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HBV

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1.

Abstract

Emergence of YMDD Motif Mutant Hepatitis B Virus during Short-term Lamivudine Therapy

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Background/Aims: The emergence of lamivudine-resistant mutant hepatitis B virus (HBV), with aminoacid substitution in the YMDD motif of DNA polymerase, has been reported in the long-term lamivudine use group. However there is no report about the emergence of mutant viruses during the short-term lamivudine therapy. The objective of this study was to investigate the emergence of YMDD mutant HBV during short-term lamivudine therapy. **Methods:** We evaluated twenty-eight chronic hepatitis B patients who were HBeAg and HBV DNA positive and treated with lamivudine 100mg p.o. daily for 12 weeks. First, we investigated the emergence of YMDD mutants by nested polymerase chain reaction (PCR) method developed by Chayama et al in 19 patients who lost HBV DNA during lamivudine therapy but showed HBV DNA re-emergence 2 weeks after the end of therapy. Second, DNA subcloning and sequencing of HBV DNA polymerase including YMDD motif was undertaken in one patient's serial blood samples at 0, 8, 12 weeks to confirm the results of nested PCR. **Results:** YMDD motif mutation was detected in 17(90%) out of 19 patients at the end of therapy and the type of mutations were YIDD only. At the end of therapy, mutant was predominant in 5 patients, both mutant and wild type were similar in proportion in 3 patients, and wild type was predominant in 9 patients. When we carried out nested PCR serially with samples of 0, 2, 4, 8, 12, 14 weeks after initiation of therapy in 5 patients who were mutant predominant at 12 weeks, YIDD mutant began to be detected from 2 weeks in 4 patients and from 4 weeks in one patient. However, rapid turnover from mutant to wild type happened after the end of therapy, so only wild type was detected in 3 patients and wild type became predominant in 2 patients at 2 weeks after the end of therapy. All the sequencing results of serial blood samples in one patient were consistent with nested PCR data. **Conclusions:** The presence of YMDD motif HBV polymerase mutant may be possible before administration of lamivudine in Korean chronic hepatitis B patients. Nested PCR assay would be an useful method to detect YMDD mutant. (Korean J Hepatol 1999;5:173 - 183)

Key Words : Chronic hepatitis B, Lamivudine, HBV DNA polymerase, YMDD mutant

■ : , 134 , .
 1999 .

100 mg
 12 18 ,
 (Lamivudine, (-)-L-2',3'-dideoxy- 10 38(24- 60) .
 3'-thiacytidine, 3TC) 1 HBeAg HBV DNA
 (cytosine nucleoside analogue) 가 anti-HCV .
 human immunodeficiency virus(HIV) 12 가
 B 16 (57%) ALT가 22
 (HBV) (RNA dependent (79%) HBV DNA가 . 4
 DNA polymerase) HBV DNA가
 1-4 B 2 HBV DNA가
 . 6 . HBV DNA가
 B 22 13 2
 HBV가 , HBV DNA가
 HBV YMDD motif 12 HBV
 (549 - 552th) 가 DNA 6
 .5-9 2 13
 550 methionine 19 . 13
 isoleucine YIDD valine , 6 , 39(26- 60) .
 YVDD , alanine amino-
 YMDD transferase (ALT) 123.8 ± 44.9(47- 190)
 . 10 11 IU/L HBV DNA 3305.3 ± 5325.4(24-
 6 17000) pg/mL (Table 1).

HBV YMDD motif 2.
 19 , 2, 4,
 B 8, 12 , 2
 HBV YMDD motif . 19
 12
 nested PCR
 nested PCR 12
 1. 5
 1998 1 1999 2 28 2, 4, 8, 12
 가 Glaxo-welcome (Zeffix[®]) 3 2 nested

가 2-3 volume absolute ethanol
 -70 5 . 4 , 15,000 rpm
 15 1ml
 70% ethanol pellet 4 , 13,000 rpm
 10
 가 .
 Pellet 30 ul 가 - 20

HBV DNA PCR
 (automatic sequencing)
 . PCR HBV DNA
 pGem-Teasy vector (Promega, Madison,
 U.S.A.) ligation *Escherichia coli* XL1
 blue transformation plasmid
 . 5 clone auto-
 matic sequencer (Amersham pharmacia Bio-
 tech, Uppsala, Sweden)

2) Nested Polymerase Chain Reaction

YMDD

(Ssp for YIDD
 , Alw44I for YVDD)
 . YIDD
 viral DNA 10 ul (template)
 BF108 BR112 94 5
 (denaturation) 35
 (94 ;1 , 58 ;1 , 72 ;1.5) 72
 7 (extension) .
 PCR 1 ul primer YNSsp1, BR109
 PCR
 . 10 ul PCR SspI
 2% agarose gel
 . YVDD
 PCR BF107, TMPpu101
 , PCR
 TMApaL1, BF111 PCR
 YIDD . PCR
 Alw44I 2% agarose gel
 . PCR
 HBV DNA subclon-
 ing YMDD
 B , 5 4 2 , 1 4
 YIDD
 가 .

1. Nested PCR YMDD motif
 Nested PCR YMDD motif ,
 19 YMDD
 12 19 17 (89.5%) YMDD
 17
 YIDD YVDD
 . 12 17
 PCR
 band
 ,
 가 5 , 가 3 ,
 가 9 (Table 1).
 12
 5 , 2 , 4 ,
 8 , 12 (), 2
 nested PCR ,
 5
 , 5 4 2 , 1 4
 YIDD
 가 .

3) Nested PCR subcloning HBV

Nested PCR
 patient 2 , 8 , 12

2
 3 , 2
 (Table 2).

Table 2. Longitudinal Data of HBV DNA and Emergence of YIDD Mutant in Patients Who Showed Predominance of YIDD Mutant at 12 Weeks after Lamivudine Therapy

		Baseline	2 weeks	4 weeks	8 weeks	12 weeks	14weeks
Patient 1	DNA	130	5.8	-	-	-	7.6
	Mutant	Wild	Wild	W>>Mi	W>>Mi	W<<Mi	W>>Mi
Patient 2	DNA	400	-	-	-	-	190
	Mutant	Wild	W>>Mi	W>>Mi	W=Mi	W<<Mi	Wild
Patient 3	DNA	24	-	-	-	-	26
	Mutant	Wild	W>>Mi	W>>Mi	W>>Mi	W<<Mi	Wild
Patient 4	DNA	82	-	-	-	-	1300
	Mutant	Wild	W>>Mi	W>>Mi	W>>Mi	W<<Mi	Wild
Patient 5	DNA	81	-	-	-	-	3.1
	Mutant	Wild	W>>Mi	W>>Mi	W=Mi	W<<Mi	W>>Mi

DNA: HBV DNA level (pg/mL)

Mutant: YIDD type mutant (Wild: wild only, W>>Mi: mutant predominant, W=Mi: similar proportion of wild and mutant type, W<<Mi: mutant predominant)

Figure 1. Longitudinal data of nested PCR after restriction enzyme application in the patient 2.

2. Nested PCR subcloning HBV

Nested PCR patient 2, 8, 12

clone 5 (automatic sequencing) nested PCR (Figure 1),

(Table 3). 8 YIDD

clone 5, YIDD 2

12 YIDD

5 clone 3, YIDD 2

12 YIDD

4

5 clone YMDD가 1, YIDD

4 YMDD motif nested PCR

3. ALT HBV DNA Mutant

Patient 1 ALT

2

HBV DNA

2

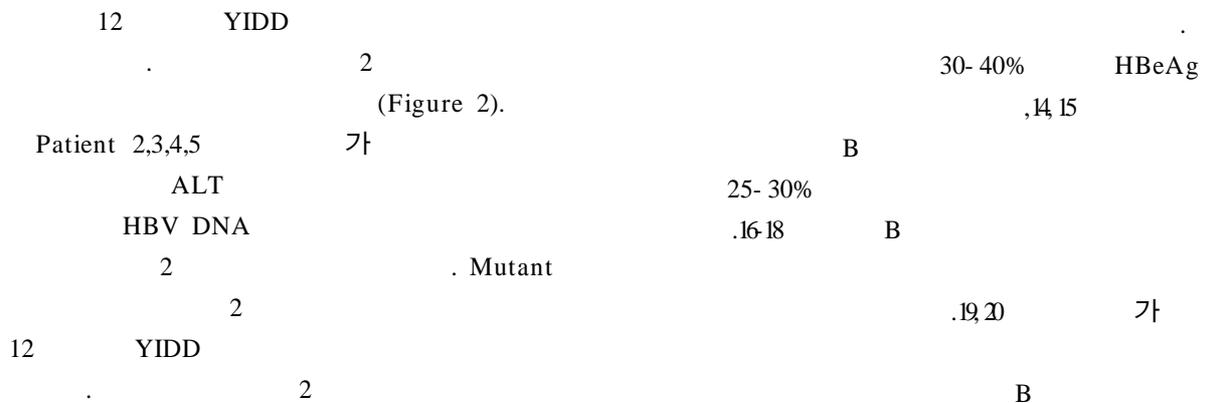
YIDD 4

Table 3. Comparison between Nested PCR Results and Automatic Sequencing Result of HBV DNA Including YMDD Motif

Sampling time	Nested PCR result	Subcloning and automatic sequencing of HBV DNA(5 clones for each sample)
Baseline	YMDD wild only	clone A-1 5'-----T----TAT/ATG/GAT/GAT-----A-3' YMDD
		clone A-2 5'-----T----TAT/ATG/GAT/GAT-----A-3' YMDD
		clone A-3 5'-----T----TAT/ATG/GAT/GAT-----A-3' YMDD
		clone A-4 5'-----T----TAT/ATG/GAT/GAT-----A-3' YMDD
		clone A-5 5'-----T----TAT/ATG/GAT/GAT-----A-3' YMDD
8 weeks	Wild type = Mutant	clone B-1 5'-----C----TAT/ATT/GAT/AAT-----G-3' YIDD
		clone B-2 5'-----T----TAT/ATT/GAT/AAT-----A-3' YIDD
		clone B-3 5'-----T----TAT/ATG/GAT/AAT-----A-3' YMDD
		clone B-4 5'-----T----TAT/ATG/GAT/AAT-----A-3' YMDD
		clone B-5 5'-----T----TAT/ATG/GAT/AAT-----A-3' YMDD
12 weeks	Wild type << Mutant	clone C-1 5'-----T----TAT/ATT/GAT/GAT-----G-3' YIDD
		clone C-2 5'-----T----TAT/ATT/GAT/GAT-----G-3' YIDD
		clone C-3 5'-----T----TAT/ATT/GAT/GAT-----G-3' YIDD
		clone C-4 5'-----T----TAT/ATT/GAT/GAT-----G-3' YIDD
		clone C-5 5'-----T----TAT/ATG/GAT/GAT-----G-3' YMDD

(Figure 3).

Figure 2. Longitudinal data of serum ALT and HBV DNA and nested PCR results after restriction enzyme application in patient 1.



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