Prevalence of hepatitis B genotype and viral basic core promoter and precore mutations among teenagers in Macao: relationship with hepatocellular carcinoma development

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ABSTRACT

Chronic hepatitis B virus (HBV) infection is a global problem and over 75% of cases are reported in the Asia Pacific region. Infection can lead to progressive liver disease, cirrhosis and hepatocellular carcinoma (HCC). Previous studies suggest the prevalence of HBV carriers in Macau to be approximately 10% of the population. This study aims to investigate the prevalence of HBV genotypes among HBV-positive teenagers in Macao and the prevalence of base core promoter (BCP) and precore (PreC) mutations in the viral genome. In addition, through monitoring aminotransferase and α-fetoprotein, it aims to investigate relationships among HBV genotypes, BCP/PreC mutations and HCC development. This study recruited 1991 teenagers in Macau in 2008, and the PreS1/S2, BCP and PreC region of the HBV genome from 34 HBsAg-positive subjects were amplified and sequenced to determine HBV genotype and presence of HCC-associated mutations. Results suggested that the average rate of HBV infection among secondary school teenagers in Macao is low, and HBV genotype B and C viruses were found to predominate in Macao. The BCP/PreC mutations A1762T, G1764A, G1896A and C1766T were identified in 2.9–11.7% of subjects. However, no significant relationship was observed between HBV genotype, BCP/PreC mutations and HCC development.

KEY WORDS: Carcinoma, hepatocellular.
Genotype.
Hepatitis B.
Mutation.

Introduction

Hepatitis B virus (HBV) is a major global health problem affecting more than 350 million people worldwide, with 75% of cases reported in the Asia-Pacific region.[1–3] Previous studies suggest that overall HBV surface antigen (HBsAg) carrier rates are 10–15% in this region;[4] thus, the prevalence of HBV carriers in Macau is estimated to be approximately 10% of the population.

Chronic HBV infection can lead to a wide spectrum of clinical problems ranging from an asymptomatic carrier state to the development of liver cirrhosis or hepatocellular carcinoma (HCC), with increased levels of serological markers including alanine aminotransferase (ALT) and α-fetoprotein (AFP).

Currently, eight HBV genotypes (A–H) are circulating worldwide, but genotypes B and C are the major types responsible for HBV in Asia.[5] Previous studies suggest that chronic HBV patients with certain HBV genotypes show greater risk of developing HCC;[6–10] however, the data on severity of liver disease associated with genotype is controversial.

Mutations in the basic core promoter (BCP; A1762T and G1764A) and precore (PreC; G1896A) region of the HBV genome enhance HBV replication and reduce expression of HBeAg.[11–15] However, there is inconsistency in the correlation between BCP/PreC mutations and HCC development.[9,16,17]

This study aims to investigate the prevalence of HBV genotypes among HBV-carrying teenagers in Macao and the prevalence of BCP/PreC mutations in the viral genome. In addition, through monitoring the serological markers ALT and AFP, it investigates the relationship between HBV genotype, BCP/PreC mutations and HCC development.

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Materials and methods

Sample collection

A total of 1991 students were recruited from 10 secondary schools in Macau in 2008. Informed consent was obtained from their parents. Peripheral blood samples were collected and serum was separated on the same day.

Diagnostic tests for HBsAg and HBV surface antibodies (anti-HBs) were performed on all participant samples. Among the 1991 students, 34 (1.7%) were HBV-positive, and genotype was identified in each case.

Genotyping

Viral DNA was extracted using the QIAamp DNA blood

Table 1. Clinical background, HBV genotype and BCP/PC mutation profiles of the study group.

			HBV genotype		
		Overall	A	B	C
		(*n*=34)	(*n*=3, 8.8%)	(*n*=11, 32.4%)	(*n*=20, 58.8%)
Gender	Male	15 (44.1%)	2 (66.7%)	4 (36.4%)	9 (45.0%)
	Female	19 (55.9%)	1 (33.3%)	7 (63.6%)	11 (55.0%)
HBV viral load (mean±SD, log copies/mL)		5.5±2.7	2.9±0.4	6.5±2.5	5.3±2.7
Serological markers	ALT (mean±SD, IU/mL)	23.2±16.4	12.0±5.6	24.3±20.2	25.8±16.1
	AFP (mean±SD, ng/mL)	2.3±1.4	1.45±0.2	2.39±1.1	2.42±0.2
BCP/PreC mutations	A1762T	3 (8.8%)	0	1 (9.0%)	2 (10.0%)
	G1764A	4 (11.7%)	0	1 (9.0%)	3 (15.0%)
	G1896A	3 (8.8%)	0	1 (9.0%)	2 (10.0%)
	C1766T	1 (2.9%)	0	0	1 (5.0%)
Reference range: ALT 5–56 IU/mL; AFP <20 ng/mL.					

mini kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. The PreS1/S2 region of the HBV genome was amplified and sequenced by the primer pairs preS1F (5′-AGGTRGGAGYGGGAGCATTCGG-3′) and preS1R (5′-CCTGAACTGGAGCCACCAGCAGG-3′), as described previously.[18] The HBV genotype was then determined by analysing the 277 bp sequence through the NCBI Viral Genotyping Tool (www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi).

Viral load quantification

To determine HBV viral load, 10 µL each DNA extract was subjected to a real-time PCR method, as described previously.[19]

Mutation detection

A nested PCR method was designed to cover the flanking region of the BCP and PreC regions. Primer set H1s and H2a (H1s: 5′-ACTCTTGGACTYTCAGCAATG-3′; H2a: 5′-GTCAGAAGGCAAAAAAGAGAG-3′) was used for the first round of PCR, while the nested primer set H3s and H4a (H3s: 5′-TCTCAGCAATGTCAACGACCG-3′; H4a: 5′-AGAGAGTAACTCCACAGAWGCTC-3′) was used for the second. The amplified products were then sequenced using the nested PCR primer set. Single nucleotide polymorphisms (SNPs) were analysed by comparison with different HBV genome sequences (Genotype A: Accession No X02763, Genotype B: Accession No D00330, Genotype C: Accession No AB03356).[20]

Statistical analysis

Results were expressed as mean±standard deviation (SD). The two-tailed Student's *t*-test was used to illustrate the relationship between HBV genotype and the level of ALT (IU/mL), AFP (ng/mL) or HBV viral load (log copies/mL). The two-tailed Student's *t*-test was also used to determine the correlation between the level of ALT (IU/mL), AFP (ng/mL) or HBV viral load (log copies/mL) and the different HBV BCP/PreC mutations (A1762T, G1764A, G1896A). $P<0.05$ was considered statistically significant.

Results

Of the 1991 students studied, 34 (1.7%) were HBV-seropositive (either HBsAg or anti-HBs). All were ethnic Chinese (mean age: 17.4±2.3 years; 15 [44.1%] male, 19 [55.9%] female) and 20 (58.8%) were carrying the HBV genotype C. Genotypes A and B were identified in three (8.8%) and 11 (32.4%) subjects, respectively.

In monitoring HBV viral load, the genotype B group (6.46±2.47 log copies/mL) showed the highest mean viral titre, while the genotype C group (5.34±2.72 log copies/mL) showed a slightly lower mean viral load. Subjects with genotype A (2.86±0.44 log copies/mL) had the lowest viral load ($P<0.001$).

Overall ALT and AFP levels were 23.2±16.4 IU/mL and 2.3±1.4ng/mL, respectively (within the normal reference range). No significant difference was seen between ALT

Table 2. Relationship between BCP/PreC mutation and serological markers.

BCP/PreC mutation		No. samples	ALT (IU/mL)	AFP (ng/mL)	HIV viral load (log copies/mL)	*P* value
A1762	Wild-type	31	22.6±14.2	2.3±1.5	5.6±2.7	>0.1
	A1762T mutant	3	39.0±37.0	2.5±0.2	4.75±2.8	>0.1
G1764	Wild-type	30	23.1±14.2	2.4±1.6	5.7±2.7	>0.1
	G1764A mutant	4	31.5±33.8	2.1±1.1	4.2±2.6	>0.1
G1896	Wild-type	31	22.7±14.2	2.3±1.6	5.4±2.7	>0.1
	G1896A mutant	3	38.7±37.2	2.6±0.7	6.7±2.7	>0.1

level in the genotype B and C groups (genotype C: 25.8±16.1; genotype B: 24.3± 20.2). Genotype A subjects had the lowest ALT levels (12.0±5.6) compared to those in the genotype B and C groups. The AFP level in the genotype B (2.4±1.1 ng/mL) and genotype C (2.4±1.7 ng/mL) groups was higher than in genotype A (1.4±0.2 ng/mL) subjects.

Mutations (A1762T, G1764A, G1896A and C1766T) in the HBV genome were identified, and A1762T, G1764A and G1896A were found in the genotype B and C groups, while C1766T was only found in genotype C. These BCP/PreC mutations were identified more commonly in genotype C samples (10–15%). No BCP/PreC mutation was identified in genotype A samples (Table 1).

The BCP/PreC mutant samples generally showed higher ALT and AFP levels compared to the wild-type samples. However, no significant differences in ALT and AFP level were observed (Table 2).

Discussion

This surveillance study is the first to consider HBV genotypes among teenagers in Macao. The HBV prevalence found is considerably lower than the 10% estimated prevalence predicted by the World Health Organization.[2,3] This suggests that teenagers represented the major risk group in Macao in 2008. The high prevalence of genotype C in this study also suggests that it is the prevalent HBV genotype in Macao. Other HBV genotypes (e.g., genotypes A and B) are also found in Macao, and is consistent with the situation in other cities in southern China (e.g., Hong Kong).[20–22] This may be explained by close proximity and frequent population migration among the cities in the region.

Genotype C is thought to be associated with the progression of HCC and liver cirrhosis, while Asians infected with HBV genotype C have a higher risk of developing HCC and liver cirrhosis.[23] As HBV genotype C is a common finding among HBV-infected Chinese in Macao, the present study investigated the relationship between the HBV genotypes and HCC by looking at ALT and AFP levels and HBV viral load. Levels of ALT and AFP were unremarkable among the subjects infected with different HBV genotypes; however, those with genotype B and C infections had viral loads $>10^5$ copies/mL. This suggests a 30-fold increase in risk of developing HCC with the presence of the BCP mutations A1762T and G1896A.[24] The mean HBV viral load ($>10^4$ copies/mL) found in the genotype A group also suggests a strong connection with HCC development.[25] However, the results of this study do not suggest a significant relationship between HBV genotypes and HCC development.

It has been reported that the A1762T, G1764A and G1896A mutations are related to HCC development.[24,26–28] Although the mutation rate for A1762T, G1764A and G1896A in genotype C in the present study was higher than that seen in other genotypes, no significant difference was observed between BCP/PreC mutation rate and HBV genotype. However, from the data presented here, Chinese subjects who carry HBV genotype B or C with BCP/PreC mutations should be monitored closely for the development of HCC.[15] □

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