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• *RAPID COMMUNICATION* •

Clinical characteristics and distribution of hepatitis B virus genotypes in Guangxi Zhuang population

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Abstract

AIM: To investigate the distribution of HBV genotypes and their YMDD mutations in Guangxi Zhuang population, China, and to study the relationship between HBV genotypes and clinical types of HB, ALT, HBV DNA, HBe system as well as the curative effect of Lamivudine (LAM) on hepatitis B.

METHODS: A total of 156 cases were randomly chosen as study subjects from 317 patients with chronic hepatitis B (CHB). HBV genotypes were determined by PCR-microcosmic nucleic acid cross-ELISA. YMDD mutations were detected by microcosmic nucleic acid cross-nucleic acid quantitative determination. HBV DNA was detected by fluorescence ratio PCR analysis. LAM was given to 81 cases and its curative effect was observed by measuring ALT, HBV DNA load, HBeAg, and HBeAg/HBeAb conversion rate.

RESULTS: HBV genotypes B, C, D, and non-classified genotypes were found in Guangxi Zhuang population. accounting for 25.6%, 47.4%, 58.3%, and 16.0%, respectively. Seventy-four cases were CD-, CB-, BD-mixed genotypes (47.7%). Forty-six (29.5%) cases had YMDD mutations. Genotype B was mostly found in mild and moderate CHB patients. Genotypes C, D and mixed genotype mostly occurred in severe CHB cases. Genotypes D and CD HBV-infected patients had higher ALT and HBV DNA than patients with other types of HBV infection. There was no significant difference among the genotypes in YMDD mutations, clinical types, ALT and HBV DNA level. Non-classified types geno had a significantly lower positive rate of HBeAg than other genotypes (χ^2=12.841, P<0.05). There was no significant difference in ALT recovery rate, HBV DNA load, HBeAg, and HBeAg/HBeAb conversion rate, 48 wk after LAM treatment between groups of genotypes D, CD, and non-classified type.

CONCLUSION: Genotypes B, C, and D, non-classified and mixed genotype of HBV are identified in the Guangxi Zhuang population. Variations in genotypes are associated with clinical severity and serum ALT levels, but not with YMDD mutation or HBV DNA load. Therapeutic effects of LAM on clinical parameters are not influenced by differences in genotypes. Further studies are needed to gain an in-depth understanding of the relationship between HBV genotypes and serum HBeAb and HBeAg.

Key words: Hepatitis B virus; Chronic hepatitis; Genotype; YMDD mutation; Lamivudine; Zhuang nationality

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INTRODUCTION

Guangxi Zhuang Autonomous Region is an endemic area of HBV infection. To examine the distribution of HBV genotypes and their associations with clinical characteristics of hepatitis B (HB) in Zhuang population, a total of 156 cases selected randomly from chronic hepatitis B (CHB) 317 patients without any previous antiviral treatment were studied, and some cases were given Lamivudine (LAM) treatment. The results reported are as follows.

MATERIALS AND METHODS

General data of patients

A total of 156 patients, 89 males and 67 females, aged 15-70 years, were selected randomly from CHB 317 patients from out-patient and in-patient departments of our hospital during January 2001 to June 2003. These patients received no previous LAM treatment or any antiviral treatment within a year. Their serum HBsAg, HBeAg or HBcAb and HBV DNA were positive. Those

infected with HCV, HDV, and HIV were excluded. All cases fulfilled the diagnostic criteria modified at the Tenth Viral Hepatitis Conference of Chinese Medical Association[1].

Detection of HBV genotypes

HBV DNA genotypes were detected by PCR-microcosmic nucleic acid cross-ELISA. One hundred microliters of serum samples was mixed with a reaction solution in a 0.5 mL centrifuge tube, heated to 100 °C for 15 min, and centrifuged at 12 000 *g* for 5 min. Twelve-microliter supernatant from the previous step was centrifuged at 10 000 *g* for 10 s before being placed in a PCR reactor, pre-denatured at 94 °C for 2 min, amplified for 35 cycles (at 94 °C for 50 s, at 53 °C for 50 s, and at 72 °C for 65 s), followed by extension at 72 °C for min and a final extension at 98 °C for 10 min. The samples were chilled immediately in an ice-bath for 10 min. Package was pinked at the microcosmic openings at 37 °C for 14 min in $NaHCO_3$ solution (pH 9.6). Nonspecific conjugate at the microcosmic openings was sealed by seal reagent. Ninety microliters of (three drops) hybridization liquid, 20 μL denatured output, 23 μL each nucleic pink (one kind was added to one opening) were added to different reaction openings, mixed up lightly and put into 50 °C water bath for 60 min, and poured completely. Then, 200 μL (eight drops) lotion was added at 37 °C for 3-5 min, poured completely and baptized once again. One hundred microliters of enzyme-antibody liquid (two drops) was added and kept at 37 °C for 30 min, poured and dried by water absorber, 200 μL (eight drops) lotion was added and kept at room temperature for 3-5 min, baptized twice, then color reagents A and B (one drop) were added, respectively, kept at room temperature in the dark for 10 min. Then, 2 mol/L H_2SO_4 solution (one drop) was added and the photoabsorption degree value (A) was detected at 450 nm. HBV DNA was considered positive, if P/N≥2.1 and negative if P/N<2.1. Positive result in hybridization indicated the virus gene

Detection of YMDD mutation

Reagent kits were provided by Biomedicine Diagnosis and Research Center of Basic Medicine Department of the First Military Medical University. YMDD mutations were detected by PCR. Twenty-five specimens were tested in Biomedicine Diagnosis and Research Center of Basic Medicine Department of the First Military Medical University, and the other 131 specimens were tested according to the manual of the kits and results were evaluated by experts at the Central Laboratory of our hospital.

Quantitative determination of HBV DNA

FX990 micro-fluorometer and HBV-PCR fluoroscopy reagent kits (detection limit is 103 copies/mL) were used to detect HBV DNA according to the instructions of the kits and results were evaluated by experienced experts.

Detection of HBVM

HBVM was detected by ELISA. Reagent kits were provided by Zhongshan Bioengineering Co., Ltd, Guangdong, China. The function of the liver was examined by the method of Laishi, and the normal level of ALT was lower than 40 U/L

Treatment

According to the common clinical practice[2], LAM (10 mg/d.p.o.) was given to 30 patients with single genotypes B, C, or D, and 35 patients with mixed genotypes CD, CB, or DB, and 16 non-classified types. HBV DNA and ALT were high, and HBeAg was positive in all cases selected. Twenty-eight cases had YMDD mutation. Liver function, HBVM and HBV DNA load were detected every 4 wk, for 48 wk.

Statistical analysis

The incidence rate of YMDD mutations, the rate of various clinical types of HB, the abnormal rate of ALT, the high loading rate of HBV DNA, and the positive rate of HBeAg in different genotypes of HBV were compared before and after LAM treatment by χ^2 using SAS statistical software. $P<0.05$ was considered statistically significant.

RESULTS

HBV genotypes in 156 CHB patients

Genotypes in 156 selected cases included B, C, D, mixed, and non-classified types. Genotypes A, E, and F were tested but not discovered. Genotype D accounted for 58.3% (91/156), type C 47.4% (74/156), type B 25.6% (40/156), and non-classified type 16.0% (25/156), mixed types (CD, CB, and BD) 47.4% (74/156). Single genotypes (B, C, or D) accounted for 36.5% (57/156, Table 1)

Table 1 Distribution of HBV genotypes in 156 cases

Patterns of genotype	A	B	C	D	E	F	CD	CB	BD	Non-A–F
n	–	9	15	33	–	–	43	16	15	25
Incidence rate (%)	–	5.8	9.6	21.2	–	–	27.6	10.3	9.6	16.0

Relationship between different HBV genotypes and YMDD mutations, clinical types of CHB, ALT, and HBV DNA, and HBe system

Natural YMDD mutations occurred in 46 cases (29.5%). Genotype B had no YMDD mutation. Genotypes had a different incidence rate of YMDD mutations, but there was no significant difference among them. Genotype B was seen mostly in mild and moderate CHB patients, while genotypes C, D and mixed types of CD, BD occurred mostly in severe CHB patients. The abnormal rate of ALT and the high loading rate of HBV DNA in genotypes D, and CD were higher than those in other genotypes, but there was no significant difference. The positive rate of HBeAg in non-classified genotype was low, and there was a significant difference between non-classified and other genotypes ($\chi^2 = 12.841$, $P<0.05$, Table 2).

Table 2 Relationship between different HBV genotypes and YMDD mutations, clinical types of CHB, ALT, and HBV DNA level, HBe system

Genotypes	*n*	Rate of YMDD mutation (%)	Clinical type of CHB		Clinical test		
			Mild and moderate CHB (%)	SevereCHB (%)	Abnormal rate of ALT (%)	HBV DNA >105 copies/mL (%)	HBeAg(+)/HBeAb(–)
B	9	–	8 (88.9)	1 (11.1)	3 (33.3)	4 (44.4)	7/2
C	15	6 (40.0)	4 (26.7)	11 (73.3)	8 (53.3)	9 (60.0)	10/5
D	33	7 (21.2)	14 (42.4)	19 (57.6)	23 (69.7)	22 (66.7)	23/10
CD	43	15 (34.9)	19 (44.2)	24 (55.8)	32 (74.4)	30 (69.8)	28/15
CB	16	6 (37.5)	9 (56.3)	7 (43.7)	9 (56.3)	10 (62.5)	12/4
BD	15	4 (26.7)	7 (46.7)	8 (53.3)	8 (53.3)	9 (60.0)	9/6
Non-A–F	25	8 (32.0)	15 (60.0)	10 (40.0)	12 (48.0)	10 (40.0)	8/17
χ^2		2.788	11.272		9.858	7.448	12.841
P		>0.05	>0.05		>0.05	>0.05	<0.05

Table 3 Curative effect after 48 weeks of LAM treatment

Group	*n*	Recovery rate of ALT (%)	HBV DNA level <103 copies/mL (%)	HBeAg negative conversion rate (%)	HBeAg/HBeAb conversion rate (%)
Single genotypes	30	23 (76.7)	24 (80.0)	16 (53.3)	11 (36.7)
Mixed genotypes	35	28 (80.0)	29 (82.9)	18 (51.4)	12 (34.3)
Non-classified genotypes	16	13 (81.3)	14 (87.5)	10 (62.5)	6 (37.5)
χ^2		0.168	0.411	0.561	0.065
P		>0.05	>0.05	>0.05	>0.05

Observation of curative effects of LAM

ALT and HBV DNA level in all genotypes decreased in different degrees, 4 wk after LAM treatment. There was no significant difference in ALT recovery rate, HBV DNA level, HBeAg, and HBeAg/HBeAb conversion rate 48 weeks after treatment between the groups (Table 3). Twenty-eight cases showed natural mutations of single mixed and non-classified genotypes. After 48 weeks, HBV DNA level decreased in 22 cases to a level <103 copies/mL and ALT became normal. Rebound of HBV DNA level and base line of ALT were not found. HBeAg became negative in 15 cases and 10 cases had HBeAg/HBeAb conversion 48 weeks after the treatment.

DISCUSSION

Research data indicate that HBV can be divided into eight genotypes[3], ranging from A to H. Genotype A is frequently found in northwest Europe and Africa. Genotypes B and C are common in Asia, while genotype D is prevalent in the Mediterranean and Near East. Genotype E is restricted to Sub-Saharan Africa, and F is localized in American aboriginal population. Genotype G has been found in France and USA, and genotypes A –H[4] have been found in patients with HBV infection in San Francisco. The distribution of HBV genotypes is related to immigration[5–7] and race background of the carriers. In China, genotypes B and C are predominant, while genotypes A and D are rare. But type D has a high percentage in minority nationalities. In our study, HBV genotypes were determined in 156 Zhuang CHB patients by PCR-microcosmic nucleic acid cross-ELISA. Genotypes B–D and non-classified type were identified. HBV genotype D was mostly found (58.3%), followed by genotypes C (47.4%), and B (25.6%). Non-classified type (16.0%) was significantly higher than that reported in Taiwan[8]. The distribution of genotypes B and C is consistent with most reports[9–11] in China. Type D is the predominant genotype in Zhuang population, which is different from other reports[11]. It may be related to the geographical and ethnic characteristics of Zhuang population. There was a high rate (47.4%) of mixed

genotypes. The mixed infection occurred between genotypes B and C or B and D, but mostly between genotypes D and C, which is also different from other reports[9] in China. The reasons may be as follows: (1) super infection and mixed infection, patients are easily treated, (2) gene mutation, detection methods, recombination of different genotypes[12]. Our study did not rule out the possibility of recombination of different genotypes. Further studies are needed to explore whether it is related to the special genobackground of Zhuang population or random mechanism.

YMDD motif, located in the C-domain of the catalytic site of the polymerase gene, is also the binding site of the antiviral drug LAM. Long-term treatment with LAM induces mutation of the YMDD motif; however, it was reported recently that YMDD mutations are occurring naturally[13-16]. Original research considered that the natural occurrence of YMDD mutant strains is associated with a great amount of HBV existing in patients[13]. Further research revealed that YMDD mutations occur spontaneously as they gain fitness without any particular cause[17]. The detection rate (29.5%) of mutant strains in our study is in accordance with that reported by Zhang *et al*[15]. showing that wild YMDD mutant strains exist in HBV DNA. There were differences of incidence rate of HBV YMDD wild mutant strains in HBV genotypes D, C, non-classified, and mixed genotypes of CD, BC, and CB. Although no significant difference was found among these genotypes, we cannot exclude the relation between YMDD mutations and HBV genotypes because of the limited samples in our study.

The virus gene controls antigen expression, leading to different genotypes and disease spectrum after infection. However, the conclusions are controversial. It was reported that genotype A is related to chronic active hepatitis, genotype D is related to acute self-limited hepatitis[18], and HBV-D is related to chronic symptomatic carriers[19]. All chronic HBV infections found in Jeju Island are genotype C[20]. But most scholars[21–23] found that genotype C is related to severe hepatitis or the aggravation of illness. Yuen *et al*[24] reported that genotype B has a higher mortality than genotype C because of decompensation of liver function. Our study showed that genotype A was not found in 156 CHB patients, while genotype D was predominant. This may be associated with uneven distribution of genotypes and simple clinical type, or the ethnic background. Genotype B was mainly found in chronic mild and moderate HBV patients, while genotypes C and D were mainly in chronic severe HBV patients. They seemed to increase with the progression of the illness. But there was no statistical significance. Studies on whether mixed genotype infection can worsen liver diseases are available[25–27], but the conclusion is controversial. In our study, mixed genotype CD and BD infections were mainly found in patients with chronic severe HBV infection, indicating that mixed genotype infection can lead to liver disease.

More attention has been paid to the relationship between HBV genotype and HBe, ALT, and HBV DNA. In our study, genotypes of CD and D had higher levels of ALT and HBV DNA than other genotypes, indicating that different genotypes are related with the level of ALT and HBV DNA. Compared to HBeAg-positive rate, there was a significant difference between non-classified and other genotypes ($P<0.05$), indicating that HBV genotype is related with HBe. Further studies are needed.

LAM is one of the first-line medicines for CHB patients[28,29]. Whether the effect of LAM treatment is influenced by HBV genotype is a topic of the researchers. Some studies showed that the effect of LAM treatment is not influenced by HBV genotype[30–32]. In our study, the effect of LAM treatment was not influenced by HBV genotype, suggesting that determination of HBV genotype cannot predict antiviral effect before LAM treatment[30]. Among the 28 cases with YMDD mutations, after 48 weeks of LAM treatment, HBV DNA levels in 22 cases decreased to below 103 copies/mL, and ALT became normal, and HBeAg became negative in 15 cases and 10 cases had HBeAg/HBeAb conversion, showing that LAM has a short-term effect in patients with YMDD wild mutation. This may be associated with the fact that wild viral strains are in the dominant side, while YMDD mutational strains are in the weak side and have a lower duplication activity and weaker pathogenicity.

In short, there are some unique characteristics in the distribution of HBV genotypes of Guangxi Zhuang population. This may be related to the geographic location and ethnics. HBV genotypes are not correlated with YMDD mutation, ALT, and HBV DNA level. The effect of LAM is not influenced by these factors. Further studies are needed to examine the relationship between the characteristics of HBV genotype, the mutation of pre-C-zone and different genotypes, as well as the conversion and prognosis of the disease.

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