determine quantitative HBV DNA at month 3 of treatment. Results are expressed as mean log$_{10}$ values (HBV DNA copies/mL).

Detection of HBV variants. A 779-bp fragment of HBV DNA polymerase gene, including the YMDD motif, was amplified by PCR. The amplified products were purified and sequenced, as described elsewhere [12].

Statistical analysis. Student’s t test, Mann-Whitney U rank sum test, and Fisher’s exact 2-sided test were used to analyze quantitative and qualitative differences between groups. P < .05 was considered to be statistically significant. All data analyses were done by using SPSS for Windows (version 9.0; SPSS).

Results

HBV DNA became negative in 26 patients (74%) and was associated with normal ALT levels in 22 (63%) at the end of 1 year of lamivudine therapy. Three of 4 patients negative for serum HBV DNA and with abnormal ALT levels after 1 year of treatment became HBV DNA positive and developed YMDD variants during prolonged therapy (2 patients by month 15 and 1 by month 18). Three patients (20%) lost HBsAg, and 2 (13%) seroconverted to anti-HBe. No patients lost HBsAg.

HBV DNA polymerase variants were detected in 15 (44%) of the 34 patients studied at year 1. Of these, 9 were HBV DNA positive, as determined by bDNA, and 11 had elevated ALT levels at 1 year (3 with ALT levels higher than at baseline). Thus, YMDD variants were detected in 9 (69%) of 13 nonresponders and in 6 (27%) of 22 responders ($P = .020$).

At month 3 of therapy, serum HBV DNA levels became undetectable in 20 cases (57%), with a median decrease of 3.95 ± 1.16 (range, 6.43–0.22). HBV DNA levels at month 3 were lower among responders than among nonresponders (3.55 ± 0.97 vs. 5.69 ± 2.05; $P = .003$), with a median decline of 4.16 ± 1.06 in virologic responders and 2.88 ± 1.77 in nonresponders ($P = .04$; figure 1). Maintenance of response to therapy was associated with the absence of HBV DNA at month 3, which was undetectable in 17 (77%) of 22 responders and in 3 (23%) of 13 nonresponders ($P = .002$).

Quantitative HBV DNA testing at month 3 of therapy that shows a negative result has a sensitivity of 73% and a specificity of 88% for the early prediction of a maintained response to lamivudine. The positive predictive value of the test is 95%, with an accuracy of 74%.

Baseline HBV DNA levels were higher in the 15 patients with YMDD variants after 1 year of treatment than in the 19 patients without the variants (8.42 ± 1.16 vs. 7.58 ± 1.01; $P = .033$). HBV DNA levels at month 3 were also higher in patients with YMDD variants than in those without (4.55 ± 1.69 vs. 3.70 ± 1.5, respectively; $P$ was not significant). However, no differences were detected in HBV DNA decline (3.86 ± 1.52 vs. 3.80 ± 1.32; $P$ was not significant) during the first 3 months of therapy.
therapy between those with or without YMDD variants at year 1 (figure 2).

Overall, quantitative HBV DNA became negative at month 3 of treatment in 6 (40%) of the 15 patients with YMDD variants and in 14 (74%) of the 19 without the variants at year 1 \((P = .041)\). Despite the emergence of YMDD variants, 6 patients were considered to be maintained responders, of whom 4 (67%) had undetectable HBV DNA levels at month 3 of treatment. In patients without YMDD variants, HBV DNA was negative at month 3 in 87% of the responders and in 25% of the nonresponders \((P = .03)\).

Negative HBV DNA by quantitative testing at month 3 of therapy has a sensitivity of 60% and a specificity of 73% for the early prediction of the emergence of YMDD variants. The accuracy of the test is 42%, with a positive predictive value of 64%.

Discussion

Two studies have suggested that the monitoring of virus load can predict the later emergence of lamivudine-resistant HBV strains during therapy \([10, 11]\). Virus loads \(>4.0 \log_{10}\) HBV DNA copies/mL were detected after 3 months in all patients who seroconverted to HBeAg, whereas HBV DNA levels became undetectable in patients who did not seroconvert during 18 months of therapy \([10]\). In our study, we found a correlation between HBV DNA levels at month 3 of lamivudine therapy and maintenance of response to therapy. In addition, there were small differences in HBV DNA loads at month 3 between patients with and without YMDD variants, but we were able to define a specific HBV DNA level \(<1000 \text{ copies/mL}\) as a predictor of the emergence of YMDD variants. HBV DNA levels \(>1 \times 10^3 \text{ copies/mL}\) at this time indicated no response to lamivudine despite continuous therapy. This will be one of the most useful applications of quantitative HBV DNA testing, since it permits the selection of patients who will benefit from prolonged lamivudine therapy, especially for patients who are anti-HBe positive.

Our results suggest that early quantitative HBV DNA testing could identify different virologic outcomes in patients receiving lamivudine therapy. Those patients who became HBV DNA negative by month 3 were more likely to maintain response to prolonged lamivudine therapy than were those who were HBV DNA positive at 3 months. Patients who were HBV DNA positive by 3 months had a lower chance of achieving a virologic response, despite extended therapy, and had a risk for the emergence of YMDD variants.

This study also made an interesting observation about patients with undetectable HBV DNA and elevated ALT levels at 1 year of therapy and who continued with lamivudine therapy. Most such patients (75%) became HBV DNA positive, despite therapy, which suggests that elevated ALT levels after 1 year of lamivudine treatment are associated with a future loss of response. If these results are confirmed by more studies, these patients could benefit from the addition of other antiviral drugs to maintain response.

Our results also demonstrated that quantitative HBV DNA testing has a 95% rate for positive prediction of the maintenance of the response to lamivudine and a 64% rate for positive prediction of the emergence of YMDD variants. Therefore, quantitative HBV DNA testing is very useful in making an early prediction of maintenance of virologic response.

The real-time PCR provides a rapid and accurate means of studying HBV DNA levels and the emergence of YMDD variants, 2 important factors in determining therapeutic strategies for patients with chronic HBV receiving lamivudine therapy \([13, 14]\). The appearance of new drugs effective against HBV, such as adefovir and entecavir, could be especially beneficial for lamivudine nonresponders, and quantitative HBV DNA testing will be very useful in different therapeutic approaches to optimize hepatitis B treatment \([15]\).

Figure 2. Hepatitis B virus (HBV) DNA profile of 34 patients with chronic hepatitis B, according to emergence of HBV variants after 1 year of lamivudine therapy. Data are mean HBV DNA levels \((\log_{10} \text{ copies/mL})\) for 15 patients with ( ) and 19 patients without ( ) YMMD variants at 1 year.

References


