



Prevalence of Inter-Genotype Recombinant Hepatitis B Viruses in a Highly Epidemic Region of Northwest China

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Abstract

Hepatitis B is among the most important infectious diseases in China. The prevalence of hepatitis B virus (HBV) infection is high in Northwest China, particularly in Wuwei City, Gansu Province. To investigate genetic diversity and genotype distribution in this region, HBV genomes were completely sequenced from 78 HBV-infected individuals from the Physical Examination Center of Wuwei City and analyzed with MEGA (version 5.05) and SimPlot program (version 3.5.1). The results indicated that genotype C (47.44%, 37/78) was the most frequently observed. The nearly half of the individuals were infected by five phylogenetically distinct B/C or C/D inter-genotype recombinant variations of HBV. Our studies indicate that highly diversified HBV viruses are epidemic in this local population. In my study may provide an important reference for further HBV clinical studies, which presents the HBV genomic background will contribute to the establishment of a reliable virus evolution history and provide vital genomic baseline references.

Keywords: Epidemiology; Hepatitis B virus; Recombinant

Introduction

Hepatitis B Virus (HBV) infection is prevalent in China. A nationwide serological survey in 2006 showed that the morbidity of HBsAg was 7.2% overall and varied among different regions in the population of those aged 1 to 59 years old. In Northwest China, the prevalence of HBV infection is much higher than in other regions [1]. The carriage rates of HBsAg ranged from 4.6% to 13.4% in Wuwei City, Gansu Province, according to a recent population-based cross-sectional study [2,3]. However, genotype distribution and genetic diversity of HBV have not been comprehensively investigated in this region. We set out to sequence and analyze HBV genomes from 78 HBV carriers in Wuwei City. To our surprise, three genotypes of HBV are epidemic in the local population, and 42% of the surveyed subjects were infected by five phylogenetically distinct B/C and C/D inter-genotype recombinant HBV variations. The reason for the high genetic diversity of HBV may be the ethnic diversity, including the Han nationality and 12 other minorities, which favored the transmission of genetically

distinct HBV strains during different historical periods and population migrations.

Materials and Methods

Study Subjects

From September 2012 to September 2013, 78 serum samples were collected from asymptomatic HBV carriers with viral loads higher than 10^5 copies/mL from the Physical Examination Center of Wuwei City Gansu Province, China.

The serum samples were stored at -80°C immediately after collection. HBV DNA was extracted from 200 μL of serum sample with a viral DNA extraction kit by following the manufacturer's directions (Tiangen, Beijing, China). DNA pellets were dissolved in 20 μL of distilled water, and the complete genome of HBV from each of the samples was amplified and sequenced with experimental procedures described in our previous report [4]. The acquired HBV complete genome sequences were deposited to Gen Bank (Gen Bank Accession numbers are KC774243-KC774279, KC774378-KC774392, KC774435-KC774460). Approval was

obtained from the Fourth Military Medical University institutional ethics committee before the study, and written informed consent for participation in this study was obtained from each individual.

Phylogenetic Analysis

The HBV complete genome sequences were aligned using MEGA (version 5.05) with gap open and extension penalties of 10. The phylogenetic tree was constructed by the Maximum Likelihood (ML) method within MEGA (version 5.05). The reliability of the pairwise comparison and phylogenetic tree analysis was assessed by boot-strap re-sampling with 1000 replicates.

Recombination Investigation

Recombination was searched with the SimPlot and BootScan methods in SimPlot program (version 3.5.1). The length of the window was set to 300 variable sites, and the step size was set to 10 nucleotides. A Kimura (2-parameter) distance model was utilized to estimate the distances between HBV complete genome sequences. In the BootScan method, bootstrap replicates were set as 100, and the recombinants were supported by the 70% bootstrap values.

Results

Demographic Information

Seventy-eight asymptomatic carriers ranged in age from 6 to 51 years old (mean =28.47±12.86, M/F ratio=40/38). Among the 78 subjects, 31 subjects (39.7%) were HBeAg-positive. Twenty-eight (35.9%) had elevated serum Alanine Transaminase (ALT) levels (mean=43.81±41.12). Twenty-four (30.8%) had elevated serum glutamic-oxaloacetic transaminase (mean=39.33±40.91). All were detected with viral load $\geq 10^5$ copies/mL, which were subjected to complete HBV genome amplification. The complete HBV genomes were successfully amplified and sequenced from each case.

HBV Genotypes and Serotypes

Phylogenetic analysis of all complete genomes with GenBank reference sequences identified three distinct clusters corresponding to HBV genotypes B, C and D. Among the 78 sequences generated in this study, genotype C (47.44%, 37/78) was the most frequently observed, followed by genotype D (33.33%, 26/78) and genotype B (19.23%, 15/78). In addition, all the sequences clustered into genotype B were within the Ba sub-genotype, and all the sequences clustered into genotype C were within Ce sub-genotype. In serotype analysis, subtypes adr_q⁻, adr_q⁺, adw₂, ayr and ayw₂ were observed in these sequences. Subtype adr_q⁺ (122K+160R+177V) (42.31%, 33/78) was the most frequently observed, followed by subtype ayw₂ (122R+160K+127P+159G) (33.33%, 26/78) and adw₂ (122K+160K+127P) (21.79%, 17/78).

Genetic Recombination Within HBV Complete Genome

A total of 33 recombinants were detected with Sim Plot and Boot scan methods in Sim Plot Software among the 78 HBV complete genomes. All fifteen genomes clustered into genotype B in the phylogenetic analysis were identified as B/C inter-genotype recombinants, while eighteen out of the twenty-six genomes clustered into genotype D were identified as C/D inter-genotype recombinants. Moreover, the fifteen B/C recombinants can be further classified into three groups based on the criteria set by Yang and colleagues [5]. Five B/C-II recombinants were found with fragments of nt1219-nt1645, which originated from genotype Band Genotype C, respectively. Three B/C-III recombinant strains were identified with nt1244-nt1799, which originated from genotype Band genotype C, respectively. Seven B/C-IV recombinant strains were identified with nt1740-nt2443, which originated from genotype C and genotype B, respectively. Similarly, seventeen out of eighteen C/D recombinants were identified as C/D-I recombination with nt10-nt799, which originated from genotype D and genotype C, respectively. The remaining recombinant was a C/D-II recombinant with nt10-nt1484, which originated from genotype D (Figure 1).

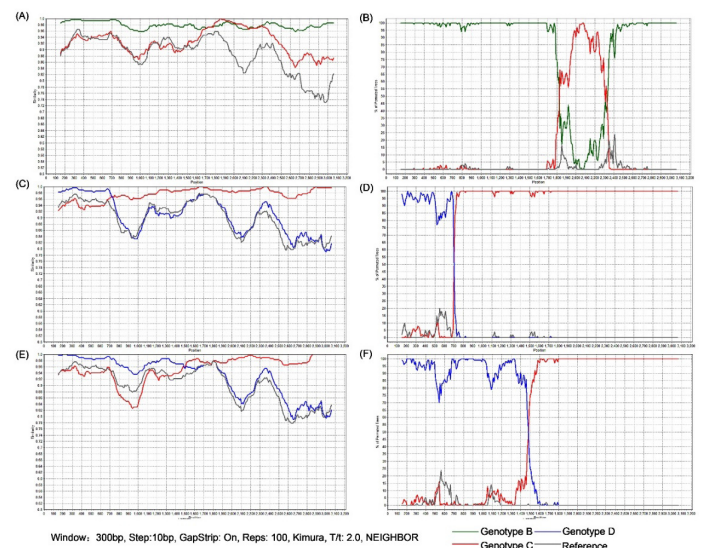


Figure 1: (A)(B) SimPlot and BootScan result of the B/C (breakpoint at nt1740/nt2440) inter-genotype recombinant. (C)(D) SimPlot and BootScan result of the C/D (breakpoint at nt10/nt800) inter-genotype recombinant. (E)(F) SimPlot and BootScan result of the C/D (breakpoint at nt10/nt1480) inter-genotype recombinant.

Discussion

The HBV that infects humans shows genetic and antigenic heterogeneity and is currently classified into nine genotypes

that differ from each other in nucleotide sequences by $\geq 8\%$ in complete genomes or $\geq 4\%$ in surface (S) antigen-coding regions [6]. In addition, recombination between genotypes also generates novel variants that contribute to genetic diversity [7]. The genesis of inter-genotypic recombinants not only impacts HBV evolution but may also impose tremendous challenges on vaccination and antiviral therapies against HBV infection.

Wuwei City is on the Silk Road to west Asia in ancient China, and current residents are of the Han nationality as well as 12 different minorities. Except for its high morbidity of chronic HBV infection (nearly 800 cases per 100,000 people), our studies indicate that highly diversified HBV viruses are epidemic in this local population. In particular, five phylogenetically distinct B/C and C/D inter-genotype recombinant HBV infected 42% of surveyed subjects. In addition, distinct from that observed in the east regions of China where genotypes B and C are the predominant epidemic HBV, genotype D is one of the predominant genotypes in Wuwei City. It was reported previously that genotype D was mainly distributed in Qinghai-Tibet Plateau in China [8]. Interestingly, twenty-one out of twenty-six subjects infected by genotype D HBV in the study live in the plateau area of Wuwei City, which is on the edge of the Qinghai-Tibet Plateau. The high genetic diversity and prevalence of inter-genotype recombinant variants in Wuwei city could be due to the fact that the city is located on a historical trade road that favors the transmission of diversified HBV variants. As well, it could be that the current residents (ethnic groups) might have migrated from many different places over different historical periods.

Geographical distribution of HBV genotypes was investigated by Zhou and colleagues in a multi-center clinical study in the other regions of Northwest China, including Gansu Province [8]. The genotype distribution pattern revealed that our current study in Wuwei City is generally consistent with that by Zhou and colleagues in a non-identified region of Gansu Province. However, 15 of 19 genotype D strains were found to be inter-genotypic recombinants with genotype C in Zhou's report and none of other inter-genotypic recombinant variations was identified. Genotype B and 18 of 26 genotype D were found to be recombinants with genotype C. The proportion of recombinants and its recombination breakpoints in the present study for C/D recombinants was similar to that reported by Zhou and colleagues [8]. Interestingly, none of

the recombinant variants were detected in the Genotype C group, suggesting that this genotype might be more conservative and acted only as a donor of genetic fragments for recombination with other genotypes [5,8]. Direct repeat 1 (DR1) sequence (1830 nt) in the X gene was considered to be a hot spot for inter-genotypic recombination among different strains [9]. However, most of the breakpoints were located outside of this region, especially for C/D recombinants, indicating that the inter-genotype recombination of HBV genomes may occur via multiple distinct mechanisms [10].

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