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Clinical Outcome and Virologic Profiles of Severe Hepatitis B Exacerbation

Due to YMDD Mutations

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Short title: Severe Hepatitis B Exacerbation due to YMDD Mutants

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Abstract

Aim: To study the outcome and the virologic profiles of severe hepatitis exacerbations due to YMDD mutants in lamivudine-treated patients.

Patients and Methods: Eighteen lamivudine-treated patients with severe hepatitis exacerbations due to YMDD mutants were recruited. Laboratory and clinical parameters were monitored. Viral genotypes and YMDD mutations were determined.

Results: None of the 18 patients had YMDD wild-type during exacerbations. Three (16.7%) and 15 (83.3%) patients had genotypes B and C respectively. Elevated bilirubin levels and prolonged prothrombin time were found in 11 (61.1%) and 6 patients (33.3%) respectively. Three patients (16.7%) had adverse outcome with the development of ascites and/ or encephalopathy. One of these patients required liver transplantation and one died. Both patients had evidence of cirrhosis before treatment and HBeAg seroreversion from anti-HBe positivity. The remaining 16 patients (88.9%) have no evidence of pre-existing cirrhosis. 37.5% patients had normal alanine aminotransferase levels at the last follow-up. The median HBV DNA level at the last follow-up was significantly lower than the pre-treatment level ($p=0.009$).

Conclusions: Though the majority of patients with severe hepatitis exacerbations

due to YMDD mutants had uneventful course, early liver transplantation should be considered in patients with pre-existing cirrhosis and HBeAg seroreversion.

Background

Lamivudine is one of the first line agents for the treatment of chronic hepatitis B (CHB) infection. The profound suppressive effect on hepatitis B virus (HBV) replication in both Asian and Caucasian patients, and the near absence of side effects are the two main reasons for its widespread use [1, 2]. The prolonged use of lamivudine, however, is associated with the development of drug resistant HBV virus mutations at the viral polymerase gene, namely YMDD mutations (tyrosine-methionine-aspartate-aspartate) changing to either YIDD (*isoleucine*) or YVDD (*valine*). The frequency of YMDD mutations increases with the duration of lamivudine treatment (15%, 38%, 56% and 67% for the first four years respectively) [2-5]. It has been shown both in in vitro and in vivo studies that compared with YMDD wild-type, YMDD mutants have less replication competence and are associated with less aggressive liver disease [6-11]. Nevertheless, severe hepatitis exacerbations due to YMDD mutants have been reported. These exacerbations are sometimes associated with hepatic decompensation and mortality [12-14]. To date, data on the mortality rate of severe hepatitis exacerbations due to YMDD mutants and the factors associated with mortality are unknown.

The aims of the present study were to study the outcome and the virologic profiles of severe hepatitis exacerbations due to YMDD mutants in

lamivudine-treated patients.

Patients and Methods

From January 1999 to December 2002, all patients on lamivudine having severe HBV exacerbations admitted to Queen Mary Hospital, The University of Hong Kong, Hong Kong were prospectively assessed for the present study. Severe hepatitis exacerbation was defined as an increase in alanine aminotransferase (ALT) levels $> 10 \times$ upper limit of normal (ULN) after excluding other causes of ALT elevation including other viral hepatitis (A, C, D, E), drug-induced hepatitis, and alcoholic hepatitis. Eighteen patients fulfilled these criteria; all had YMDD mutations. Of these 18 patients, 8 patients who were on lamivudine from our previous clinical trials [1, 15] had regular follow-up in our Hepatitis Clinic, Queen Mary Hospital, The University of Hong Kong, Hong Kong, both before and throughout the course of lamivudine treatment. Pretreatment liver biopsies were performed in these 8 patients under trial protocols. They developed YMDD mutants at a median time of 8.5 (range 6.2 – 23.2) months after lamivudine therapy. The remaining 10 patients were referred to our hospital for further management when they developed the severe exacerbations. The intervals between the initiation of lamivudine therapy and the emergence of YMDD mutants for these 10 patients were

unknown. All 18 patients were maintained on lamivudine treatment till the time of writing. The complete blood count, liver biochemistry, HBV DNA levels, hepatitis B e antigen (HBeAg)/ antibody to HBeAg (anti-HBe) status before lamivudine treatment were recorded.

Patients having severe exacerbations were closely monitored by checking the complete blood count, prothrombin time and liver biochemistry every 2 days till discharge or death. The HBV DNA levels were measured by Digene Hybrid Capture II assay (Digene Corporation, Gaithersburg, MD) (lower limit of detection 0.14×10^6 copies/ml). The development of clinical complications including ascites, encephalopathy and variceal bleeding, was noted. "Adverse outcome" is defined as exacerbations resulting in development of the clinical complications mentioned above and/ or necessity of liver transplantation and/ or death. Patients recovering from the hepatic decompensation were regularly followed up every 4 weeks for the first 12 weeks and subsequently at 12 weekly intervals. The liver biochemistry, HBeAg/ anti-HBe status and HBV DNA levels were measured during every follow-up.

A line probe assay (INNO-LiPA HBV DR, Innogenetics NV, Belgium) was used to determine the YMDD mutations. The methodology was described in our previous study [16]. The assay can detect mixed populations of YMDD wild-type and YMDD

mutations when the proportion of either viral strain is higher than 5% of the total population.

The HBV genotypes were determined by enzyme-linked immunosorbent assay (ELISA) with type specific antibody and subtypes of Ba and Bj in genotype B were assayed by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) as described previously [17, 18].

The core promoter and precore mutations were determined in patients who were anti-HBe positive by direct sequencing. Briefly, serum HBV DNA, in a final volume of 200ul, was extracted from 200 µl of serum, using the QIAGEN DNA blood minikit (QIAGEN GmbH, Germany). The HBV genome at nucleotide 1653 – 1974 containing precore and core promoter regions was amplified by PCR using the primers HBV1 (5'-cataagaggactcttgact-3') and HBV2 (5'-ggaaagaagtgcagaggc- 3') in a GeneAmp 9700 PCR system (Applied Biosystems, US) with the following conditions: 95⁰C for 10 mins, followed by 94⁰C for 30s, 54⁰C for 30s and 72⁰C for 1 min up to 40 cycles and a final extension 72⁰C for 10 mins. The 322 bp PCR products were purified by ethanol precipitation with ammonium acetate in a final volume of 20 µl. Two microliter of the purified PCR products was used in a thermocycle sequencing with the DYEnamic ET Terminator Cycle Sequencing Kit as directed (Amersham Biosciences, Uppsala, Sweden) using

HBV2 as the primer. Automated sequencing was performed with an ABI prism 3700 DNA Analyzer.

Statistical analysis

The difference in the paired HBV DNA levels at the baseline and at the last follow-up was tested by Wilcoxon signed ranks test. The difference in the ALT levels before lamivudine treatment, during lamivudine treatment and at the last follow-up was tested by Kruskal Wallis test. P value of less than 0.05 is considered to be statistically significant.

Results

Clinical outcome of severe exacerbations

The demographic data, liver biochemistry, HBV DNA, HBeAg/ anti-HBe status, strains of YMDD mutants, and the outcome of the severe hepatitis exacerbations are listed in Table 1.

Elevated bilirubin levels and prolonged prothrombin time were found in 11 (61.1%) and 6 patients (33.3%) respectively (Table 1). Eight patients (44.4%) and six patients (33.3%) had bilirubin levels elevated to more than 2 X ULN and prothrombin time prolonged by more than 3 seconds respectively. Three patients (16.7%) had adverse outcome with the development of ascites; one of these three

patients also developed encephalopathy. None had variceal bleeding.

Two patients (11.1%) had irreversible hepatic decompensation. Adefovir dipivoxil was given 4 weeks after the severe hepatitis exacerbation in one patient in whom spontaneous recovery was unlikely to occur. However, because of the irreversible hepatic decompensation, liver transplantation was finally performed. The histology of the explanted liver showed that the liver was cirrhotic with regeneration nodules and fibrous septa. There was also submassive necrosis of the liver with loss of hepatocytes. Another patient died of hepatic decompensation 3 weeks after admission, while compassionate use of adefovir dipivoxil and liver transplantation were being arranged. These two patients were the oldest (age 47 and 51 years) of the 18 patients (Table 1). The HBV DNA levels of these two patients were not especially high. However, both patients had ultrasonographic evidence of small cirrhotic liver, splenomegaly and ascites before the start of lamivudine. They also had biochemical evidence of cirrhosis before treatment with low albumin levels, high bilirubin levels and low platelet count (Table 1). In contrast, the pretreatment histology of the 8 patients with pretreatment biopsies and who had uneventful course showed only mild to moderate inflammation without evidence of fibrosis or cirrhosis. Another feature was that both patients were anti-HBe positive before the severe exacerbation and had HBeAg seroreversion during the severe exacerbations.

These were the only two patients with HBeAg seroreversion. For the other 16 patients, 15 were HBeAg positive and one was anti-HBe positive at the time of severe exacerbations.

ALT levels and HBV DNA levels before, during and after severe exacerbations

The median duration of follow-up from the time of severe exacerbation to the last follow-up for the 16 patients with uneventful course was 19.2 (range 1.3 – 53.9) months. Thirteen patients remained HBeAg positive, 2 patients had HBeAg seroconversion to anti-HBe (at months 33 and 42 months after the severe exacerbations) and 1 patient was anti-HBe positive all along. Six out of these 16 patients (37.5%) had normal ALT levels at the last follow-up. Among the 13 patients with the ALT results available before lamivudine treatment, during lamivudine treatment and at the last follow-up, there was no difference in the median ALT levels between these time points [61 (range 27 – 1189) vs. 47 (range 30 – 70) vs. 59 (range 17 – 227) respectively, $p=0.37$]. Six patients (37.5%) had relatively low HBV DNA levels that were below the detection limit of the Digene Hybrid Capture II assay ($<0.14 \times 10^6$ copies/ml) at the last follow-up. Among the 15 patients whose HBV DNA levels both before lamivudine treatment and at the last follow-up were available, the median HBV DNA level at the last follow-up was significantly lower

than at baseline [1.7×10^6 (range $<0.142 - 806.3 \times 10^6$) vs. 263.8×10^6 (range $<0.142 - 11,000 \times 10^6$) respectively, $p=0.009$].

Virologic profiles in severe exacerbations

Three patients (16.7%) had HBV genotype B (all with Ba subtype) and 15 patients (83.3%) had HBV genotype C.

None of the 18 patients had detectable YMDD wild-type during the severe exacerbations indicating that the YMDD mutants [either single (YIDD or YVDD) ($n=15$) or mixed (YIDD & YVDD)($n=3$)] became the predominant viral strains at the time of exacerbations. The changes in the viral populations of the 16 patients with uneventful course 3 months after the severe hepatitis exacerbations were also determined. There were no changes in the viral populations in 11 patients (68.8%) (4 with YIDD alone, 5 with YVDD alone and 2 with YIDD & YVDD). Two patients (12.5%) (1 with YVDD alone, 1 with YIDD & YVDD) had the reappearance of YMDD wild-type in the presence of YMDD mutants. Two patients (12.5%) with YVDD mutants during the severe exacerbations had the addition of the YIDD mutants. The remaining patient (6.3%) changed the viral population from YIDD alone to YVDD alone.

Among the 18 patients, 16 were HBeAg and 2 were anti-HBe positive when

lamivudine was started. One patient had HBeAg seroconversion before the severe exacerbation. In total, 15 patients were HBeAg positive and 3 patients were anti-HBe positive at the time of severe exacerbations. Among the 3 anti-HBe positive patients, 2 patients had serum 1, 3 and 6 months and 1 patient had serum 1 month before the severe hepatitis exacerbations that were available for the determination of core promoter and precore mutations. The first 2 patients had the same core promoter mutations (A1762T & G1764A) and precore wild-type at the three time points. The last patient also had the same core promoter mutations and precore wild-type detected one month before the exacerbation. YMDD mutations were also determined in the 2 patients (one with and one without HBeAg seroreversion) with available serum at 1, 3 and 6 months prior the severe hepatitis exacerbations. For the patient with HBeAg seroreversion, the YMDD mutant (YIDD) was already present 6 months before the severe exacerbation. The patient without HBeAg seroreversion had YMDD wild-type at 3 and 6 months before the exacerbation; YMDD mutation (YVDD) was only present 1 month before the severe exacerbation.

Discussion

The results of this study provide essential information on the clinical outcome

and virologic profiles of patients with severe exacerbations due to YMDD mutations.

These findings are important for patient management.

According to Liaw and his colleagues, in a study of 55 patients, acute exacerbations occur in 41% of patients with YMDD mutants at a median interval of 24 weeks after the emergence of the YMDD mutants [12]. In these patients, 9.4% develop hepatic decompensation. The present study examined only patients with severe exacerbations (ALT > 10 X ULN). Significant prolongation of prothrombin time was observed in 33.3% patients and adverse outcome with the development of ascites and/or encephalopathy occurred in three patients (16.7%). Of these, two patients had irreversible hepatic decompensation (11.1%) (one required liver transplantation, one died). These findings suggest that severe exacerbations due to YMDD mutants can cause considerable morbidity and mortality though the majority of patients run an uneventful course.

The HBV DNA levels in the two patients with irreversible hepatic decompensation were not especially high (Table 1) indicating the viral load by the time of presentation was not an important prognostic factor. However there were two features that were special for both patients. Both patients had ultrasonographic (small sized liver, splenomegaly and ascites), biochemical/hematological (albumin levels of 32 and 33 g/L, bilirubin levels of 25 μ mol/L and platelet count of 56 and 99

X 10^6 /L) and histologic (in one patient) evidence of pre-existing cirrhosis. Malik and Lee also describe two deaths occurring in a small number of patients with advanced HBV disease on lamivudine therapy [19]. In contrast, the eight patients of the present study with pretreatment liver biopsy and with uneventful course did not have evidence of fibrosis or cirrhosis. In all the 16 patients without irreversible hepatic decompensation, the pretreatment albumin levels were normal or near normal (range 39 – 49 g/L) (Table 1).

Another relevant point is that both these patients with irreversible hepatic decompensation had HBeAg seroreversion during the severe exacerbations. This has two implications. Firstly, the seroreversion probably signified a very severe immunologic reaction. Secondly, in a proportion of Asian chronic hepatitis B patients, the process of HBeAg seroconversion and the accompanying immune-related damage may worsen the liver function [20, 21]. This may set the stage for more severe outcome during a subsequent acute exacerbation. Early transplantation with adefovir dipivoxil cover should be considered for patients with pre-existing cirrhosis and HBeAg seroreversion during the severe exacerbations due to YMDD mutations.

For the 16 patients with uneventful course, 37.5% had normal ALT levels and 37.5% had HBV DNA levels $< 0.14 \times 10^6$ copies/ml at the last follow-up (Table 1).

Among the 15 patients who had available HBV DNA levels both before lamivudine treatment and at the last follow-up, the median HBV DNA level at the last follow-up was significantly lower than that pre-treatment level ($p=0.009$). It indicated that the majority of patients who recovered from the severe exacerbations still have a much lower viral activity than that at the baseline. Nevertheless, at least 62.5% patients still had elevated ALT levels and HBV DNA levels $> 10^5$ copies/ml. Treating this subgroup of patients with adefovir dipivoxil or other newer nucleoside analogues that are effective against YMDD mutants may be of benefits.

Akuta and his colleagues show that there is no difference in the chance of emergence of YMDD mutations between patients with genotypes B and C [22]. This has been confirmed in another study from our center [23]. In the present study, of the 18 patients with severe exacerbations due to YMDD mutants, 16.7% and 83.3% were genotypes Ba and C respectively. In our locality, the percentages of HBV patients with genotypes B and C are around 28% and 61% respectively [24]. Thus, genotypes also do not appear to play any role in predisposing patients to severe exacerbations due to YMDD mutants, though a large study is required to confirm this.

In all the 18 patients at the time of the exacerbations, the YMDD wild-type virus was not detected by the LiPA which is capable of detecting mixed populations

of YMDD mutants and wild-type if either viral strain contributes more than 5% in the population [16]. This phenomenon suggests that severe exacerbations during lamivudine therapy are unlikely to be due to YMDD wild-type. According to Zhou et al., the viral load in the body increases gradually while YMDD mutants are gradually replacing YMDD wild-type in the viral population [25]. In our previous study, patients with YMDD mutants in the absence of YMDD wild-type are more likely to have HBV DNA breakthroughs [16]. In the present study, we further documented that the viral population remained unchanged 3 months after the severe exacerbations in 68.8% of the patients. In addition, 2 patients had the reappearance of YMDD wild-type 3 months after the severe exacerbations. The replacement of the YMDD mutants and emergence of different viral strains after exacerbation have been reported in several studies. Liaw and his colleagues show that clearance of YMDD mutants can occur after severe exacerbations [12]. Yeh and his colleagues also show that new distinct mutants may arise during prolonged lamivudine therapy [26]. In addition, mutations of the 'a' determinant of the viral envelope gene together with YMDD mutations in liver transplant recipients receiving lamivudine and hepatitis B immune globulin have been described by Bock and his colleagues [27]. Further longitudinal studies are required to examine the clinical importance of all these possible virologic changes after severe exacerbations due to YMDD mutants.

In conclusion, the present cohort study suggested that majority (88.9%) of patients with severe hepatitis exacerbation due to YMDD mutants had uneventful course. Early use of adefovir dipivoxil and/ or liver transplantation should be considered in patients with pre-existing cirrhosis and HBeAg seroreversion during the exacerbations because these patients had a higher chance of irreversible hepatic decompensation.

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Table 1: Clinical details of the 18 patients with severe hepatic exacerbations due to YMDD mutations

			Before lamivudine							Severe hepatic exacerbation										Last follow-up				
Patients	Sex	Age	Albumin	Bilirubin	ALT	Platelet	HBV DNA	HBeAg	Months after lam	Mutants	Albumin	Bilirubin	ALT	Platelet	PT	HBV DNA	HBeAg	Duration (months)	Albumin	ALT	Bilirubin	HBV DNA	Duration of FU	
1	1	21	49	17	77	161	244	+	36.6	I/V	50	37	1376	161	11.3	170	+	#	46	146	25	<0.14	86.6	
2	2	24	na	na	na	na	900	+	17.2	I	35	408	1011	151	27.1	872.7	+	2.0	37	24	28	<0.14	18.5	
3	2	21	45	12	37	213	3000	+	62.4	I/V	43	9	1208	213	13.3	561.8	+	#	46	118	9	1.7	63.9	
4	1	26	49	11	51	266	45.4	+	48.3	V	44	14	868	285	12	980	+	12.4	45	59	17	7.95	85	
5	1	28	41	6	42	237	77	+	27.7	I	44	45	778	177	11.5	7900	+	9.9	49	62	13	1.0	79.6	
6	2	28	48	8	91	186	1943	+	55.3	V	40	15	1321	192	12.2	57	+	5.6	44	43	9	<0.14	87.9	
7	1	30	47	8	27	154	1306	+	64.1	I	39	41	1395	156	11.4	392	+	1.9	41	38	12	6.9	93.4	
8	1	31	na	na	na	na	1189	+	46.1	I	44	620	2005	122	19	642.8	+	2.3	46	206	17	<0.14	50.7	
9	2	31	49	11	147	155	11000	+	28.1	V	40	22	1136	140	10.9	0.18	+	20.7	42	22	11	11.4	56.9	
10	1	35	45	11	61	238	74.8	+	41.1	I/V	41	24	976	164	11.3	1900	+	27	44	32	20	<0.14	95	
11	1	35	na	na	na	na	Na	+	12.0	V	48	15	1980	212	12.4	4	+	#	48	79	15	29.2	14.3	
12	1	37	45	11	39	273	134.9	+	90.7	V	39	17	575	263	12	594	+	#	41	145	21	806.3	95.3	
13	1	41	47	7	41	177	263.8	+	35.9	V	41	9	990	157	11.6	304.7	+	30.2	44	17	10	<0.14	86.6	
14	1	42	45	22	1189	175	<0.14	+	20.7	I	39	42	900	56	15.7	936	+	#	44	227	28	155.5	30.2	
15	1	46	39	14	78	76	330	-	27.8	V	40	13	1067	66	10.8	1.44	-	#	43	83	13	33.8	31.5	
16	1	46	44	17	634	na	100	+	16.8	V	46	293	3910	73	18.8	106	+	2.3	44	52	22	11.4	19.1	
17*,\$	1	47	33	25	30	99	0.22	-	7.8	I	33	602	1000	87	37.5	1639	+	x	x	x	x	x	16.5	
18*,\$	1	51	32	25	35	56	498	+	39	I	38	870	1199	72	45.7	24	+	x	x	x	x	x	39.9	

*Patient with liver transplantation, † Patient who died, § Patients with HBeAg seroreversion, x not applicable

duration of hepatic exacerbation not applicable because of persistent elevated ALT levels

Bold values indicate bilirubin levels elevated to > 2 X upper limit of normal (normal < 19 µmol/L) and prothrombin time greater than 3 seconds of controls

+: positive; -: negative; na: not available; lam: lamivudine; PT: prothrombin time; I: YIDD; V: YVDD; Sex: 1=Male, 2=Female; FU: follow-up

Units: Albumin (g/L); Bilirubin (µmol/L); ALT (U/L); Platelet (X 10⁹/L); HBV DNA (X 10⁶ copies/ml); PT (seconds); Duration of follow-up (months)