HBV serum markers of 49164 patients and their relationships to HBV genotype in Fujian province of China

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Background: There is a high prevalence of Hepatitis B virus (HBV) infection in China. The aim of this article is to reveal the HBV prevalence and its outcome in southern China by analyzing the HBV serum markers and their relationship to HBV genotype. **Methods:** HBV serum markers, HBV-DNA and Alanine aminotransferase (ALT) of 49,164 patients were detected in Fujian Province of China, and HBV genotyping was conducted using melting curve method. **Results:** 23.46% patients (11,535/49,164) were found HBsAg-positive and the concentration of ALT and HBV-DNA were positively correlated with HBsAg. The concentration of ALT and HBV-DNA markedly decreased when HBeAg turned

negative or HBsAg concentration dropped to undetectable. The concentration of HBsAg and HBV-DNA were higher in genotype C than in genotype B and mixed genotype B/C, and the concentration of ALT in genotype C and B was higher than in mixed genotype B/C. **Conclusions:** The positive rate of HBsAg of Fujian hospitalized patients is higher than in the previous reports. The concentration of HBsAg and HBV-DNA of genotype B is different from that of genotype C, and the concentration of HBsAg could be valuable in predicting the curative efficacy in HBV-infected patients. J. Clin. Lab. Anal. 27:130–136, 2013. © 2013 Wiley Periodicals, Inc.

Key words: hepatitis B virus; serum marker; genotype

INTRODUCTION

The prevalence of HBV infection has decreased in the past three decades (1) due to extensive inoculation of hepatitis B virus (hepatitis B virus, HBV) vaccine and the changes of living conditions and lifestyles in China. On the other hand, the detection technology of HBV serum markers has been constantly updated: from reversed passive hemagglutination assay (RPHA) 30 years ago to enzyme-linked immunosorbent assay (ELISA) 20 years ago and to currently widely used magnetic enzyme-linked immunoassay (MEIA) and electrochemiluminescence assay (ECLA). Improved ability to detect HBV serum markers is mainly reflected in the detection sensitivity and specificity that made it possible to the quantitative detection of HBV surface antigen (HBsAg), antibodies to HBV surface (Anti-HBs), HBV e antigen (HBeAg), antibodies to HBV e (Anti-HBe) and antibodies to HBV core

antigen (Anti-HBc). The accurate quantitative detection of serological markers is significant for assessing the current situation of immunization programs (2), monitoring the HBV infectivity and curative efficacy of interferon and other anti-viral drugs. The quantization results of five serological markers of the children in Beijing showed that there was an obvious change in positive modes of HBV serological marker due to the extensive vaccination of HBV (2).

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In addition, HBV genotyping was highly valuable. Several epidemiological surveys showed that HBV genes were mainly genotype B and genotype C in mainland China, mainly genotype B in the South, and genotype C in the North (3). There was an obvious relationship between HBV genotype and the outcome and curative efficacy of the patients with chronic hepatitis B (4, 5).

A few years ago, the First Affiliated Hospital of Fujian Medical University started to use the magnetic enzyme-linked immunoassay method (MEIA) for quantitative detection of HBV serological markers of all hospitalized patients and some outpatients. This article analyzed the results of HBV serum markers, HBV genotyping results and clinical data in some patients from February 2009 to December 2010 in order to further reveal the HBV prevalence and its clinical outcomes in southern China.

MATERIALS AND METHODS

Patients

Sera of 49,164 patients were collected from February 1, 2009 to December 1, 2010 in various clinical divisions including Department of Internal Medicine, Surgery Department, Department of Gynecology and Obstetrics, Pediatrics (39,398 in total) and Outpatient Department (5,399 in total), hepatic center (2,614 hospitalized patients in total), and medical examination center (1,753 in total) in the First Affiliated Hospital of Fujian Medical University. There were 26,933 men (54.78%) and 22,231 women (45.22%) with a mean age of 46.8 ± 18.5 years (range: 1 h after birth–107 years). Serum markers were detected twice or more for 7,037 patients. Serum markers were detected in 58,240 in total (including the patients detected for once only). For 2,113 patients [1,621 male (76.72%) and 492 female (23.28%)], the concentration of HBsAg was measured after diluting the serum (HBsAg >250 IU/ml). Some patients received repeated quantization of HBsAg and 4,277 cases were quantitated after dilution.

Detection of HBV Serum Markers

HBsAg, Anti-HBs, HBeAg, Anti-HBe, and Anti-HBc were detected by an automated magnetic enzyme linked immunology analyzer (Abbott I2000, Abbott Laboratories, Chicago) with original kits supplied by Abbott. The samples with concentration of HBsAg exceeding the detection limit (250 IU/ml) were further diluted and the result was multiplied by a dilution factor (1:500).

TABLE 1. Primers Used in Melting Curve Method

Genotype	Primer sequence (5' → 3')	Position	Amplified fragment length (bp)
B	Forward: AGTTAATC ATTACTTCCAGACGC	nt 2,717–2,739	130
	Reverse: CTGTAGAT CTTGTTCCCAAGAAT	nt 2,846–2,824	
C	Forward: TGTTC GACTACTGCCTCAC	nt 91–110	273
	Reverse: GGACAAA TTGGAGGACAAGAG	nt 363–343	

Measurement of Alanine aminotransferase (ALT)

The kit (Ningbo Meikang Company, Ningbo, China) was used in a biochemical analyzer (Olympus AU2700, Olympus Corporation, Tokyo, Japan) on the principle of Enzyme Kinetics with standard substance and quality-control substance from American Bio-Rad Laboratories (Hercules).

Quantization of HBV–DNA

Real-time fluorescent quantitative PCR method based Shenzhen PG kit (Shenzhen PG Biotech Company, Shenzhen, China) and Roche Lightcycler 480 (Roche Corporation, Basel, Switzerland) were used. The quality-control substance was from National Center for Clinical Laboratory (Ministry of Health, Beijing, China).

HBV Genotyping

HBV genotyping method was developed by our laboratory (6), as described below.

Reagents

Viral genome DNA extraction kit was purchased from Shanghai Generay Biotech Company (Shanghai, China), and SYBR® Premix Ex Taq™ and ROX Reference Dye (50×) were purchased from Dalian Takara Biological Company (Dalian, China).

Primer design

Two pairs of primers were synthesized by Dalian Takara Biological Company according to the whole genome sequences of genotype B and genotype C (Table 1).

Virus DNA extraction and PCR amplification

Virus DNA was extracted using viral genome DNA extraction kit and stored at -20°C . A total of 50 μl

TABLE 2. HBV Serum Marker Detection of 49,164 Patients

Serum marker	Positive patient	Ratio (%)	Serum marker	Positive patients	Ratio (%)
HBsAg(+), other markers may be positive or negative	11,535	23.46	Anti-HBs(+) Anti-HBc(+)	10,961	22.29
HBsAg(+) HBeAg(+) Anti-HBc(+)	2,219	4.51	Anti-HBs(+) Anti-HBe(+) Anti-HBc(+)	7,429	15.11
HBsAg(+) Anti-HBe(+) Anti-HBc(+)	6,540	13.30	HBsAg(+) Anti-HBs(+)	301	0.61
Anti-HBs(+)	7,061	14.36	HBeAg(+) Anti-HBe(+)	215	0.44
Anti-HBc(+)	3,788	7.70			
HBsAg(+) and other markers are all negative	589	1.20			

PCR reactive system consisted of 25 μ l 2 $\times$ SYBR Premix Ex Taq, 1 μ l genotype B primers and 1 μ l genotype C primers (10 μ mol/l), 1 μ l ROX Reference Dye (50 $\times$), 4 μ l DNA template, and 16 μ l ddH₂O. PCR reactive condition: 95°C for 30 s and then 95°C for 5 s, 60°C for 31 s with 40 cycles and fluorescence was detected at 60°C. SYBR Green I was selected for fluorescent channel detection. H₂O was designed as a blank control group. Type B, C, and mixed type B/C after sequencing and verification were used as positive standard substances and detected with specimen with identical batch. The analysis was performed on the ABI PRISM[®] 7000 fluorescent quantization PCR apparatus (Life Technologies Corporation, Carlsbad).

HBV genotyping

The results were analyzed by the software of ABI PRISM[®] 7000 and the temperature represented T_m value (melting-out temperature) of double-stranded DNA at crest in melting curve. The T_m depended on the length of DNA and G/C content. Genotype was determined by T_m value in the melting curve. The amplified fragment of B-specific primer was 130 bp and T_m was 79.9 $\pm$ 0.5°C; the amplified fragment of C-specific primer was 273 bp and T_m was 84.6 $\pm$ 0.4°C when the crest occurred at 79.9 $\pm$ 0.5°C and 84.6 $\pm$ 0.4°C, the genotype was mixed type B/C.

Statistical Analysis

SPSS 13.0 statistical software was used to analyze data. Measurement data were presented as median to skewed distribution; rank sum test was adopted to conduct group comparison about serological concentration; rank correlation was used to analyze and explore the relationship of different serological indicators.

RESULTS

HBV serum markers

We found that 23.46% patients (11535/49164) were positive for HBsAg; 4.51% patients (2219/49164) were positive for HBsAg, HBeAg and Anti-HBc; 13.30% patients (6540/49164) were positive for HBsAg, Anti-HBe and Anti-HBc. Anti-HBs and Anti-HBc double positive were found in 22.29% patients (10961/49164); Anti-HBs, Anti-HBe and Anti-HBc positive were in 15.11% patients (7429/49164); patients with positive Anti-HBs or Anti-HBc or HBsAg were 14.36% (7061/49164), 7.70% (3788/49164), 1.20% (589/49164) respectively. Some “rare modes” appear with increasing frequency, patients with positive HBsAg and Anti-HBs were 0.61% (301/49164); patients with positive HBeAg and Anti-HBe were 0.44% (215/49164) (Table 2).

After excluding the patients in hepatic center, outpatient department (mainly for hepatic disease) and those who applied for health examination (mainly for HBV carriers), the patients with positive HBsAg were 14.96% (5893/49164); the patients with positive HBsAg, HBeAg, and Anti-HBc were 2.41% (949/49164); the patients with positive HBsAg, Anti-HBe, and Anti-HBc were 11.41% (4494/49164).

Comparison of ALT and HBV–DNA level in Patients with HBsAg(+) Anti-HBe(+) Anti-HBc(+)

54.27% patients (2439/4494) had 250 IU/ml or higher HBsAg in all patients with positive HBsAg, Anti-HBe and Anti-HBc; the patients with HBsAg lower than 250 IU/ml were 45.73%(2055/4494). The median concentration of Alanine aminotransferase (ALT) was 32.0 U/l and the average copy number of HBV–DNA was 1.05 $\times$ 10³ copies/ml in patients with positive HBsAg, Anti-HBe, and Anti-HBc after excluding the patients in hepatic center, clinic service, and those who applied for health examination.

The patients with positive HBsAg, Anti-HBe, and Anti-HBc were grouped according to the level of HBsAg. The

TABLE 3. Comparison of ALT and HBV–DNA Level in Patients with HBsAg(+) Anti-HBe(+) Anti-HBc(+)

	Total		HBsAg > 250 IU/ml		HBsAg < 250 IU/ml		Z	P
	The median concentration	Min~Max	The median concentration	Min~Max	The median concentration	Min~Max		
ALT (U/l)	32.0	6~1,540	36.0	6~1,540	26.0	8~818	–10.535	<0.01
HBV–DNA (copies/ml)	1.05×10^3	$<1.0 \times 10^3$ $\sim 6.78 \times 10^7$	2.13×10^4	$<1.0 \times 10^3$ $\sim 6.78 \times 10^7$	$<1.0 \times 10^3$	$<1.0 \times 10^3$ $\sim 4.67 \times 10^7$	–9.755	<0.01

median concentration of ALT (36.0 U/L) in the group with 250 IU/ml HBsAg and above was higher than that (26.0 U/L) in the group with less than 250 IU/ml HBsAg; the average copy number of HBV–DNA (2.13×10^4 copies/ml) in the group with 250 IU/ml HBsAg and above was higher than that ($<1.0 \times 10^3$ copies/ml) in the group with less than 250 IU/ml HBsAg. The differences were statistical significance for these indicators ($P < 0.01$; Table 3).

The patients with positive HBsAg, Anti-HBe, and Anti-HBc from various clinical divisions, especially were 20.33% in Chinese medical science/ dermatology, 19.01% in chemotherapy ward, 16.11% in radiation ward, 14.56% in surgery of liver, gall and pancreas, 13.61% in digestive internal medicine.

Comparison of ALT and HBV–DNA level in patients with HBsAg(+) HBeAg(+) Anti-HBc(+)

The patients with 250 IU/ml HBsAg and above were 96.00% (911/949) in all patients with positive HBsAg, HBeAg, and Anti-HBc; the patients with less than 250 IU/ml HBsAg were 4.00% (38/949). The median concentration of HBeAg was 406.7 (S/CO), the median concentration of ALT was 32.0 U/L and the average copy number of HBV–DNA was 1.93×10^6 copies/ml in patients with positive HBsAg, HBeAg, and Anti-HBc, excluding the patients in hepatic center, outpatient department, and those who applied for health examination.

The median concentration of HBeAg (451.5) (S/CO) in patients with 250 IU/ml HBsAg and above was obviously higher than that (10.4) (S/CO) in patients with

less than 250 IU/ml HBsAg; the median copy number of HBV–DNA (2.46×10^6 copies/ml) in patients with 250 IU/ml HBsAg and above was higher than that (1.18×10^3 copies/ml) in patients with less than 250 IU/ml HBsAg ($P < 0.05$). (Table 4).

The concentration of HBeAg was positively correlated with the copy number of HBV–DNA in all patients with positive HBsAg, HBeAg, and Anti-HBc ($r = 0.731$, $P < 0.01$) and the concentration of HBeAg was negatively correlated with the concentration of ALT ($r = 0.14$, $P < 0.01$).

The further analysis found that the concentration of HBeAg was more positively correlated with the copy number of HBV–DNA in the patients with positive HBsAg, HBeAg, and Anti-HBc (HBsAg > 250 IU/ml) ($r = 0.713$, $P < 0.01$) and the concentration of HBeAg was also negative with the concentration of ALT ($r = 0.151$, $P < 0.01$). There was no correlation among the concentration of HBeAg and ALT and the copy number of HBV–DNA in the patients with positive HBsAg, HBeAg, and Anti-HBc (HBsAg < 250 IU/ml) ($P > 0.05$).

The patients with positive HBsAg, HBeAg, and Anti-HBc were found in various clinical divisions and five divisions with maximum distribution including department of gynaecology and obstetrics (13.57%), orthopedics (10.21%), otorhinolaryngology (8.14%), digestive internal medicine (5.32%), and neurosurgery (5.21%).

Detection of HBsAg in diluted samples

4277 samples were diluted for the detection of HBsAg and the HBsAg ranged 0~125,000 IU/ml, the

TABLE 4. Comparison of ALT and HBV–DNA Level in Patients with HBsAg(+) HBeAg(+) Anti-HBc(+)

	Total		HBsAg $\geq$ 250 IU/ml		HBsAg < 250 IU/ml		Z	P
	The median concentration	Min~Max	The median concentration	Min~Max	The median concentration	Min~Max		
HBeAg	406.7	1.1~1,944.6	451.5	1.2~1,944.6	10.4	1.1~1,261.3	–5.383	<0.01
ALT (U/l)	32.0	5~1,463	32.0	5~1,463	30.0	8~556	–0.333	0.739
HBV–DNA (copies/ml)	1.93×10^6	$<1.0 \times 10^3$ $\sim 5.43 \times 10^8$	2.46×10^6	$<1.0 \times 10^3$ $\sim 5.43 \times 10^8$	1.18×10^3	$<1.0 \times 10^3$ $\sim 2.0 \times 10^8$	–3.4533	<0.01

TABLE 5. Comparison of ALT and HBV-DNA Level of Patients with Different HBsAg Level

Concentration of HBsAg (IU/ml)	ALT (U/l)		HBV-DNA(copies/ml)	
	The median concentration	Min~Max	The average copy number	Min~Max
250~1,000	60	16~1,132	2.18×10^3	$<1.0 \times 10^3 \sim 5.42 \times 10^6$
1,001~5,000	54	17~1,840	5.10×10^3	$<1.0 \times 10^3 \sim 7.50 \times 10^7$
50,001~10,000	60	16~931	6.82×10^4	$<1.0 \times 10^3 \sim 3.03 \times 10^8$
10,001~50,000	101.5	18~1,950	1.74×10^6	$<1.0 \times 10^3 \sim 3.16 \times 10^8$
>50,000	112	19~910	1.03×10^7	$1.15 \times 10^3 \sim 1.68 \times 10^9$
Z	147.523587		1.06×10^3	
P	<0.01		<0.01	

median concentration was 3,545 IU/ml; the concentration of ALT was 8~2,125 U/L and the median concentration was 87.5 U/L; the copy number of HBV-DNA was $<1.0 \times 10^3 \sim 1.70 \times 10^9$ copies/ml and average copy number was 1.49×10^5 copies/ml. Rank sum correlation suggested that the concentration of HBsAg was positively correlated with that of ALT ($r = 0.250$, $P < 0.01$) and with copy number of HBV-DNA ($r = 0.450$, $P < 0.01$). The concentration of ALT and the copy number of HBV-DNA were listed in five groups in Table 5 according to the concentration of HBsAg. It demonstrated that the concentration of ALT and the copy number of HBV-DNA were related with HBsAg. The concentration of ALT and the copy number of HBV-DNA increased with HBsAg. Rank correlation analysis showed that ALT was positively correlated with HBsAg ($r = 0.132$, $P < 0.01$) and HBV-DNA was also positively correlated with HBsAg ($r = 0.407$, $P < 0.01$).

2,113 patients who received diluted HBsAg quantitation including those with positive HBsAg, Anti-HBe, and Anti-HBc (564) and those with positive HBsAg, HBeAg, and Anti-HBc (696). In patients with HBsAg, Anti-HBe and Anti-HBc positive, HBsAg was 0.05~125,000 IU/ml and the median concentration was 2,272.5 IU/ml. In patients with HBsAg, HBeAg and Anti-HBc positive, HBsAg was 2~125,000 IU/ml and the median concentration was 5,402.5 IU/ml.

Rank sum test showed that the concentration of HBsAg in patients with positive HBsAg, HBeAg, and Anti-HBc was higher than that in patients with positive HBsAg, Anti-HBe, and Anti-HBc with statistical significance ($Z = 10.815$, $P < 0.05$); the median concentration of ALT was 108.0 U/l, which was higher than that in patients with positive HBsAg, Anti-HBe, and Anti-HBc (63.5 U/L, $Z = -7.882$, $P < 0.05$); the average copy number of HBV-DNA was 7.22×10^3 copies/ml, which was obviously higher than that in patients with positive HBsAg, Anti-HBe, and Anti-HBc (1.36×10^4 copies/ml, $Z = -12.106$, $P < 0.05$).

Rank correlation demonstrated that the concentration of HBsAg was positively correlated with the copy number of HBV-DNA in patients with positive HBsAg, Anti-HBe, and Anti-HBc ($r = 0.247$, $P < 0.01$); the concentration of HBsAg was also positively correlated with the copy number of HBV-DNA in patients with positive HBsAg, HBeAg, and Anti-HBc ($r = 0.457$, $P < 0.01$).

Dynamic detection of HBsAg and HBeAg

932 patients who received dynamic detection for the concentration of HBsAg. Of them, HBsAg reduced to undetectable level in 76 patients and 143 patients whose HBeAg turned negative. The concentration of ALT and the copy number of HBV-DNA markedly decreased when HBeAg turned negative and the concentration of HBsAg reduced to undetectable level (Table 6).

Analysis of HBV Genotypes

HBV genotyping was carried out in 263 patients. 134 patients infected with genotype B; 87 patients infected with genotype C; 42 patients had mixed HBV genotype B/C infection. The level of HBsAg, ALT, and the copy number of HBV-DNA was different in the patients with different genotype (Table 7). The concentration of HBsAg and the copy number of HBV-DNA in genotype C were higher than that in genotype B and mixed genotype B/C; the concentration of ALT in genotype C and genotype B was higher than that in mixed genotype B/C.

DISCUSSION

There was a high prevalence of HBV in China, and Fujian was one of the hardest hit provinces in the south-east coastal regions (1). The prevalence trend of HBV has changed remarkably in the last several decades due to the widespread use of HBV vaccine and antiviral drugs (7). In addition to special large-scale investigation, regular screening and related analysis of HBV serum marker of

TABLE 6. Comparison of ALT and HBV-DNA Before and After the Concentration of HBeAg and HBsAg Changing

	HBeAg (before turning into negative)		HBeAg (after turning into negative)		Z	P
	The median concentration	Min~Max	The median concentration	Min~Max		
ALT (U/l)	58	3~1,242	33	2~931	-6.609	<0.01
HBV-DNA (copies/ml)	2.34×10^3	$<1.0 \times 10^3 \sim 5.43 \times 10^8$	$<1.0 \times 10^3$	$<1.0 \times 10^3 \sim 2.80 \times 10^7$	-9.242	<0.01
	HBsAg (before decreasing)		HBsAg (reduced to undetectable level)		Z	P
	The median concentration	Min~Max	The median concentration	Min~Max		
ALT (U/l)	94	40~1,242	71	18~474	-2.063	0.039
HBV-DNA (copies/ml)	3.814×10^3	$<1.0 \times 10^3 \sim 7.8 \times 10^8$	1.05×10^3	$<1.0 \times 10^3 \sim 2.72 \times 10^5$	-4.815	<0.01

numerous clinical patients can help us explore the current situation of HBV infection conveniently. The positive rate of HBsAg of Fujian hospitalized patients is 14.96% (excluding the patients at hepatic center, outpatient department, and medical examination center, for most of them were already known with positive HBsAg when applying for the test of five serum markers), which is obviously higher than that reported by Ye et al. in 2008 (8).

The relationship between various serum markers of HBV was compared on the basis of quantitative analysis, suggested that the concentration of ALT and the copy number of HBV-DNA in patients with 250 IU/ml HBsAg and above were higher than that in patients with less than 250 IU/ml HBsAg regardless of "positive HBsAg, HBeAg, and Anti-HBc" or "positive HBsAg, Anti-HBe, and Anti-HBc" status. This result combined with further data of ALT and HBV-DNA in patients with diluted suggested that the concentration of HBsAg was obviously correlated with ALT and HBV-DNA. Therefore, as the most important serum marker of HBV, accurate concentration of HBsAg combined with the result of ALT and HBV-DNA can better reflect hepatic injury and HBV status. Interestingly, the concentration of HBeAg is positively correlated with copy number of HBV-DNA in all patients with positive HBsAg, HBeAg, and Anti-HBc, but negatively correlated with the concentration of ALT. The results are consistent with that in patients with 250 IU/ml

HBsAg and above. No relationship was found between the concentration of HBeAg, ALT, and the copy number of HBV-DNA in the patients with positive HBsAg, HBeAg, and Anti-HBc positive with less than 250 IU/ml HBsAg, which is worth further exploration whether it results from the sample sizes or other reasons.

The relationship between HBV serum marker and genotype was also analyzed suggesting that the concentration of HBsAg was higher in genotype C than that in genotype B and mixed genotype B/C and the concentration of ALT in genotype C and genotype B was higher than that in mixed genotype B/C. Moreover, the copy number of HBV-DNA in genotype C was higher than that in genotype B and mixed genotype B/C, which was consistent with the former reports (9, 10). HBV genotype is related to the outcome of hepatitis. The concentration of HBV is obviously higher in genotype C (which causes more severe hepatic injury) than in another two genotypes. So it is significant for monitoring the concentration of HBsAg regularly in order to estimate treatment and prognostics definitely.

The detection of serum marker in HBV patients of Fujian province and the relationships among ALT, the copy number of HBV-DNA and genotype generally reflect the relationships among HBV serum marker, hepatic injury, replication of virus and genotype, which is valuable in understanding the clinical characteristics of HBV carriers

TABLE 7. Comparison of HBsAg, ALT, and HBV-DNA Level of Patients with Different Genotypes

	Genotype B		Genotype C		Genotype B/C	
	The median concentration	Min~Max	The median concentration	Min~Max	The median concentration	Min~Max
HBsAg (IU/ml)	6,742.5	6.35~>125,000	8,123.4	113.79~>125,000	7,632.1	2.15~>125,000
ALT (U/l)	220.4	9~1,897	178.26	9~1,733	112.86	11~714
HBV-DNA (copies/ml)	1.62×10^7	$1.02 \times 10^3 \sim 3.11 \times 10^8$	3.05×10^7	$1.05 \times 10^3 \sim 4.09 \times 10^8$	1.79×10^5	$1.02 \times 10^3 \sim 6.18 \times 10^6$

and patients in China and provide the data for outcome and estimation of curative efficacy in HBV infected patients.

CONFLICT OF INTEREST

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

REFERENCES

1. Chen YS, Wang XX, Shang PH, Liang XF. The study of tendency of hepatitis B virus surface antigen in Chinese population. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* 2007;1(1):36–39.
2. Wang JX, Xiao F, Wang YL, Wang SH, Wang LP. Analysis of the serum markers in children hepatitis B virus infection. *Zhonghua Wei Sheng Wu He Mian Yi Xue Za Zhi* 2009;29:281–282.
3. Wang Z, Huang Y, Wen S, Zhou B, Hou J. Hepatitis B virus genotypes and subgenotypes in China. *Hepatol Res* 2007;37(S1):S36–S41.
4. Akuta N, Kumada H. Influence of hepatitis B virus genotype on the response to antiviral therapies. *J Antimicrob Chemother* 2005;55(2):139–142.
5. Chen CH, Lee CM, Lu SN, et al. Clinical significance of hepatitis B virus (HBV) genotypes and precore and core promoter mutations affecting HBV e antigen expression in Taiwan. *J Clin Microbiol* 2005;43(12):6000–6006.
6. Shang HY, Cheng ZJ, Ou QS. Establishment of melt curve analysis for genotyping of hepatitis B virus. *Lin Chuang Jian Yan Za Zhi* 2011;29(3):225–227.
7. Chen XJ, Pan YX, Cheng YZ. Detection and distribution characteristics of serological markers of hepatitis B in neonates, infants and preschool children in Zhejiang province. *Chin J Lab Med* 2011;34(3):200–205.
8. Ye B, Yang DG, Zheng SF. Retrospective analysis of hepatitis B virus serological markers in 70,582 inpatients. *Lin Chuang Jian Yan Za Zhi* 2010;33(10):918–923.
9. You J, Zhuang L, Chen HY, et al. Advances in the researches on hepatitis B virus genotyping and its clinical significance. *Shi Jie Hua Ren Xiao Hua Za Zhi* 2007;15(9):921–928.
10. Su MH, Jiang JN. Hepatitis B virus genotypes and its mutations and curative effect of anti-virus therapy. *Guo Ji Liu Xing Bing Xue Chuan Ran Bing Xue Za Zhi* 2007; 34(3):212–216.