RESEARCH COMMUNICATION

High Prevalence of HBV in Tibet, China

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Abstract

Hepatitis B virus (HBV), distributed throughout the world, is classified into seven geographically separated genotypes designated A to G. Since the prevalence of HBV infection in isolated ethnic Tibetan populations in China, and the HBV genotypes involved have been hither to remained unclear, we collected 262 blood samples from four isolated villages in the east and west regions of Tibet. The prevalence of HBV infection was estimated by EIA for HBV Ag and HBV Ab. The HBV genotypes were determined by a PCR-microwell plate hybridization method using plasma DNA. The prevalence of HBV Ag and HBV Ab positives was 19.1% (50/262 cases) and 29.0% (76/262 cases), respectively. We detected only the C genotype (20/20 cases), this being known as a predominant type of HBV among Mongoloid populations in Asia. The results revealed, for the first time, that Tibetan villagers have a high rate of infection with HBV of C genotype, in line with the available data for chronic hepatitis and liver cancer.

Key words: HBV - Prevalence - Genotype - Tibet

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Introduction

Human hepatitis B virus (HBV) is a circular, partially double-stranded DNA virus of the hepadna family, 3200 nucleotides in length (nt). The viral genome contains four major open reading frames (ORF), encoding the envelope (preS1, preS2 and surface antigen HBsAg), core (preCore precusor protein, HBeAg and HBcAg), polymerase (HBPol) and X (HBX) proteins, respectively (Tillais et al., 1985). HBV infections cause hepatitis of varying severity, with sexual contact and blood contamination as common routes of transmission (Bowyer and Sim., 2000). Natural infection is acquired from HBV carrier mothers during either the perinatal or neonatal period. Such early infantile infections with HBV are asymptomatic and result in HBV carriers in a population (Vardas et al., 1999; Bowyer and Sim., 2000).

HBV strains are classified into seven genomic groups (or genotypes), designated as A to G, based on the nucleotide sequence of the HBV preS region (Usuda et al., 2000), with characteristic variation in human populations across the world. Genotype A is pandemic but most prevalent in North-west Europe, North America and Central Africa. Genotype B is mainly found in South East Asia, Indonesia and Papua new Guinea, while C predominates in Central and East Asia including Korea, China and Japan. Genotype D is more or less pandemic, but most common in the Mediterranean and the Middle East to India. Genotype E is predominant in Africa, F in South America and Polynesia, and G in North America and France (Bowyer and Sim., 2000; Stuyver et al., 2000; Nakamo et al., 2001).

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HBV is highly prevalent in Asian countries, including China, Japan, Thailand, Korea, and Indonesia with infection rates ranging from 5% to 10% in the general population (Nakamo et al., 2001). The peak prevalence rate of HBV Ag in China is as high as 19.0%, while the overall prevalence of HBV Ag in China is 10% (Zhou et al., 2000). We have found many cases of chronic hepatitis and liver cancer among Tibetans in China. However there has hither to been no study to clarify the etiological background of these liver diseases with respect to HBV infection. We have report a high prevalence of HBV infection among Tibetans with determination of the genotype.

Materials and Methods

Study subjects

Two hundred and sixty-two peripheral blood samples were collected with heparin as an anticoagulant from four isolated villages after obtaining informed consent. Forty (from 24 females and 16 males, ranging from 15 to 84 years old) from village LB and 62 (from 36 females and 26 males, ranging from 6 to 69 years old) from village LM in the East of Tibet; 55 samples (from 11 females and 44 males, ranging from 11 to 45 years old) from village RZ and 105 samples (from 31 females and 74 males, ranging from 10 to 60 years old) from village RG in the West of Tibet (Table 1). The plasmas were separated within three days and stored at -30 °C until HBV viral DNA was extracted from 100 µl plasma using the guanidine HCl method (Bowtell, 1987).

Serological assay

Using commercial enzyme immunoassays (EIA, Abbott Laboratory), the plasma was used to determine hepatitis markers, including Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody (HBsAb) and Hepatitis B core antibody (HBcAb).

Identification of HBV genotypes

The genotypes of HBV were identified by a PCR-Microwell plate hybridization method (HBV genotype kit, GSL, Tokyo, Japan), amplifying 160 base pair (bp) PCR products using primers specifically designed for the preS1 region of HBV DNA (2902 to 3091 nucleotide positions).

The PCR amplification was performed in 50 µl reaction mixture containing HBV viral DNA extracted from 100 µl plasma using AmpliTaq Gold DNA polymerase (Roche Molecular System, Somerville, New Jersey, USA). The conditions were as follows: pre-heating at 50 °C for 5 minutes; DNA polymerase activation at 95 °C for 10 min; 15 cycles of denaturation at 95 °C for 20 s, annealing at 65 °C for 10 s, and extension at 72 °C for 10 s; 55 cycles of denaturation at 90 °C for 20 s, annealing at 60 °C for 10 s, and extension at 72 °C for 10 s; a final extension at 72 °C for 5 min running in a GeneAmp PCR system 9600 (Perkin-Elmer, Norwalk, Connecticut, USA).

The amplified PCR products were denatured and added to microwell plates coated with 8 biotinylated probes specific for HBV genotypes, seven wells for HBV genotypes A to G and a control well to monitor PCR amplification. The plates were washed twice, and streptavidin-peroxidase conjugate solution was added followed by the substrate solution containing hydrogen peroxide and tetramethylbenzidine. Absorbance was measured at 450 nm, and a positive result was concluded when the corresponding relative genotypic probe was greater than 0.300.

Statistical analysis

Differences in HBV infection among Tibetan village were compared. Statistical significances (p-values) were evaluated by the chi-square test.

Results

Prevalence of HBV among Tibetans

We found 50 persons (19.1%) with HBsAg (current infection) and 76 (29.0%) with either HBcAb or HBsAb (past infection) among 262 Tibetan donors (Table 2). In village LB, the rates for HBV Ag and HBV Ab were 37.5% and 45.0%, respectively, demonstrating the highest prevalence of HBV infection among LB, LM, RZ, and RG. Differences from the alter villages were significant for both men and women.

There was no significant variation in the rates for HBV Ag and HBV Ab infections between the sexes. The HBsAg positive rates in people younger than 20 years old were 8.6% and 12.2 for women and men, respectively, lower than those

Table 1. Demographic Distribution of Tibetan Study Subjects

	Women (years old)			Men (years old)			
	<20	20-39	≥40	<20	20-39	≥40	Total
East Tibet							
LB	2	10	12	1	8	7	40
LM	18	11	7	10	9	7	62
West Tibet							
RZ	5	6	0	36	5	3	55
RG	10	14	7	43	16	15	105
Total	35	41	26	90	38	32	262

Table 2. Prevalence of HBV Infection among Tibetans

	Eastern Tibet				Western Tibet					
	LB		LM		RZ		RG		Total	
	n	rate (%)	n	rate (%)	n	rate (%)	n	rate (%)	n	rate (%)
HBsAg (+)										
Women	9	37.5*	3	8.3	3	27.3	6	19.4	21	20.6
<20 Years	1	50.0	0	0.0	1	20.0	1	10.0	3	8.6
20-39 Years	2	20.0	3	27.3	2	33.3	5	35.7	12	29.3
≥40 Years	6	50.0	0	0.0	0	0.0	0	0.0	6	23.1
Men	6	37.5**	2	7.7	4	9.1	17	23.0	29	18.1
<20 Years	1	100.0	0	0.0	1	2.8	9	20.9	11	12.2
20-39 Years	2	25.0	0	0.0	1	20.0	6	37.5	9	23.7
≥40 Years	3	42.9	2	28.6	2	66.7	2	13.3	9	28.1
Total	15	37.5***	5	8.1	7	12.7	23	21.9	50	19.1
HBsAb (+) or HBcAb (+)										
Women	10	41.7#	5	13.9	2	18.2	10	32.3	27	26.5
<20 Years	1	50.0	2	11.1	0	0.0	5	50.0	8	22.9
20-39 Years	7	70.0	1	9.1	2	33.3	2	14.3	12	29.3
≥40 Years	2	16.7	2	28.6	0	0.0	3	42.9	7	26.9
Men	8	47.1	8	30.8	14	31.8	19	25.7	49	30.6
<20 Years	0	0.0	3	30.0	11	30.6	10	23.3	24	26.7
20-39 Years	5	62.5	3	33.3	2	40.0	4	25.0	14	36.8
≥40 Years	3	42.9	2	28.6	1	33.3	5	33.3	11	34.4
Total	18	45.0#	13	21.0	16	29.1	29	27.6	76	29.0

*: significantly greater rate of HBsAg positivity in LB compared with LM. (Chi-square test)
**: significantly greater rate of HBsAg positivity in LB compared with LM and RZ. (Chi-square test)
* **: significantly greater rate of HBsAg positivity in LB compared with the other villages. (Chi-square test)
#: significantly greater rates of HBsAb or HBcAb positivity in LB compared with LM. (Chi-square test)

in the other age groups (20 to 40 years old and elder than 40 years) (Table 2).

The HBV infection rates in the three different age groups (less than 20 years old, 20 to 40 years old, and elder than 40 years) are given in Table 3. The average rate for HBsAg positives was 19.1%, although the prevalence rate among group 2 and 3 was higher than group 1.

HBV genotype in Tibetans

Forty samples of plasma DNA obtained from village LB were rendered for HBV genotyping by a PCR-Microwell plate hybridization method as shown in Figure 1. All sample investigated showed C genotype of HBV. There were no cases with genotypes A, B, D, E, F or G.

Table 3. Distribution of HBV Infection among Tibetans of Different Ages

	Group 1 (< 20 years)	Group 2 (20-39 years)	Group 3 (≥40 years)
HBsAg (+)			
Women	3 (8.6)	12 (29.3)	6 (23.1)
Men	11 (12.2)	9 (23.7)	9 (28.1)
Total	14 (11.2)	21 (26.6)*	15 (25.9)*
HBsAb (+) or HBcAb (+)			
Women	8 (22.9)	12 (29.3)	7 (26.9)
Men	24 (26.7)	14 (36.8)	11 (34.4)
Total	32 (25.6)	26 (32.9)	19 (32.8)

*: significantly greater rate of HBsAg positivity compared with group 1. (Chi-square test)

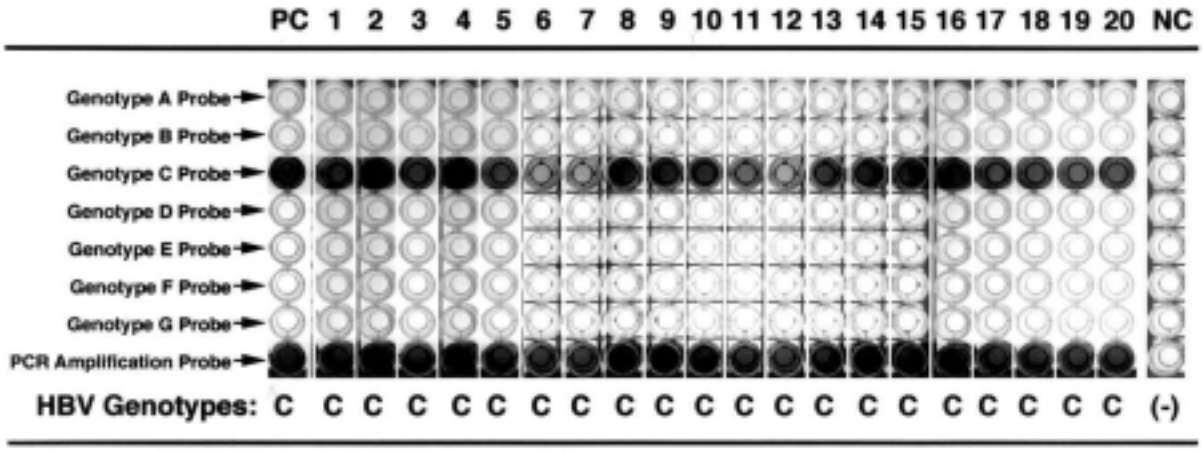

Figure 1. Determination of HBV Genotypes among Tibetan Donors. The HBV Genotypes were Identified by a PCR-Microwell Plate Hybridization Method using Plasma DNA. From top to botton in the left margin, the 7 HBV genotype probes (A to G) used in the study; the PCR Amplification Probe was induced to Monitor Amplified PCR Products of HBV DNA. PC is a Positive Control HBV DNA of the C Genotype. NC is a Negative Control DNA without HBV infection. Numbers 1 to 20 Represent 20 Cases of HBV DNA Obtained from Village LB in Eastern Tibet.

Comparison of HBV genotypes among the ethnic groups in the world

The worldwide ethnic distribution of HBV genotypes was compared using data from other reports (Usuda et al., 1999; Bowyer and Sim., 2000; Nakamo et al., 2001; Bowyer et al., 1997; Arauz-Ruiz et al., 1997; Blitz et al., 1998; Norder et al., 1993;), as illustrated in Figure 2. The twenty cases of HBV genotype C obtained in this study were included for comparison (Figure 2, Tibet), with clear similarity to its prevalence in other Asian Mongoloid peoples, particularly Koreans, but dissimilar to the A and D genotypes found in Indians and Nepalese (Figure 2).

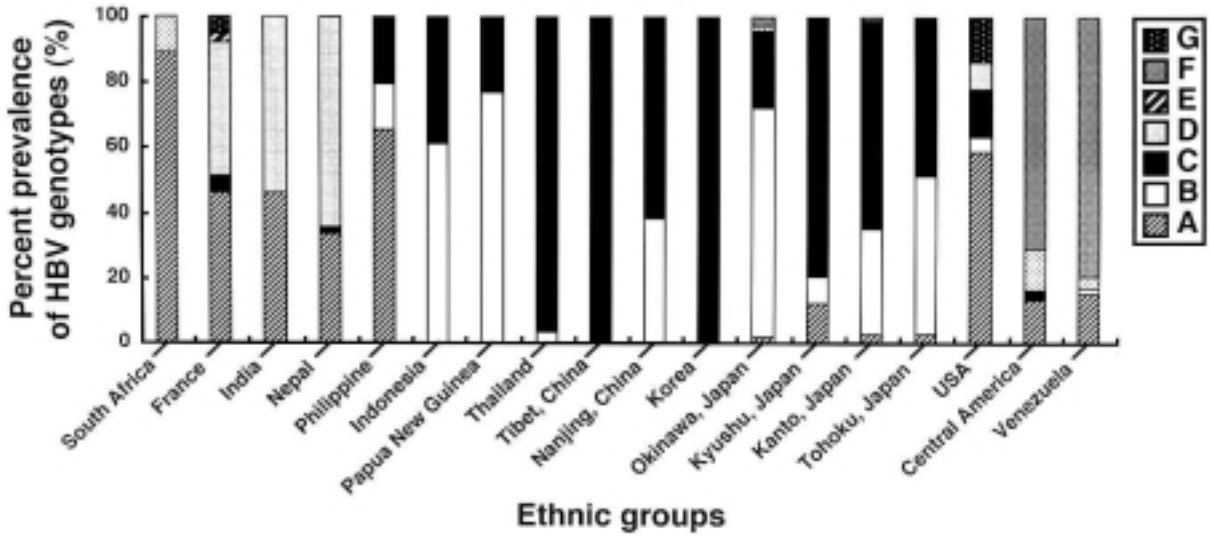

Figure 2. Distribution of HBV Genotypes among Various Ethnic Groups. The Worldwide Ethnic Distribution of HBV Genotypes was Compared using Reported Results (Usuda et al., 1999; Bowyer and Sim., 2000; Nakamo et al., 2001; Bowyer et al., 1997; Arauz-Ruiz et al., 1997; Blitz et al., 1998; Norder et al., 1993;). For fourteen ethnic groups, including the Tibetan results obtained in this study: South Africa (n=27), France (n=39), India (n=13), Nepal (n=45), Philippine (n=64), Indonesia (n=31), Papua New.Guinea (n=52), Thailand (n=30), China (Tibet, n=20; Nanjing, n=42), Korea (n=27), Japan (Okinawa, n=72; Kyushu, n=123; Kanto, n=106; Tohoku, n=206), USA (n=82), Central America (n=31) and Venezuela (n=59).

Discussion

The population geography of Tibet is unique with inhabitation of a high plateau averaging more than 3,500 meters, isolated from neighboring countries by high mountains. It has been reported that patients with chronic hepatitis and liver cancer are frequently encountered in local hospitals, but the actual prevalence of HBV and the predominant genotype in Tibet have hither to not been reported. We therefore investigated HBV infection in four isolated villages, more than 200 kilometers from Lhasa. None of the participants had a history of HBV vaccination.

The average positive rate of HBsAg (19.1%) among Tibetans was much higher than the world average (<5.0%) (Mbayed et al., 1998) (Table 2). In village LB, the rates of HBV Ag and HBV Ab were 37.5% and 45.0% respectively, very much greater than the rate for village LM was of 8.1% and 21%. The average rate for HbsAg in China is 10.0% (She et al., 1988; Zhou et al., 2000).

HBV infection is known to have a perinatal / neonatal transmission, linked to socio-economic status and age (Norder et al., 1993). It is therefore important to clarify how the HBV prevalence rate varies with age and sex. Among Tibetan natives. We have found the HBsAg positive rates in adults to be significantly higher than in children or adolescents. There was no significant difference between men and women in each age group indicating that the age-dependent increase of HBV infection may be due to the birth cohort effect.

It is reported that the HBV genotypes are ethnically clustered (Bowyer and Sim., 2000; Stuyver et al., 2000; Nakamo et al., 2001;) and we identified only genotype C in the LB village in Tibet, this being the predominate genotype in Asian Mongoloids, including peoples in Thailand, China, Korea and Japan (Bowyer and Sim., 2000; Nakamo et al., 2001; Bowyer et al., 1997; Arauz-Ruiz et al., 1997; Blitz et al., 1998; Norder et al., 1993;) (Figure 2).

Ethnic clustering of the HBV genotype is of particular importance with regard to HBV vaccines and their application. Some failures in vaccine programs seem to reflect an ethnic incapacity to confer immunity rather that mutations of the HBV genotype. Chronic hepatitis B is known to be associated with HBV of genotype A, while acute hepatitis B is linked with genotype D in Western countries (McMillan et al., 1996). In Asian countries, where HBV of genotypes B or C is common, patients infected with C appear to contract more severe hepatitis (Lindh et al., 1999). These results strongly suggest that the HBV genotype and the host immune response are related to different clinical symptoms.

HBV infection is known to be associated with the human leukocyte antigen (HLA) class I and class II alleles (Stuyver et al, 1999; Bowyer et al., 2000; Stuyver et al, 1999). Therefore, an understanding of genetic polymorphism of HLA genes as well as HBV genotypes may be helpful in interpreting virus-host interactions and the outcome of disease in different ethnic groups (Bowyer et al., 2000; Yashiki et al, 2001). HLA and HBV gene sequences warrant further investigation in this context.

In conclusion, the present investigation of the prevalence of HBV infection in four isolated villages in Tibet, demonstrated a high rate, especially in LB in the East. This is the first report of a high prevalence of HBV infection with genotype C among the native Tibetan population.

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Zhao Shuming was born in Sichuan Province, China in 1965. After graduating from Third Military Medical University, he trained internal medicine for 4 years in the University hospital, laboratory medicine for 3 years as Master of Medicine in Third Military Medical University and Clinical Biochemistry for 3 years as Doctor of Medicine in Chongqing Medical University. Since 1999, he has been Associated Professor in the Department of Transfusion, Southwest Hospital, Third Military Medical University in Chongqing, P.R. China.

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