MAJOR ARTICLE

Prevalence and Clinical Correlates of YMDD Variants during Lamivudine Therapy for Patients with Chronic Hepatitis B

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YMDD variants of hepatitis B virus (HBV) emerge in some patients with chronic hepatitis B who receive lamivudine. YMDD variants were examined in 794 patients in 4 controlled studies of 1 year's duration. The long-term effects of YMDD variants were examined in a subset of patients treated up to 4 years. YMDD variants were detected by polymerase chain reaction (PCR) and restriction fragment-length polymorphism assays. After 1 year, YMDD variants were detected in 81 (24%) of 335 patients. In these patients, the median serum HBV DNA concentration at 1 year was <20% of the baseline level, and serum alanine transaminase (ALT) levels and liver histologic findings had significantly improved. In patients with YMDD variants who were treated for up to 4 years, median HBV DNA and ALT levels showed improvements. Sex, baseline body mass index, and HBV DNA level were associated with emergence of YMDD variants. Patients with YMDD variants losing clinical response with a significant increase in the HBV DNA and ALT levels may require additional therapy.

Viral quasi species in chronic viral infections may contain variants that evade host immune responses or resist antiviral-agent inhibition [1–7]. Such resistant variants that emerge during HIV infection are associated with disease progression [8]; however, in patients with chronic hepatitis B, the link between emergence of variants and progression of liver disease is less predictable [9].

Hepatitis B virus (HBV) replication requires the activity of HBV DNA polymerase [10], the molecular target of antiviral nucleoside analogues [11]. Spontaneous polymerase mutations occur naturally. HBV polymerase does not have a proofreading mechanism [12, 13]; therefore, the fidelity of replication is imperfect. Prolonged therapy with the nucleoside analog lamivudine selects for HBV variants with amino acid changes in the YMDD motif (tyrosine [Y], methionine [M], aspartate [D], and aspartate [D] amino acid residues at position 552 of the HBV DNA polymerase) of the polymerase protein [14] that have >10,000-fold reduced susceptibility to lamivudine in vitro [15, 16]. Compared with wild-type HBV, however, YMDD variants have reduced affinity for nucleotide substrates

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[17], circulate at lower serum titers [18], and appear to be functionally impaired, with potentially less capacity for causing disease.

Controlled clinical trials involving patients receiving lamivudine therapy provided an opportunity to investigate the frequency of YMDD variants as well as the impact of these variants on virologic, biochemical, and histologic outcomes associated with therapy [19]. In this report, we assess the prevalence of YMDD variants during lamivudine therapy, the effect of these variants on drug efficacy and safety, and host variables that influence their emergence.

PATIENTS AND METHODS

Patient populations. Data from 4 multicenter, controlled, phase 3 trials [7, 20–22] were combined to yield a study population comprising 967 patients, 558 of whom received lamivudine monotherapy for 1 year (table 1). Entry criteria included chronic hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) seropositivity for >6 months, HBV DNA detectability (by the Abbott Genostics solution hybridization assay), a serum alanine aminotransferase (ALT) level <10 times the upper limit of normal, age of >18 years, and no evidence of hepatic decompensation. Most patients in the Asian multicenter study B3009 [7] were enrolled into a follow-up study, study B3018 [23, 24], for up to 208 weeks; we included them to assess YMDD variants in patients undergoing extended lamivudine therapy. Studies were approved by local ethics committees, and all patients provided written, informed consent before enrolling in the follow-up study.

Analysis of HBV variants. In the 4 phase 3 studies, HBV variant analyses were performed on serum samples at study week 52 or at the end of treatment and 12–16 weeks after completion of treatment. Patients in whom YMDD variants were detected at week 52 were tested to determine when the variants first appeared. HBV variants were determined by PCR amplification of DNA extracted from serum samples with a restriction fragment-length polymorphism assay [25] that can detect variant or wild-type virus in a mixed population if either is present as ⩾5% of the total population.

Statistical methods. Data from the 4 phase 3 studies were integrated to examine the effect of YMDD variants on the following protocol-defined end points: histologic response (⩾2 point decrease between baseline and week 52 in histologic activity index [HAI]), HBeAg seroconversion (loss of HBeAg and acquisition of antibody [anti-HBe]), serum ALT level (⩾2 consecutive normal ALT measurements obtained ⩾7 days apart, in patients with elevated baseline ALT levels), serum HBV DNA (loss of detectable serum HBV DNA, as determined by solution hybridization assay), and safety parameters (serious adverse event [SAE], as defined by World Health Organization criteria) after 1 year of lamivudine therapy. "Continued" HBV DNA or ALT responses were defined as HBV DNA or ALT responses maintained subsequently with no 2 consecutive detectable HBV DNA measurements or abnormal ALT values and with undetectable HBV DNA or normal ALT values at week 52.

Results were presented as the proportion of patients with a response displayed by treatment group (the placebo group, the lamivudine variant group, and the lamivudine nonvariant group). Exploratory logistic regression analyses were prepared

Table 1. Clinical studies of patients with hepatitis B who received lamivudine therapy.

Study	Study group and criteria	Location of study	Study design	Treatment arm
B3009 [7]	358 Patients who were not previously treated for hepatitis B; enrollment required abnormal liver histologic findings but permitted normal baseline alanine aminotransferase levels	Hong Kong, Singapore, Taiwan	Randomized, double blind, placebo controlled	Lamivudine (100 mg once daily) for 12 months (n = 143); lamivudine (25 mg once daily) for 12 months (n = 142); placebo for 12 months (n = 73)
A3010 [20]	141 Patients who had not previously been treated for hepatitis B	United States	Randomized, double blind, placebo controlled	Lamivudine (100 mg once daily) for 12 months (n = 70); placebo for 12 months (n = 71)
B3010 [21]	230 Patients who had not previously been treated with IFN for hepatitis B	Europe, Canada, South Africa, Australia, New Zealand	Randomized, partially blinded	Lamivudine (100 mg once daily) for 12 months (n = 84); IFN-α (10 MU 3 times weekly; 2 months of placebo, followed by 4 months of IFN-α; n = 70); lamivudine–IFN-α combination (2 months of lamivudine, followed by 4 months of combination therapy; n = 76)
AB3011 [22]	238 Patients whose illness failed to respond to IFN-α therapy	United States, Europe	Randomized, placebo controlled, partially blinded	Lamivudine (100 mg once daily) for 12 months (n = 119); placebo for 12 months (n = 56); lamivudine–IFN-α combination (2 months of lamivudine, followed by 4 months of combination therapy; n = 63)
B3018 [23, 24]	331 Patients; continuation of protocol for patients who completed study B3009	Hong Kong, Singapore, Taiwan	Randomized, double blind, placebo controlled	Lamivudine (100 mg once daily) for 12 months (n = 133); lamivudine (25 mg once daily) for 12 months (n = 132); placebo (n = 66)[a]

[a] Patients who received lamivudine in B3009 were randomized to continue therapy or to switch to placebo; patients who received placebo in B3009 received lamivudine (100 mg) in B3018. Four-year data refer to the 58 patients assigned to receive 5 years of therapy with lamivudine (100 mg).

Table 2. Hepatitis B virus (HBV) genotypes found after 1–4 years of lamivudine therapy.

Study	No. (%) of patients			
	HBV DNA negative	Infected with wild-type HBV	Infected with mixed HBV type[a]	Infected with YMDD-variant HBV[b]
Integrated 1-year data[c]	63 (19)	191 (57)	33 (10)	48 (14)
A3010[d]	9 (20)	21 (48)	1 (2)	13 (30)
B3010[d]	13 (21)	29 (48)	8 (13)	11 (18)
AB3011[d]	34 (34)	38 (38)	10 (10)	17 (17)
B3009[d,e]	7 (5)	103 (79)	14 (11)	7 (5)
B3018, study year[f]				
2 (n = 74)	17 (23)	26 (35)	7 (9)	24 (32)
3 (n = 51)	10 (20)	14 (27)	10 (20)	17 (33)
4 (n = 43)	5 (12)	8 (19)	12 (28)	18 (42)

[a] Both YMDD-variant HBV and wild-type HBV were detectable.
[b] Only YMDD-variant HBV was detectable.
[c] Combined data after 1 year of therapy in A3010, B3010, AB3011, and the lamivudine (100 mg) group in B3009.
[d] After 1 year of therapy.
[e] Study of therapy with lamivudine, 100 mg/day.
[f] Data refer to patients randomized to receive 5 years of therapy with lamivudine (100 mg).

to assess efficacy responses observed in the lamivudine variant and nonvariant groups. Because the groups being compared in the integrated 1-year analysis were not randomized, baseline variables were adjusted in comparing the variant and nonvariant subgroups. In addition, pairwise correlations between efficacy parameters and the presence or absence of variants were presented to explore which efficacy parameters were affected by YMDD variants. Similar analyses were performed to compare the lamivudine-variant group to the placebo group. We also performed logistic regression analyses to explore the effects of baseline and demographic variables as predictors of YMDD-variant development.

Samples of HBV DNA were categorized as wild type, YMDD variant, mixed (wild-type and YMDD variants detected), or DNA-negative (HBV DNA undetectable by PCR). For most analyses, YMDD-variant and mixed categories were combined as YMDD-variant, whereas wild-type and DNA-negative categories were combined as nonvariant.

RESULTS

Incidence of YMDD Variants

Serum samples were available for analysis from 794 of the 967 study patients. Of the 558 patients who received lamivudine monotherapy in the 1-year phase 3 studies, 468 (84%) were analyzed for the presence of HBV variants at week 52. The overall 1-year incidence of YMDD variants in the lamivudine group (n = 426) was 24%, ranging in individual studies from 15% to 32%, with the lowest in the Asian trial [7] (table 2). YMDD variants, which were identified only in patients receiving lamivudine monotherapy (never in recipients of placebo, interferon monotherapy, or 6 months of lamivudine-interferon combination therapy), were seldom detected before 36 weeks of therapy.

Among the 93 patients treated with lamivudine (100 mg) for 2 years, YMDD variants were detectable in 31 (42%) of the 74 with data available for analysis at week 104 [23]. Among the 58 patients randomized to receive 4 years of lamivudine, YMDD variants were detectable in 53% and 70% after 3 and 4 years, respectively [24]. In the only study comparing lamivudine doses of 25 mg and 100 mg [7], the 1-year incidences

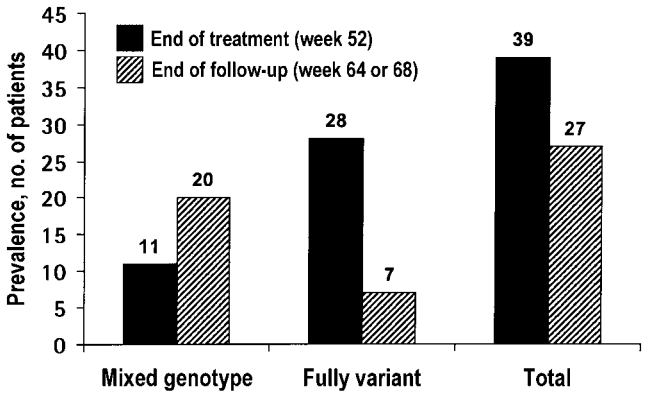

Figure 1. Proportion of patients infected with mixed wild-type and YMDD-variant hepatitis B virus (HBV) or YMDD-variant HBV that was detected at the end of 52 weeks of lamivudine therapy and at the end of 12 or 16 weeks' posttreatment follow-up (integrated phase 3 data).

Table 3. Effects of YMDD variants on protocol-defined responses (1-year integrated data).

Response	Placebo group	Lamivudine group	
		YMDD-variant HBV	Nonvariant HBV
HAI response	48/156 (31)	43/84 (51)[a]	199/324 (61)[a]
HBeAg seroconversion	16/196 (8)	8/100 (8)	75/363 (21)[b]
Continued ALT response	25/173 (14)	28/96 (29)[c]	192/279 (69)[d]
Continued HBV DNA response	22/194 (11)	15/99 (15)[e]	188/346 (54)[d]

NOTE. Data are no. of patients with response/no. of patients who received agent or placebo (%). ALT, alanine aminotransferase; HAI, histologic activity index; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.

[a] $P < .003$, compared with the placebo group.
[b] $P < .006$, compared with the YMDD-variant group.
[c] $P < .005$, compared with the placebo group (adjusted for baseline factors).
[d] $P < .001$, compared with the YMDD-variant group.
[e] $P = .04$, compared with the placebo group (adjusted for baseline factors).

of YMDD variants were 14% (19 of 133 patients) and 16% (21 of 131 patients), respectively.

YMDD variants were detectable in fewer patients at the end of a 12–16-week posttreatment follow-up period compared with the end of therapy (figure 1). Moreover, most patients with YMDD variants at the end of follow-up had mixed wild-type/YMDD-variant HBV, whereas fully YMDD variant populations were more common at the end of therapy. Thus, when lamivudine therapy was stopped, YMDD-variant HBV tended to revert to wild-type HBV.

Effects of YMDD Variants on Efficacy End Points

HBeAg seroconversion. The emergence of YMDD variants during 1 year of lamivudine therapy did not preclude HBeAg seroconversion in phase 3 studies; however, the rate in the YMDD-variant group was indistinguishable from that in placebo recipients (table 3). During 1 year of lamivudine therapy, the nonvariant group had a significantly higher HBeAg seroconversion rate than did the YMDD-variant group (21% vs. 8%; $P < .006$), a difference that continued during 4 years of lamivudine therapy in study B3018 (figure 2).

Serum ALT level. During 1 year of lamivudine therapy, the median serum ALT level was lowest in lamivudine-treated patients in the nonvariant group, and, in lamivudine-treated patients with YMDD variants, it remained less than that for placebo recipients (figure 3). These differences persisted as therapy extended to 4 years (figure 4). "Continued" ALT responses were maintained in lamivudine recipients in the YMDD-variant group (29%) less frequently than in lamivudine recipients in the nonvariant group (69%; $P < .001$) but more frequently than in placebo recipients (14%; $P < .005$; table 3).

During 1-year studies, the median decrease in the ALT level was substantially greater in lamivudine recipients regardless of whether YMDD variants were present (41%) or absent (60%), compared with placebo recipients (15%). Improved ALT values persisted in most patients, even with the extended presence of YMDD variants. Among all patients receiving lamivudine in study B3018 who acquired YMDD variants, the median ALT level at the end of 2 years of therapy was less than the baseline level and within the normal range (table 4); however, the magnitude of improvement diminished as the duration of exposure to YMDD variants increased. Even among the subset of study B3018 patients treated for 3 years who harbored YMDD variants for ⩾2 years, the median ALT level remained less than the baseline level (figure 5).

Serum HBV DNA. In 1-year studies, the median baseline

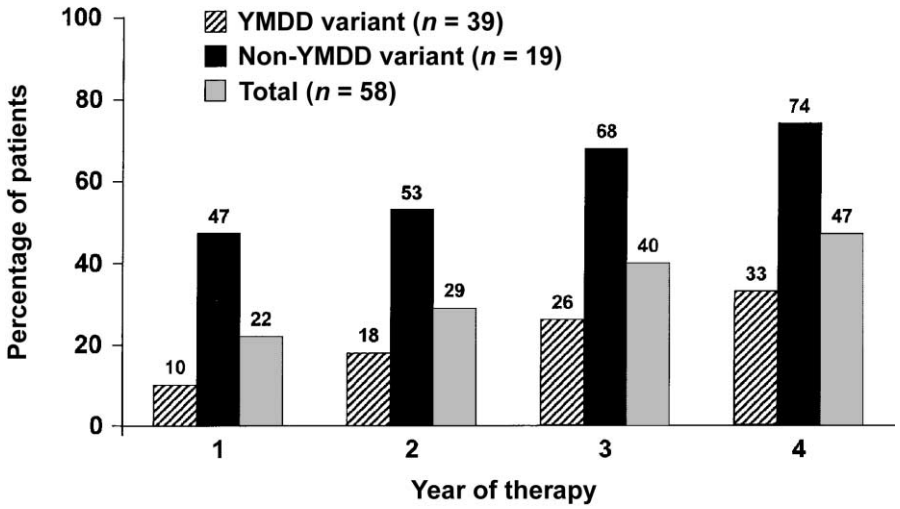

Figure 2. Proportion of patients with hepatitis B e antigen seroconversion at the end of 1–4 years of therapy with lamivudine (100 mg), analyzed with respect to whether YMDD-variant hepatitis B virus was detectable (Asian extended treatment study).

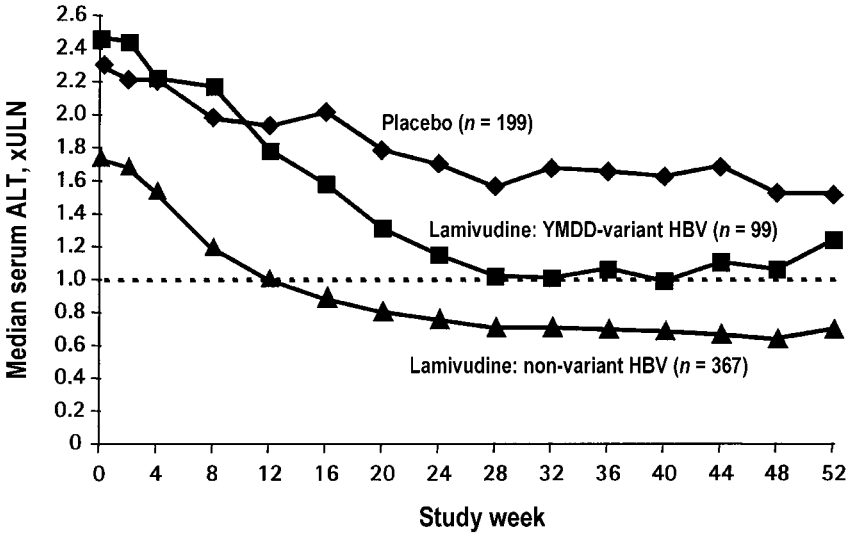

Figure 3. Median serum alanine aminotransferase (ALT) level during 1 year of lamivudine therapy in patients with and without detectable YMDD-variant hepatitis B virus (HBV) infection at the end of the year (integrated phase 3 data). ULN, upper limit of normal.

HBV DNA value was similar in the placebo (78.5 pg/mL) and variant-free lamivudine groups (75.4 pg/mL), but it was higher (137.8 pg/mL) in the YMDD-variant group. Similarly, among 51 patients treated for 3 years, the median baseline HBV DNA value was 33 pg/mL for the variant-free group, and it was 67 pg/mL for the variant group.

After 1 year of therapy, median HBV DNA concentrations were 28.5 pg/mL in placebo recipients, 22.0 pg/mL in the YMDD-variant group, and 1.2 pg/mL (less than the assay's detection limit) in the nonvariant group. The median 1-year decrease in the serum HBV DNA level was 46% in the placebo group, 81% in the YMDD-variant lamivudine group, and 97% in the nonvariant lamivudine group. Continued HBV DNA responses were maintained in the YMDD-variant group (15%) less frequently than in the nonvariant lamivudine group (54%; $P < .001$) but more frequently than in the placebo group (11%; $P = .04$; table 3).

In study B3018, after 2 years of therapy, 88% of patients in the YMDD-variant group had HBV DNA levels that were less than the baseline level. Even among the subset of patients treated for 3 years who harbored YMDD variants for ⩾2 years, the median HBV DNA level remained less than the baseline level (figure 6).

Histologic response. In phase 3 studies, 1 year of lamivudine therapy improved liver histologic findings significantly (⩾2 point HAI reduction) compared with placebo, regardless of whether YMDD variants were detected ($P < .003$; table 3). Results of liver biopsies were available at baseline and at the end of 1 and 3 years of therapy for 13 patients, 9 of whom harbored YMDD variants at the end of 3 years [25]. Compared

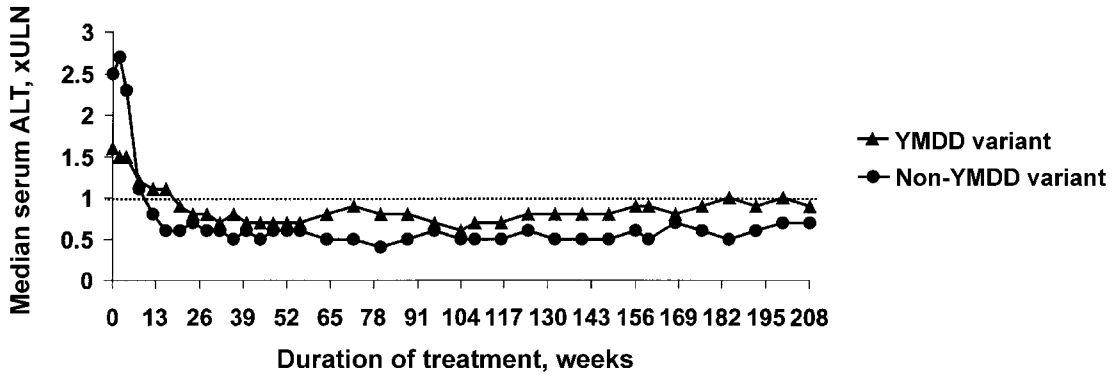

Figure 4. Median serum alanine aminotransferase (ALT) level during 4 years of lamivudine therapy (Asian extended therapy study). ULN, upper limit of normal.

Table 4. Effects of YMDD variants on alanine aminotransferase (ALT), bilirubin, and hepatitis B virus (HBV) DNA levels during 2 years of lamivudine therapy (B3018 study).

Laboratory parameter	Median value (range)	
	Nonvariant HBV group	YMDD-variant HBV group
ALT level, ×ULN		
Baseline	1.2 (0.3–10.8)	1.7 (0.4–15.5)
Week 52	0.7 (0.2–12.3)	0.7 (0.2–27.7)
Week 104	0.6 (0.2–4.5)	0.9 (0.1–9.1)
Bilirubin level, ×ULN		
Baseline	0.5 (0.2–2.5)	0.6 (0.2–1.6)
Week 52	0.5 (0.1–2.2)	0.6 (0.3–2.6)
Week 104	0.5 (0.1–3.1)	0.6 (0.3–1.6)
HBV DNA level, pg/mL		
Baseline	78.6 (≤0.8 to 990.1)[a]	79.8 (≤0.8 to 762.7)
Week 52	2.5 (≤0.8 to 580.2)	1.3 (≤0.8 to 178.2)
Week 104	0.8 (≤0.8 to 104.0)	7.3 (≤0.8 to 128.2)

NOTE. ULN, upper limit of normal.

[a] The lower limit of detection for the assay is 0.8 pg/mL.

with baseline, 3-year histologic findings in YMDD-variant patients were improved in 5 of 9 patients, unchanged in 2 of 9 patients, and worse (≥2-point HAI increase) in 2 of 9 patients. Compared with the findings of the year 1 biopsy, however, histologic findings deteriorated at 3 years in 6 of 9 patients in the YMDD-variant group.

Effects of YMDD Variants on Safety

Adverse events. In 1-year studies, the incidence of adverse events (the most frequent of which were viral respiratory infection, malaise or fatigue, abdominal discomfort, cough, and headache) was similar in lamivudine-treated patients both during treatment, with (86 [86%] of 100) or without (294 [80%] of 368) YMDD variants, and after treatment, with (28 [62%] of 45) or without (69 [58%] of 119) variants. A slightly higher frequency of malaise occurred in the YMDD-variant group (37 [37%] of 100) than the nonvariant group (81 [22%] of 368). With more-prolonged lamivudine therapy (study B3018), the presence of YMDD variants did not affect the incidence and nature of adverse events.

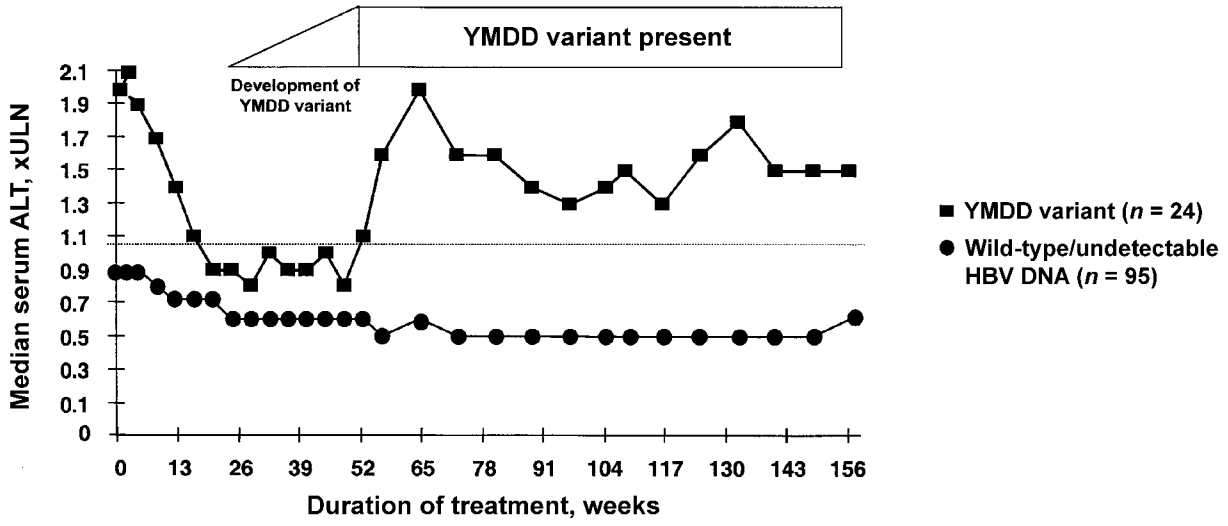

Figure 5. Median serum alanine aminotransferase (ALT) level during 3 years of daily lamivudine (100 mg) therapy (Asian extended therapy study) in the subset of study participants in whom YMDD variants developed during year 1 of therapy and persisted for ≥2 years. HBV, hepatitis B virus; ULN, upper limit of normal.

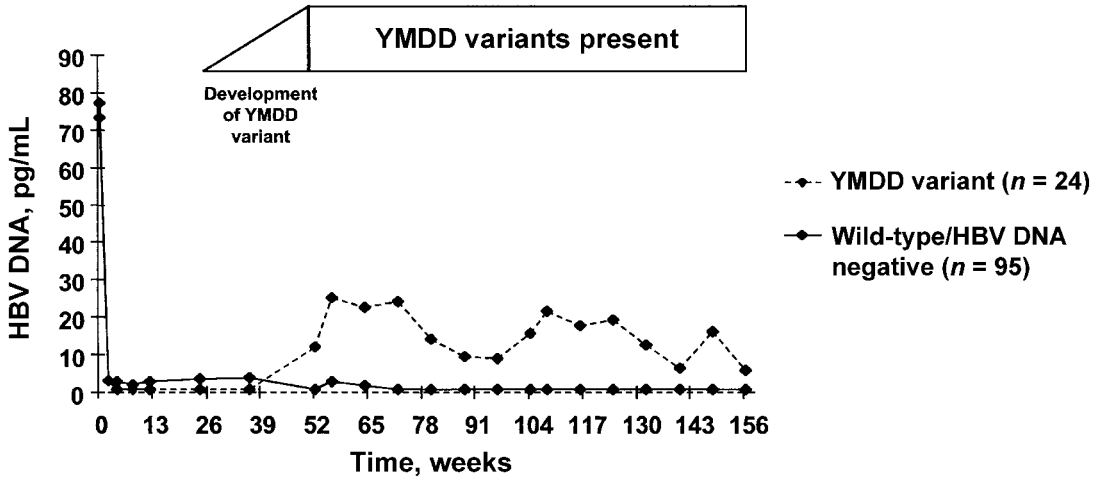

Figure 6. Median serum hepatitis B virus (HBV) DNA level, as measured by hybridization assay, during 3 years of daily lamivudine (100 mg) therapy (Asian extended therapy study) in the subset of study participants in whom YMDD variants developed during year 1 of therapy and persisted for ⩾2 years. Median HBV DNA values at week 156 were significantly lower than the baseline value ($P < .001$) for the YMDD-variant group.

Laboratory parameters. Two-fold and 3-fold ALT elevations during treatment were more common in patients with YMDD variants than in those without variants (table 5); however, no increase in the most severe category of ALT elevation (i.e., bilirubin elevation) occurred in patients with variants. ALT elevations that occurred after the completion of treatment were less common in patients harboring fully variant HBV than among those harboring either mixed HBV types or nonvariant HBV.

During year 2 of lamivudine therapy in study B3018, 4 patients with ALT elevations had concomitant bilirubin elevations and had YMDD variants at week 104. One event was associated with HBeAg seroconversion. All events resolved spontaneously.

SAEs. Of 967 patients in the 1-year trials, 12 with YMDD variants had SAEs reported: 3 ALT elevations (2 with transient hyperbilirubinemia) when YMDD variants emerged during treatment and that resolved spontaneously with continued treatment; 3 posttreatment ALT elevations coinciding with re-emergence of wild-type HBV in 2 patients (1 of whom had a transient 14.7-s prothrombin time, a bilirubin level of 2.0 mg/dL, and later seroconversion after 3 weeks of lamivudine therapy) [26]; 2 instances of asymptomatic emergence of YMDD variants without ALT elevations (reported by study investigators as SAEs); and 4 SAEs unrelated to the emergence of the YMDD variants (drug overdose, ALT elevation 14 days into treatment, elevated creatine phosphokinase level, and symptomatic cholelithiasis).

In study B3018, SAEs were reported for 16 patients with YMDD variants in the second year of lamivudine therapy: 14 patients (88%) had ALT elevations that resolved spontaneously

Table 5. Elevations in the alanine aminotransferase (ALT) level during and after lamivudine therapy.

	During therapy				After therapy			
ALT response definition	No YMDD group[a] (n = 362)	Mixed-variant group[b] (n = 45)	YMDD-variant group[c] (n = 55)	Placebo group (n = 166)	No YMDD group[a] (n = 118)	Mixed-variant group[b] (n = 12)	YMDD-variant group[c] (n = 33)	Placebo group (n = 52)
⩾2 times the baseline level	16	27	40	30	34	50	6	17
⩾3 times the baseline level	8	20	27	14	25	42	3	6
⩾2 times the baseline level and >500 U/L	3	11	13	7	17	42	3	6
⩾2 times the baseline level; bilirubin level of >2 times the ULN and ⩾2 times the baseline level	0	4	0	<1	2	8	0	2

NOTE. Data are percentage of total patients. HBV, hepatitis B virus; ULN, upper limit of normal.

[a] Lamivudine treatment group; HBV was wild-type or undetectable.

[b] Lamivudine treatment group; mixed population of wild-type and YMDD-variant HBV was detected.

[c] Lamivudine treatment group; only YMDD-variant HBV was detected.

without change in treatment (1 elevation occurred before treatment, 9 occurred during treatment [5 within 12 weeks after the first detection of YMDD variants], and 4 occurred after treatment), and 2 patients had SAEs that were unrelated to the emergence of the YMDD variants (one patient had creatine phosphokinase elevation after trauma, and the other had an adenoid carcinoma). Throughout 4 years of lamivudine therapy in study B3018, the proportion of patients with liver disease–related SAEs was similarly low in patients with and without YMDD variants. One death, which was due to acute bacterial peritonitis, occurred after the emergence of a YMDD variant.

Predictors of YMDD Variant Emergence

In 1-year trials, YMDD variants correlated positively with weight ($P = .0008$), body mass index ($P = .004$), and male sex, baseline HBV DNA level, height, and age ($P < .10$). Sex, age, ethnicity, body mass index, cirrhosis, HAI, necroinflammatory HAI, ALT level, bilirubin level, and HBV DNA level were the candidate variables with a significance level of $P = .10$ (specified for inclusion in the model) that were investigated in a stepwise regression analysis. Asian ethnicity ($P = .0008$) and female sex ($P = .01$), the only significant regressors, were associated with a lack of emergence of YMDD variants.

In a second regression model, in which ethnicity was removed as a candidate variable to identify alternative explanations for the effect of ethnicity, high baseline HBV DNA level, high body mass index, and male sex were significant, independent predictors ($P < .03$) for the emergence of YMDD variants. Male sex correlated significantly ($P < .05$) with higher baseline ALT level, HAI, necroinflammatory score, and HBV DNA level, suggesting that disease severity contributed to the effect of sex.

Detection of Clinically Important YMDD Variants

An algorithm was developed for identifying clinically important YMDD variants with standard serum ALT and HBV DNA data, without the need to test for YMDD-variant HBV. In patients who have completed $\geqslant$24 weeks of lamivudine therapy, the combined presence of an ALT level of >1.3 times the upper limit of normal and an HBV DNA level of >20 pg/mL (as determined by solution hybridization) was predictive of the presence of YMDD variants.

During 1-year studies, the sensitivity of the algorithm for identifying YMDD variants was modest (60 [60%] of 100 cases); however, the false-positive rate was only 3% (12 of 368 cases), and the positive predictive value was 77% (40 of 52 cases). Thus, the algorithm could identify patients with YMDD variants who were most in need of medical attention—that is, those with elevations in the ALT and HBV DNA levels.

This algorithm was also explored for its ability to predict longer-term clinical outcomes in the B3018 extended-treatment study. Of the study patients, 13 (22%) of 58 met the ALT and HBV DNA criteria at least once during the first 2 years of treatment, and 9 (69%) of 13 patients had YMDD variants; ALT and HBV DNA levels remained elevated in 6 (46%) of 13 patients 3 months after satisfying the algorithm, but the levels remained elevated in only 4 (31%) of 13 patients by the end of treatment year 3. In 3 (23%) of 13 patients, HBeAg loss occurred. Thus, although most HBeAg-positive patients who met the ALT and HBV DNA criteria had detectable YMDD variants, the algorithm was not a reliable predictor of subsequent changes in serologic HBV markers or necroinflammatory activity.

DISCUSSION

As supported by reduced in vitro replication competence of YMDD variants [15–17], clinical and laboratory findings suggest that YMDD variants are functionally impaired. After lamivudine therapy is withdrawn from patients who harbor YMDD variants, wild-type HBV usually reemerges within months as the dominant virus strain. Moreover, in patients with YMDD variants, the presence of persistently reduced serum HBV DNA levels suggests that YMDD variants do not replicate as effectively as wild-type HBV.

In our patients with chronic hepatitis B, YMDD variants were seldom detected before 36 weeks of lamivudine therapy, and ~50% of patients remained YMDD-variant free after 3 years. In contrast, resistance occurs within a few months in nearly all HIV-infected recipients of lamivudine monotherapy [27]. Similarly, YMDD variants emerge more rapidly in immunocompromised liver allograft recipients [28].

In most patients, improvements in liver disease persisted in the presence of YMDD variants if lamivudine therapy was continued. Serum HBV DNA was suppressed, and liver histologic findings and serum ALT levels improved significantly in patients who received lamivudine for 1 year, regardless of whether YMDD variants emerged. After 2–4 years of lamivudine therapy, serum HBV DNA and ALT levels generally remained improved, compared with pretreatment levels, even among patients who harbored YMDD variants for >2 years. These findings are supported by a recent histologic analysis, in which both necroinflammatory activity and fibrosis were improved in most patients who harbored YMDD variants after 2 years of lamivudine therapy [29].

In some patients, the extended presence of YMDD variants is associated with a slow increase in the serum HBV DNA level, a parallel increase in the ALT level, and, sometimes, deterioration of liver histologic findings [23, 24, 30]. Compared with baseline, liver histologic findings were improved in 5 of 9 patients who harbored YMDD variants after 3 years of lamivudine therapy, but histologic findings had deteriorated compared with

the findings of the year 1 biopsy in 6 of these 9 patients. Thus, after the emergence of YMDD variants, histologic responses to lamivudine may diminish, even though they do not generally disappear. Although, in our experience, YMDD variants were generally benign, other reports document that, in cohorts of liver allograft recipients, YMDD variants with enhanced replication and more-severe clinical consequences occur [28, 31].

Emergence of YMDD variants reduces or delays, but does not prevent, HBeAg seroconversion [32]. Throughout 4 years of lamivudine therapy, the rate of HBeAg seroconversion in patients who harbored YMDD variants remained lower than in patients without variants, probably a reflection of less substantial reduction in HBV replication in the variant group [19].

The similarity of adverse events in patients with or without YMDD variants, even with extended lamivudine therapy, suggests that YMDD variants have no unique pathogenic effects. Predominantly asymptomatic ALT elevations during treatment were somewhat more common among patients with YMDD variants; however, these generally coincided with the return of detectable viremia and were often associated with HBeAg seroconversion [33]. A similar transient increase in the ALT level occurs sometimes after cessation of treatment in patients who did not harbor YMDD variants, suggesting that HBV replication, rather than HBV type, is the cause.

Posttreatment ALT elevations were less frequent among patients with YMDD variants than they were among those who did not have YMDD variants, perhaps because YMDD variants may be less virulent than is wild-type HBV, as suggested by reduced replication competence in vitro [15, 17]. Indeed, most posttreatment elevations in the ALT level coincided with reemergence of wild-type HBV, even when YMDD-variant HBV predominated at the end of therapy.

Baseline virus level, disease severity, and body size were associated with the emergence of YMDD variants during the first year of lamivudine therapy. Baseline virus level, reflecting the rate of HBV replication and, therefore, mutational frequency, is a logical predictor of YMDD variants. Similarly, recent data suggest that initial virologic response after 3 or 6 months of lamivudine therapy predicts whether YMDD variants will emerge subsequently [34, 35]. In the same vein, the association between the level of HBV replication and liver injury may explain the higher frequency of YMDD variants in patients with high baseline necroinflammatory activity. Increased body size might enhance YMDD variant frequency as a result of reduced drug concentration; however, YMDD incidence was unaffected by lamivudine dose in study B3009. In terms of viral factors, whether different YMDD mutations (L180M, M204V, and M204I [36]) are associated with different clinical expressions is unknown. Although some have suggested that YMDD variants are more likely in certain HBV genotypes [37], others have failed to corroborate this association [38, 39].

When this study was conducted, routine clinical assays for YMDD variants were not available. Therefore, we sought to identify an algorithm based on routine laboratory tests to suggest the presence of phenotypically relevant (i.e., associated with recurrent hepatitis) YMDD variants in lamivudine recipients. Even now that a clinical assay for YMDD mutants is available, many clinicians continue to rely on ALT and HBV DNA values in decision-making about lamivudine resistance.

Most patients with YMDD variants retain at least partial clinical response for several years, but approaches to lamivudine resistance will change as new antiviral agents are introduced. The urgency with which new therapies are needed is greatest in patients with end-stage liver disease and after liver transplantation, in which additional clinical deterioration has a potentially fatal outcome [40, 41]. Recently approved adefovir dipivoxil, which has potent activity against YMDD-variant hepatitis B, will simplify management of infection due to such variants in the future [42–44].

In summary, in compensated chronic hepatitis B, YMDD variants increased with the duration of lamivudine treatment. Despite the emergence of variants, many patients maintained improvements in markers of HBV infection and liver injury, although there was not necessarily histologic benefit, after several years of YMDD-variant HBV infection.

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