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BRIEF ARTICLE

Relationship between hepatocellular carcinoma and hepatitis B virus genotype with spontaneous YMDD mutations

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Abstract

AIM: To investigate the relationship between hepatitis B virus (HBV) genotype with spontaneous YMDD mutations and hepatocellular carcinoma (HCC) in HBV-related cirrhosis.

METHODS: We investigated 264 liver cirrhosis patients who were not treated with antiviral drugs, including 81 patients with HCC. YMDD mutations were detected by fluorescent hybridization bioprobe polymerase chain reaction (PCR) and melting curve assay using the Diagnosis Kit for HBV YMDD Mutation. Serum HBV genotypes were detected by real-time PCR using genotype-specific TaqMan probes. Statistical analysis was performed according to data type using the *t* test, χ^2 test and unconditional logistic regression analysis.

RESULTS: In the HCC group, genotype C strains, spontaneous YMDD mutations, and genotype C strains with YMDD mutations were detected in 33 (40.74%), 13 (16.05%) and 11 (13.58%) patients, respectively. In the liver cirrhosis (LC) group, HBV genotype C strains, spontaneous YMDD mutations, and genotype C strains with YMDD mutations were detected in 33 (18.03%), 7 (3.83%) and 2 (1.09%) patients, respectively. The differences in genotype C strains, spontaneous YMDD mutations, and genotype C strains with YMDD mutations between the two groups were statistically significant (χ^2 = 15.441, *P* = 0.000; χ^2 = 11.983, *P* = 0.001; *P* = 0.000). In the HCC and LC groups, there were seven patients infected by genotype B strains with YMDD mutations and 13 by genotype C strains with YMDD mutations. Further research revealed that HCC occurred in 2 patients infected by genotype B strains with YMDD mutations and 11 infected by genotype C strains with YMDD mutations. The difference was statistically significant (*P* = 0.000). Unconditional logistic regression analysis revealed that patients infected by genotype C strains with spontaneous YMDD mutations had a 7.775-fold higher risk for the development of HBV-related HCC than patients infected by other type HBV strains (*P* = 0.013, 95%CI: 1.540-39.264).

CONCLUSION: Genotype C strains with spontaneous YMDD mutations are an independent risk factor for HCC in LC patients and are important for early warning of HCC.

Key words: Hepatitis B virus; Liver cirrhosis; Primary hepatocellular carcinoma; Hepatitis B virus genotype; YMDD mutation

Core tip: YMDD mutation is a research hotspot globally. Until recently, most research about YMDD mutation focused on the occurrence of lamivudine-related YMDD mutation and its impact on antiviral treatment. In our research, 264 hepatitis B virus (HBV)-related liver cirrhosis patients not treated with antiviral drugs, including 81 with primary hepatocellular carcinoma (HCC), were investigated for the association between infection by different HBV genotype strains with spontaneous

YMDD mutations and occurrence of primary HCC in cirrhosis patients. Infection by genotype C strains with spontaneous YMDD mutations is an independent risk factor for the development of HCC in cirrhosis patients.

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INTRODUCTION

According to the estimation of the World Health Organization, about 350 million people worldwide are chronically infected by hepatitis B virus (HBV)[1-3]. HBV infection is endemic in China. The seropositive rate of hepatitis B surface antigen is 7.18% and about 93 million people are chronically infected by HBV[4-6]. Chronic infection by HBV is the major cause of hepatocellular carcinoma (HCC)[7,8], and in addition to HCC, leads to a series of HBV-related liver diseases, including asymptomatic carrier, chronic hepatitis, and liver cirrhosis (LC)[9-11].

HBV replicates actively in host liver cells and the reverse transcriptase domain of HBV polymerases lacks a proofreading function. Thus, the mutation rate of HBV is relatively high. YMDD motif is a highly conserved sequence in domain C of HBV reverse transcriptase. YMDD mutation, also known as M204V/I mutation, is the substitution of methionine by valine or isoleucine and designated as YVDD or YIDD variant[12,13]. Until recently, most research about YMDD mutations has focused on the occurrence of lamivudine-related YMDD mutations and their effect on antiviral treatment[14-16]. During recent years, spontaneous YMDD mutations have been detected in patients with chronic HBV infection not previously treated with antiviral drugs. The relationship between spontaneous YMDD mutation and HBV-related HCC has rarely been reported. Different HBV genotype strains are formatted by accumulation of point mutations in the viral genome. Previous research has revealed that infection by genotype C strains is associated with HCC. However, the relationship between infection by different HBV genotype strains with spontaneous YMDD mutations and the occurrence of HCC in HBV-related LC patients has not been reported before.

In order to investigate the association between infection by different genotype strains with spontaneous YMDD mutation and the occurrence of HBV-related HCC, we investigated 264 cirrhosis patients not previously treated with antiviral drugs, including 81 with HCC.

MATERIALS AND METHODS

Patients

To ensure that HBV genotype and YMDD mutations could be detected by our kit, 264 HBV-related LC patients with serum HBV DNA load > 5×10^3 copies/mL, diagnosed and treated at the Department of Infectious Diseases in our hospital from May 2010 to August 2012, were selected for further research. According to the criteria "Chinese Standard for the Diagnoses and Treatment of Primary Hepatocellular Cancer in 2011" and "Prevention and Treatment Standard of Chinese Viral Hepatitis in 2000", 81 LC patients with HCC and 183 without HCC were selected and assigned to the HCC group and LC group, respectively. In the HCC group, there were 65 male patients (80.25%) and 16 female patients (19.75%). Their ages ranged from 31 to 78 years, with a mean of 53.86 ± 11.05 years. In the LC group, 129 patients (70.49%) were male and 54 patients (29.51%) were female. Their ages ranged from 22 to 79 years, with a mean of 52.66 ± 11.42 years. None of the patients had been treated previously with antiviral drugs and were not affected by other liver injury factors, such as co-infection with hepatitis A virus, hepatitis C virus, hepatitis D virus or hepatitis E virus, alcoholic hepatitis, autoimmune hepatitis, and fatty liver.

Sample collection

Fasting venous blood was collected from these patients. The serum was separated immediately and stored at -70 ℃.

Detection methods

Serum HBV DNA was quantified by real-time polymerase chain reaction (PCR) (Qiagen, Shenzhen, Guangdong Province, China). YMDD mutant types were determined by fluorescence hybridization bioprobe PCR and melting curve assay with the use of the care HBV mutation PCR assay (Qiagen, Shenzhen, China) and distinguished by melting temperature value. HBV genotype was detected by real-time PCR using genotype-specific TaqMan probe (Fuxing, Shanghai, China). Serum HBV markers were tested by enzyme-linked immunosorbent assay (Huamei, Shanghai, China).

Statistical analysis

Statistical analysis including Student's t test, χ^2 test and unconditional logistic regression analysis were performed using SPSS 17.0 software. A difference with $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

Patient characteristics are shown in Table 1. Age, sex distribution, hepatitis B e antigen (HBeAg)-positive rate, and serum HBV load did not differ significantly between the HCC and LC groups. Thirteen and seven spontaneous YMDD mutations were detected in the HCC and LC groups, respectively. In the HCC group, 33 (40.74%) patients were infected by genotype C strains and 47 (58.02%) were infected by genotype B strains. In the LC group, 33 (18.03%) patients were infected by genotype C strains

Table 1 Patient characteristics in the hepatocellular carcinoma and liver cirrhosis groups

	HCC group	LC group	t or χ^2 value	P value
No. of patients	81	183		
Age (yr; mean ± SD)	53.86 ± 11.05	52.66 ± 11.42	-0.797	0.426
Sex (male)	65	129	2.742	0.098
HBeAg positive	18	37	0.137	0.712
Serum HBV DNA loads (log10 copies/mL)	5.43 ± 1.16	5.25 ± 1.28	-1.077	0.283
Spontaneous YMDD mutation	13	7	11.983	0.001
Genotype C virus	33	33	15.441	0.000
Genotype B virus	47	146	13.518	0.000
Co-infected by genotype B and C viruses	1	4	-	-

HCC: Hepatocellular carcinoma; LC: Liver cirrhosis; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus.

and 146 (79.78%) were infected by genotype B strains. The ratio of patients infected by genotype C virus and spontaneous YMDD mutation rate in the HCC group was higher than in the LC group, and these differences were significant.

Ratio of patients infected by genotype B or C strains with spontaneous YMDD mutation

There were 2 (1.09%) and 11 (13.58%) patients infected by genotype C strains with YMDD mutation in the LC (n = 183) and HCC (n = 81) groups, respectively. The ratio of patients infected by genotype C strains with spontaneous YMDD mutation was higher in the HCC than in the LC group, and the difference was significant (P = 0.000). The constituent ratio difference of patients infected by genotype B strains with spontaneous YMDD mutations between the HCC and LC groups was not significant [2 (2.47%) *vs* 5 (2.73%), P = 1.000].

HCC in LC patients infected by genotype B or C strains with spontaneous YMDD mutation

Thirteen patients were infected by genotype C strains with YMDD mutations; 11 in the HCC group and two in the LC group. Seven patients were infected by genotype B strains with YMDD mutations; two in the HCC group and five in the LC group. The occurrence of HCC was significantly higher in patients infected by genotype C strains with YMDD mutation than in patients infected by genotype B strains with YMDD mutation (P = 0.022).

Identification of risk factors for HBV-related HCC

Unconditional logistic regression analysis was performed with the exclusion criterion $P \geqslant 0.05$. Sex, HBV genotype, and genotype C strains with spontaneous YMDD mutation were included in the regression model. As shown in Table 2, the occurrence of HCC in the male patients was 2.114 times higher than in the female patients. Patients infected by genotype C virus were 2.469 times more susceptible to HCC than those infected by genotype B virus. Risk of HCC in patients infected by genotype C strains with spontaneous YMDD mutation was 7.775-fold higher than in other patients. Other factors, including age, HBeAg status, serum HBV DNA load, spontaneous YMDD mutation, and genotype B strains with YMDD mutation, were excluded from the regression model.

DISCUSSION

Prior research has suggested that lamivudine is the major cause of YMDD mutation in HBV P-ORF. However, the mechanism remains unclear. Further research has revealed that strains with YMDD mutation also exist in patients with chronic HBV infection not previously treated with lamivudine[17-20]. Hosaka *et al*[21] have suggested that lamivudine-related YMDD mutation is an independent risk factor for HCC. Our results showed that spontaneous YMDD mutations were detected in LC and HCC patients, and spontaneous YMDD mutation rate in HCC patients was significantly higher than in LC patients. This suggests that spontaneous YMDD mutations are associated with the occurrence of HCC. Unlike the study of Hosaka *et al*[21], our unconditional logistic regression analysis showed that spontaneous YMDD mutation was not an independent risk factor for HBV-related HCC. One possible reason for this difference is that we studied the effect of spontaneous YMDD mutations, and the carcinogenicity of HBV strains with spontaneous YMDD mutations and lamivudine-related YMDD mutations may be different. This needs to be studied further.

Previous research has revealed that HBV-related LC is a leading cause of HCC[22-24]. Infection with genotype C strains can induce continuous gangrenous inflammation in the host liver and increase the risk of LC and HCC[25]. Our results showed that the infection rate of genotype C strains in the HCC group was higher than in the LC group. This suggests that infection by genotype C strains is associated with HCC. Consistent with previous reports, our unconditional logistic regression showed that the risk of HCC in patients infected by genotype C strains was 2.469 times higher than in those infected by genotype B strains, and infection by genotype C strains was an independent risk factor for HCC. This may have been caused by different genotype strains having different biological properties, pathogenicity and carcinogenicity.

Our research revealed that the ratio of patients infected by genotype C strains with spontaneous YMDD mutation was higher in the HCC than LC group. Unconditional logistic regression analysis showed that patients infected by genotype C strains with spontaneous YMDD mutations were 7.775-fold more susceptible to HCC than patients infected by genotype B strains with or without spontaneous YMDD mutations and genotype C strains

Table 2 Multivariate regression analysis for occurrence of hepatitis B virus-related hepatocellular carcinoma

Related factors	*B*	SE	Wald	*P* value	Exp (B)	95%CI
Sex (male)	0.749	0.353	4.500	0.034	2.114	1.059-4.224
HBV genotype C strain	0.904	0.335	7.257	0.007	2.469	1.279-4.764
Spontaneous YMDD mutation in genotype C strain	2.051	0.826	6.161	0.013	7.775	1.540-39.264

HBV: Hepatitis B virus.

without spontaneous YMDD mutations. This suggests that infection by genotype C strains with spontaneous YMDD mutations is an independent risk factor for HCC. Spontaneous YMDD mutation and formation of different genotype strains are the result of mutation in the HBV genome. Mutations in the reverse transcriptase domain and different HBV genotypes may result in changes in amino acid sequence and protein configuration in HBV polymerase. These may change HBV biological properties, influence the process of HBV-related diseases, and increase HBV carcinogenicity. Previous research has shown that mutated HBV may be easier to integrate into host hepatocytes. The integration may lead to chromosome mutation in host cells and increase host chromosomal instability. As a result, chromosome repeat, inversion, deletion and translocation can be detected in many HCC cells. Virus integration may activate many proto-oncogenes and cause mutations in anti-oncogenes. The activation of proto-oncogenes and repression of anti-oncogenes causes loss of control of cell proliferation and differentiation and results in the formation of cell clusters with accelerated division and malignant transformation[26,27].

To the best of our knowledge, this is the first study to show that infection by genotype C strains with spontaneous YMDD mutation is an independent risk factor for the development of HCC in patients with HBV-related LC. Our results pave the way for exploring the molecular biological mechanism of HCC and have important clinical value for early warning of HBV-related HCC.

COMMENTS

Background

YMDD motif is a highly conserved sequence in the C zone of the reverse-transcriptase domain of hepatitis B virus (HBV) polymerase. The YMDD motif is the binding site for lamivudine to interfere with the replication of HBV. YMDD mutation, also known as M204V/I mutation, is the substitution of methionine by valine or isoleucine and designated as YVDD or YIDD variant. YMDD mutation, caused by amino acid substitution, leads to a change in protein configuration, abolishes its binding affinity with lamivudine, and weakens the antiviral activity of lamivudine. The emergence of YMDD mutation can induce a series of symptoms including elevation of serum alanine aminotransferase level, positive conversion of serum HBV DNA, and lamivudine resistance. Resistance to lamivudine increases in parallel with the duration of treatment and affects clinical application of the drug. These findings have made YMDD mutation a research hotspot globally.

Research frontiers

In order to investigate the association between infection by different genotype strains with spontaneous YMDD mutation and occurrence of HBV-related hepatocellular carcinoma (HCC), 264 cirrhosis patients not previously treated with antiviral drugs, including 81 with HCC, were included in our research.

Innovations and breakthroughs

Until recently, most research about YMDD mutations have focused on the occurrence of lamivudine-related mutations and their effect on antiviral treatment. The relationship between spontaneous YMDD mutation and HBV-related HCC and the association between infection by different HBV genotype strains with spontaneous YMDD mutations and the occurrence of HCC in HBV-related liver cirrhosis patients have rarely been reported.

Applications

It is believed that our study is the first to identify infection by genotype C strains with spontaneous YMDD mutation as an independent risk factor for the development of HCC in HBV-related liver cirrhosis. These results pave the way for exploring the molecular biological mechanism of HCC and have important clinical value for early warning of HBV-related HCC.

Terminology

Spontaneous YMDD mutations: Tyrosine (Y)-methionine (M)-aspartic acid (D)-aspartic acid (D) (YMDD) mutation occurs in the absence of known mutagens, such as antiviral drugs. YMDD motif mutations can naturally occur in chronic HBV patients without antiviral treatment.

Peer review

This is a case control study. The case and control group should at least have similar characteristics in age, HBV viral load, and hepatitis status (HBV carrier, chronic hepatitis B, liver cirrhosis), otherwise the statistical analysis would be biased.

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