

SHORT REPORT

A new trend of genotype distribution of hepatitis B virus infection in southeast China (Fujian), 2006–2013

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*Received 6 May 2014; Final revision 1 November 2014; Accepted 7 January 2015;
first published online 4 February 2015*

SUMMARY

HBV genotypes have specific geographical distributions and can serve as epidemiological markers. Accumulated data have shown that the major HBV genotypes in China are B and C. Here, the HBV genotypes were examined from 6817 blood samples, which were collected from patients with chronic HBV infection in Fujian Province during 2006–2013; genotype B was identified in 3384 patients (49·6%), while genotype C was identified in 3430 patients (50·3%). The percentage of patients infected with genotype C gradually increased with age from 39·5% (patients aged <20 years) to 63·9% (patients aged >50 years), reaching a peak of 67·3% in the 45–50 years age group. These results clearly demonstrate that the genotype distribution of HBV in Fujian Province has significantly changed in recent years with almost equal numbers of genotype B and genotype C infections existing in the entire patient population, while higher incidence of genotype C infection exists in older patients, but genotype B is no longer dominant in the Fujian area as previously reported.

Key words: Genotype distribution, HBV, new trend.

Hepatitis B virus (HBV) infection is a serious global public health problem because of its high morbidity and mortality worldwide [1]. Despite the significant progress made in understanding its pathogenesis and in antiviral therapies, HBV infection is still responsible for about 1 million deaths globally due to liver failure and cirrhosis each year. Additionally, HBV infection is also the main cause of hepatocellular carcinoma (HCC, >75%) worldwide [2], which is the second leading cancer cause of death in China [3]. Several viral factors, including HBV genotype, HBV

viral DNA load and viral mutations, have been closely associated with the disease's progression, severity and prognosis [4]. Of these, HBV genotype is closely associated with therapy response and drug resistance [5]. Therefore, identification of the HBV genotype could provide extremely valuable information about the dynamics of HBV transmission in the population [6], and help clinicians optimize therapy protocols based on the specific genotype. According to the heterogeneity of HBV genome sequences, there are at least eight genotypes that have been identified (designated A–H), based upon an inter-group divergence of $\geq 8\%$ in the entire genomic sequences [4].

Accumulated data show that the prevalence of HBV genotypes varies geographically, and the

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prevalent HBV genotypes reported in the Asia-Pacific region, especially in China, are B and C. Although the HBV prevalence rate is slightly lower than in the past in Fujian Province (located in the southeast of China), it is still the region with the highest HBV infection prevalence in China. Hence, an epidemiological survey of HBV genotype distribution is very important for prevention and treatment of the disease. It has been reported that the majority of patients (63.8%) were infected by HBV genotype B, while 23.2% of patients were infected by HBV genotype C in Fujian Province, which has one of the highest incidences of HBV infection in southeast China [7, 8]. However, these reports suffered from limited population sizes, which might not be sufficient to estimate the real genotype distribution of HBV in large populations. Here, we conducted an epidemiological survey in HBV-infected patients in a large population size over the past 8 years to determine the recent trend of genotype distribution of HBV in Fujian Province, China. A total of 6817 patients with chronic HBV infection who were admitted to Mengchao Hepatobiliary Hospital of Fujian Medical University during January 2006 to December 2013 were retrospectively studied. The patients considered in this study had a wide age distribution from 1 to 88 years. HBV genotyping of all samples was performed by real-time fluorescence polymerase chain reaction (PCR) using a commercial HBV genotyping kit (Shanghai Fosun Pharmaceutical Co. Ltd, China), which maximally reduces the chances of HBV genotype misclassification. Real-time PCR mixtures consisted of 26 μ l master reaction mixture and 4 μ l DNA template in a total volume of 30 μ l. After 2 min incubation at 50 °C and 5 min incubation at 94 °C, 40 amplification cycles were performed (93 °C for 15 s, and 60 °C for 45 s) on an ABI 7500 Real-Time PCR System (Applied Biosystems, USA). C_t values obtained from the real-time PCR were adopted to identify HBV genotypes, and the identification criteria are given in Supplementary Table S1. Those patient samples, from which the identification of genotype was uncertain, were subsequently further characterized by the Sanger sequencing method. In this case, the HBV-DNA sequences were aligned with the standard sequences of genotypes B and C obtained from an international DNA database using DNAssist software v. 2.2 (dnassist.soft32.com). SPSS v. 12.0 (SPSS Inc., USA) was used for statistical analysis, and differences between groups were considered statistically significant at $P < 0.05$. The

relationships between HBV genotypes and age were examined using Pearson's correlation coefficient. The study was approved by the Ethics Committee of the Mengchao Hepatobiliary Hospital of Fujian Medical University. To estimate current distribution of HBV genotypes in Fujian Province, we collected the results obtained from HBV genotype detection, as described above, over the last 8 years. From 2006 to 2013, a total of 6869 HBV-positive patients were identified. Information regarding age or sex was missing for 52 participants (0.76%). Thus, a total of 6817 patients were analysed. From our analysis, the annual number of patients detected with HBV genotypes B or C from 2006 to 2013 was 338, 357, 882, 1165, 1154, 1048, 859, and 1014, respectively; the mean age of infected patients was 34.9 ± 12.2 years; the genotype prevalence was gender-related, and the infection rates of males were higher than those of females. The main characteristics are given in Table 1. As shown in Table 1, HBV genotype B was detected in 3384 people (49.6%) and genotype C in 3430 people (50.3%), while three people had a mixed infection with both B and C genotypes. No other genotypes (A, D, E, F, G, H) were detected in this study. There was almost an equal percentage of patients infected with HBV genotypes B (49.6%) and C (50.3%); meanwhile, HBV genotype distribution has been significantly associated with the patient's age. Interestingly, Hu *et al.* reported that the HBV genotype B-infected population was 63.8%, while genotype C was 23.2% in a cohort study of 431 patients in the same region (Fujian Province) in 2004 [7]. In comparison with that study, we could conclude that HBV genotype C infection was significantly increased (from 23.2% to 50.3%), while genotype B infection was significantly decreased (from 63.8% to 49.6%) over the past 10 years in Fujian Province. This change might be due to the increasing migration rate of people from the north, even overseas, the route of HBV transmission, the rate of HBV vaccination in different regions, as well as the different response of the HBV genotype to antiviral therapies. By carefully analysing the distribution rate of HBV genotypes B or C per year, we observed that HBV genotype B had a significantly higher prevalence (54.1%) than genotype C (45.9%) in 2006, but not in any other years (from 2007 to 2013) (Fig. 1a). Thus, the data are consistent with previous studies that reported a higher HBV genotype B infection in 2004 in Fujian Province [7], and the present study reveals that in the subsequent years the genotype B infection rate has declined.

Table 1. General features and genotype distributions of 6817 HBV-infected patients in Fujian, 2006–2013

Year	2006	2007	2008	2009	2010	2011	2012	2013	Total
Case numbers	338	357	882	1165	1154	1048	859	1014	6817
Male (%)	280 (82.8)	284 (79.6)	708 (80.3)	915 (78.5)	911 (78.9)	825 (78.7)	638 (74.3)	761 (75.0)	5322 (78.1)
Female (%)	58 (17.2)	73 (20.4)	174 (19.7)	250 (21.5)	243 (21.1)	223 (21.3)	221 (25.7)	253 (25.0)	1495 (21.9)
Outpatient (%)	73 (21.6)	179 (50.1)	584 (66.2)	499 (42.8)	518 (44.9)	479 (45.7)	349 (40.6)	345 (34.0)	3026 (44.4)
Inpatient (%)	265 (78.4)	178 (49.9)	298 (33.8)	666 (57.2)	636 (55.1)	569 (54.3)	510 (59.4)	669 (66.0)	3791 (55.6)
Genotype B (%)	183 (54.1)	174 (48.7)	424 (48.1)	595 (51.1)	574 (49.7)	503 (48.0)	416 (48.4)	515 (50.8)	3384 (49.6)
Genotype C (%)	155 (45.9)	182 (51.0)	458 (51.9)	568 (48.7)	580 (50.3)	545 (52.0)	443 (51.6)	499 (49.2)	3430 (50.3)
Genotypes BC (%)	0 (0.0)	1 (0.0)	0 (0.0)	2 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.0)
Average age (yr)	30.9 ± 11.7	32.4 ± 11.5	33.9 ± 12.1	35.0 ± 12.6	35.1 ± 12.2	35.6 ± 11.9	36.0 ± 12.6	36.2 ± 11.9	34.9 ± 12.2
Average B age (yr)	29.3 ± 11.5	29.8 ± 10.1	31.1 ± 11.1	32.5 ± 11.6	32.9 ± 12.0	33.3 ± 11.3	34.0 ± 12.3	34.7 ± 11.5	32.7 ± 11.6
Average C age (yr)	32.8 ± 11.6	34.82 ± 12.3	36.6 ± 12.3	37.8 ± 13.1	37.3 ± 12.1	37.7 ± 12.1	37.9 ± 12.6	37.7 ± 12.1	37.1 ± 12.4

To further study the influence of age on the HBV genotype distribution, we divided the patients into groups according to age in 5-year intervals (<20, 20–25, 25–30, 30–35, 35–40, 40–45, 45–50, >50 years). The percentage of patients infected by HBV genotypes B or C in different age groups are plotted in Figure 1*b*. As shown in Figure 1*b*, a significant difference in HBV genotype distribution in different age groups was observed. Genotype C was more common in older patients (>35 years, 60.4%), than in younger patients (<35 years, 42.3%); whereas, genotype B was more common in younger patients (57.7%) than in older patients (39.6%) (Supplementary Table S2). This phenomenon could be further confirmed by the mean age of HBV genotype B-infected patients (32.7 ± 11.6 years) which is significantly lower than that of genotype C-infected patients (37.1 ± 12.4 years). However, there was no significant difference in the age distribution of HBV genotypes in each year. Furthermore, the percentage of HBV genotype B infections gradually decreased with increasing age (from 60.5% in the <20 years age group to 36.1% in the >50 years age group), while the percentage of genotype C infections increased with increasing age (from 39.5% in the <20 years age group to 63.9% in the > 50 years age group). The correlation coefficient of age against the percentage of genotypes B or C was -0.935 and 0.935, respectively. Statistical analysis indicated that the correlations of age with genotypes B and C were significant ($n = 46, P < 0.01$) (Supplementary Fig. S1). As a consequence, these findings show that in the very near future, we will face a considerable number of old patients with HBV genotype C. To the best of our knowledge, this epidemiological shift observed in HBV infection has never been reported previously in a Chinese population.

The HBV genotype distributions varied considerably in different regions of China; for example, HBV genotype C rate is 98.8% in Harbin City (north-east of China), while it is only 11.5% in Zengcheng City (south of China) [7]. However, the strictly restricted geographical patterns of HBV genotype distribution might be shifted because of the extensive migrations of populations in different regions, even different countries. Fujian Province has a more developed economy and higher floating population rates, due to its port economy; the high floating populations in this region would significantly affect the prevalence rate of HBV genotypes [8], and might be responsible for the increase in HBV genotype C infections.

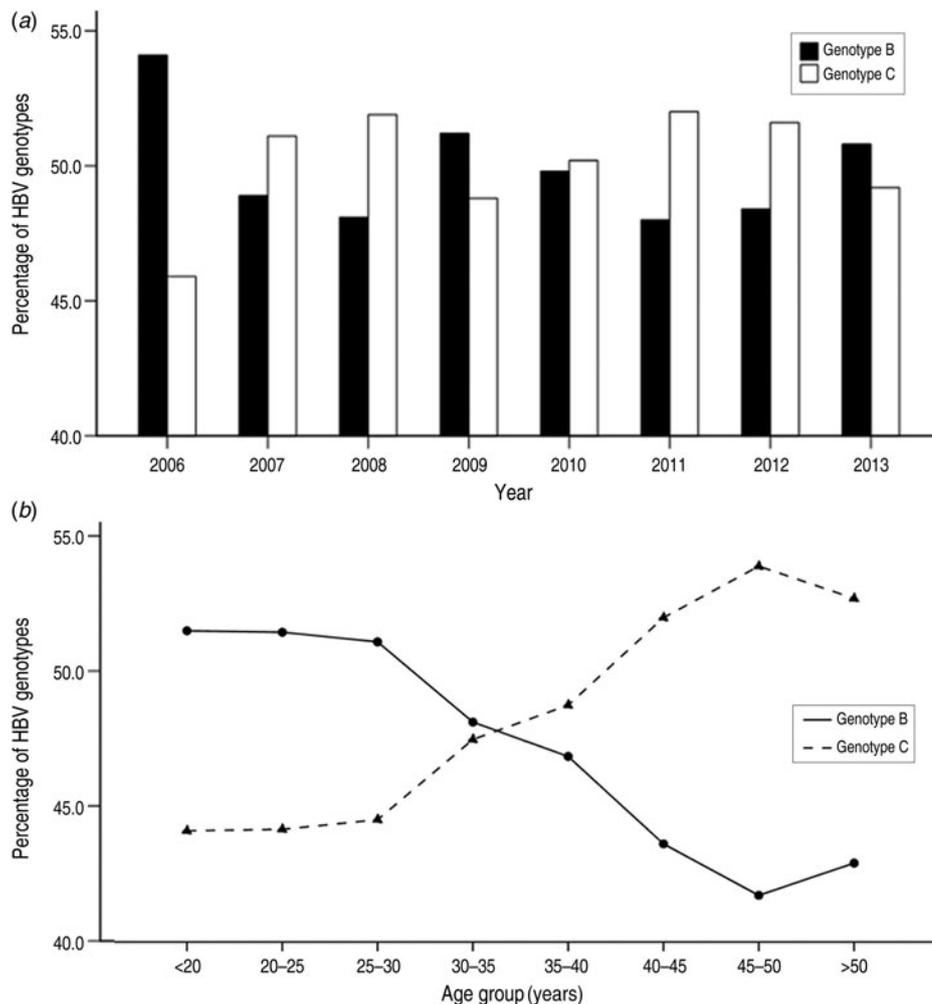


Fig. 1. Distribution of HBV genotypes B and C. (a) The distribution of HBV genotypes B and C in different years from 2006 to 2013. (b) The percentage of HBV genotypes B and C in different age groups.

Interferon alpha (IFN- α) is a widely clinically used antiviral drug for suppressing the viral replication to halt the disease progression caused by chronic HBV infection [9]. It has been reported that patients infected by HBV genotype C had a poor response to IFN- α treatment compared to genotype B [10]. Therefore, patients infected with HBV genotype C might remain infected, while patients infected with genotype B might be rescued from the IFN- α -based long-term antiviral treatment. This would cause the observed epidemiological changes in this study (such as higher HBV genotype C infection rate in older populations), especially in those patients who underwent long-term IFN- α -based antiviral therapy. This finding is in agreement with our results that HBV genotype B is still prevalent in adolescents and young patients, while genotype C is rapidly increasing in older patients.

In summary, we provide a new prevalence trend of HBV genotypes B and C in infected patients in Fujian Province (southeast China). Further studies with larger population sizes are required to evaluate the recent epidemic trend of HBV, including the spreading rate of genotypes and the epidemiological characteristics of its subtypes.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit <http://dx.doi.org/10.1017/S0950268815000059>.

ACKNOWLEDGEMENTS

This work is supported by the key clinical speciality discipline construction programme of Fujian, P.R.C.; the key project of National Science and Technology of

China (grant no. 2012ZX10002010-001-006, and grant no. 2012ZX10002016-013), the Scientific Foundation of Fuzhou Health Department (grant no. 2013-S-wp1, grant no. 2013-S-w12, and grant no. 2014-S-w19).

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