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Comparative analysis of HBV basic core promoter/pre-core gene mutations and viral quasispecies diversity in HIV/HBV co-infected and HBV mono-infected patients

Haohui Deng[1,2], Hongbo Gao[2], Yu Liu[1], Ying Xu[1], Juncheng Yang[1], Miaoxian Zhao[1], Huiyuan Liu[2*], Zhanhui Wang[1*]

[1]State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of Viral Hepatitis Research, Department of Infectious Diseases and Hepatology Unit, Nanfang Hospital, Southern Medical University, Guangzhou, P. R. China; [2]Infectious Disease Center, Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou, P. R. China

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Summary. – Human immunodeficiency virus (HIV)/hepatitis B virus (HBV) co-infection accelerates the progression of HBV-related liver diseases. HBV basic core promoter (BCP)/pre-core (preC) gene mutations may be one of the most important risk factors. In this study, a total of 230 patients were recruited, and 199 patients whose HBV BCP/preC gene were successfully amplified and sequenced, including 99 HIV/HBV co-infected and 100 HBV mono-infected patients. Next-generation sequencing was used for detection of BCP/preC mutations which were then compared in patients with different HBV genotypes and different HBeAg statuses, and 1% and 20% cutoff values were defined to evaluate the mutations. HBV quasispecies diversity was also compared in HIV/HBV co-infected and HBV mono-infected patients. Among the patients infected with HBV genotype C and HBeAg-negative status, the frequency of A1762T/G1764A double mutations was significantly lower in HIV/HBV co-infected patients than in HBV mono-infected patients (53.3% vs. 100.0%, $P = 0.008$) regardless of the 1% or 20% cutoff value level. However, A1762T/G1764A double mutations did not differ in the other groups ($P > 0.05$). Viral quasispecies diversity was lower in HIV/HBV co-infected patients than in HBV mono-infected patients ($P < 0.05$). This study revealed the characteristic of HBV BCP/preC gene mutations and viral quasispecies diversity in HIV/HBV co-infected patients.

Keywords: human immunodeficiency virus; hepatitis B virus; mutations; viral quasispecies; next-generation sequencing

Introduction

According to the literature, an estimated 400 million people are infected with hepatitis B virus (HBV), and 33 million are infected with human immunodeficiency virus (HIV) worldwide (Hoffmann and Thio, 2007; Kourtis *et al*., 2012). Since HBV and HIV share similar transmission routes, 5–20% of HIV-positive patients are also infected with HBV (Easterbrook *et al*., 2012; Kim *et al*., 2008; Thio, 2009). Several studies have revealed that HIV patients co-infected with HBV increase the risks of liver cirrhosis and hepatocellular carcinoma (Hoffmann and Thio, 2007; Kim, 2020). However, the reasons why HIV/HBV co-infection accelerates the progression of HBV-related end-stage liver disease are still unknown.

The HBV genome contains approximately 3200 nucleotides with four open reading frames (ORFs), namely, pre-core (preC)/core, polymerase, surface and x ORFs (Tiollais *et al*., 1985). Since HBV lacks proofreading capacity during replication, its rate of nucleotide mutations is much higher than that of other DNA viruses (Rajput, 2020). Therefore, large amounts of mutations are produced naturally or occur in a given replicative environment. HBV basic core promoter (BCP) A1762T/G1764A double and pre-core (preC) G1896A mutations are the

*Corresponding authors. E-mail: huiyuanliu@163.com, phone: 86-20-83710851 (Huiyuan Liu). E-mail: wangzhh@smu.edu.cn, phone: 86-20-62787314 (Zhanhui Wang).

Abbreviations: HIV = human immunodeficiency virus; HBV = hepatitis B virus; BCP = HBV basic core promoter; preC = pre-core; ORFs = open reading frames

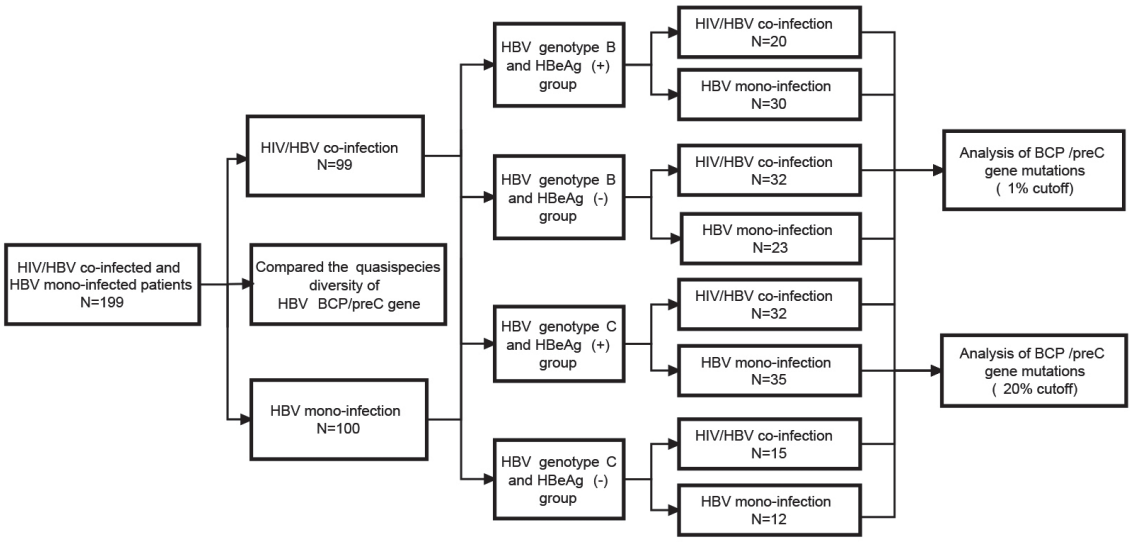

Fig. 1

Study design flow chart

Among 230 patients recruited in this study, 199 patients whose samples were successfully amplified and sequenced were enrolled in 4 groups. Patients previously exposed to antiviral drugs were strictly excluded from this study.

most common mutations in the BCP/preC gene regions. Previous studies have shown that A1762T/G1764A double mutations are independent risk factors for HBV-related end-stage liver disease, such as hepatocellular carcinoma (Mak and Kramvis, 2020; Wang *et al*., 2019). Other BCP/preC gene mutations, such as C1653T in enhancer II and T1753C in BCP, are also associated with the progression of HBV-related end-stage liver diseases (Liu *et al*., 2009). HBV BCP/preC gene mutations in HIV/HBV co-infected patients have been reported in some areas, but the results vary greatly in different studies, especially regarding BCP A1762T/G1764A double mutations (Audsley *et al*., 2010; Cassino *et al*., 2009; Li *et al*., 2017; Tangkijvanich *et al*., 2013). Thus, the difference in BCP A1762T/G1764A double mutation levels between HIV/HBV co-infected and HBV mono-infected patients has yet to be fully defined.

In China, the prevalence of HBV infection is high; accordingly, HIV/HBV co-infection is common (Zhang *et al*., 2014). However, the difference in the prevalence of HBV BCP/preC mutations between HIV/HBV co-infected patients and HBV mono-infected patients is still unknown. HBV BCP/preC mutations may be one of the most important risk factors associated with HBV-related end-stage liver diseases. It is important to investigate the difference in HBV BCP/preC mutations between HIV/HBV co-infected and HBV mono-infected patients. In addition, studies focused on HBV quasispecies diversity in HIV/HBV co-infected patients in China are currently limited. In this study, we detected the A1762T/G1764A double mutations, G1896A mutation and other BCP/preC gene mutations, additionally, compared HBV quasispecies diversity in HIV/HBV co-infected and HBV mono-infected patients in China, to better understand BCP/preC mutations and the characteristics of HBV quasispecies diversity in HIV/HBV co-infected patients.

Materials and Methods

Study subjects. A total of 230 patients were recruited, and 199 patients whose HBV S and BCP/preC genes were successfully amplified and sequenced were enrolled in this study, including 99 HIV/HBV co-infected and 100 HBV mono-infected patients. Samples were collected from 2012-2016. To compare the frequency of BCP/preC mutations in patients with different HBV genotypes and hepatitis B e antigen (HBeAg) statuses, the 199 patients were classified into 4 groups: (i) the HBV genotype B and HBeAg-positive group, including 20 HIV/HBV co-infected and 30 HBV mono-infected patients; (ii) the HBV genotype B and HBeAg-negative group, including 32 HIV/HBV co-infected and 23 HBV mono-infected patients; (iii) the HBV genotype C and HBeAg-positive group, including 32 HIV/HBV co-infected and 35 HBV mono-infected patients; and (iv) the HBV genotype C and HBeAg-negative group, including 15 HIV/HBV co-infected and 12 HBV mono-infected patients. The study design flow chart is shown in Fig. 1. All subjects were recruited from Guangzhou Eighth People's Hospital and Nanfang Hospital. Patients previously exposed to antiviral drugs were strictly excluded from this study. All the HIV genotype of HIV positive patients enrolled in this study was HIV-1. Additionally, serum samples

Table 1. Demographic, virological and clinical characteristics of HIV/HBV co-infected and HBV mono-infected patients

Characteristics	HIV/HBV co-infection (N = 99)	HBV mono-infection (N = 100)	X^2/t/Z value	P
Gender (male/female)	80/19	74/26	1.318	0.251
Median age, year (IQR)	38.0 (30.0–46.0)	35.0 (32.0–41.0)	-1.419	0.156
$CD4^+$ T cell (cell/mm^3, IQR)	63.0 (18.0–260.0)	N.A		–
TBIL level (μmol/L, IQR)	15.2 (11.1–20.1)	15.1 (11.1–18.2)	-0.923	0.356
ALT level (IU/L, IQR)	48.0 (33.0–79.0)	58.5 (32.0–97.5)	-0.742	0.458
HBV DNA (log_{10} copies/ml)	6.9 ±1.3	6.6 ±1.5	1.400	0.163
HBV e antigen (positive/negative)	52/47	65/35	3.196	0.074
Risk behaviors (%)				
IDU	3(3.0)	N.A	–	–
Heterosexuality	71(71.7)	N.A	–	–
Male homosexuality	23 (23.2)	N.A	–	–
Unknown	2 (2.0)	N.A	–	–

IDU, injection drug users; ALT, alanine aminotransferase; TBIL, total bilirubin; N.A. not available.

were included only when their hepatitis B surface antigen (HBsAg) was positive and HBV viral load was above the detection limit (HBV DNA>100 IU/ml) for successful amplification of the target in HBV genome. Informed consent was obtained from each subject at recruitment. The study protocol was approved by the Ethics Committees of Guangzhou Eighth People's Hospital (202042175).

Laboratory screening. Routine clinical examinations were performed in all study subjects. Anti-HIV-1 antibody titers were determined by an ELISA (Wantai Biological, China) and confirmed by a Western blot assay (MP Biomedicals, Singapore). HBV serological markers (HBsAg and HBeAg) were analyzed by chemiluminescence immunoassays (Abbott Laboratories, USA). Serum HBV DNA levels were measured by a TaqMan PCR assay (DaAn Gene, China). Serum alanine aminotransferase (ALT) and total bilirubin (TBIL) levels were determined with commercial kits using an AU2700 automatic biochemical analyzer (Olympus, Japan). $CD4^+$ T cell counts were performed by flow cytometer (Canto II, USA).

Viral nucleic acid extraction and PCR amplification. HBV DNA was extracted from 200 μl of serum by using Qiagen DNA blood mini kits (Qiagen, Germany). Regions containing the HBV S (nt 368–827, based on HBV B2, D00330) and BCP/preC genes (nt 1607–2068, based on HBV B2, D00330) were amplified for HIV/HBV co-infected and HBV mono-infected patients. Nested PCR was performed for all samples with HBV-specific primers designed for the HBV S and BCP/preC genes. The primer sequences are shown in Supplementary Table 1. A pair of different barcodes (6 nucleotides) was added to inner primers (BCP/preC gene) to identify each sample's sequence data. PrimeStar MAX DNA polymerase (Takara Biotechnology Co., Ltd., China) was used for nested PCR. The amplicons of the HBV S and HBV BCP/preC genes were 472 bp and 474 bp in length, respectively. PCR products were purified by a TaKaRa MiniBEST agarose gel DNA extraction kit (Takara Biotechnology Co., Ltd., China).

Library construction, next-generation sequencing and Sanger sequencing. Next-generation sequencing (NGS) was performed on the Illumina platform with HiSeq PE250 for the HBV BCP/preC gene. An NEB Next Ultra DNA library prep kit for Illumina (NEB, USA) was used to construct the library. Sanger sequencing was performed on an Applied Biosystems 3730XL DNA analyzer (Thermo Fisher Scientific, USA) for HBV S gene products.

Data analysis. NGS data for the HBV BCP/preC gene were first filtered with FASTQ Quality filter in the FASTX-toolkit, where Q30 reads less than 80% were excluded. The paired-end reads of each sample were merged by Flash V1. 2. 9, and then, the sequence data that did not match the expected length were filtered, followed by confirmation of the BCP/preC gene mutations with Geneious R10. 0. 5. C1653T, T1753C, A1762T, G1764A, T1858C, G1896A and G1899A mutations in the HBV BCP/preC gene were analyzed in this study. Based on our previous research (Deng *et al*., 2020, 2021), a positive cutoff value of 1% was defined. To compare our data to previous studies (Sanger sequencing), a 20% cutoff value was used to reanalyze the NGS data (Alidjinou *et al*., 2018; Lowe *et al*., 2016; Rybicka *et al*., 2014). The reference sequences of different HBV genotypes were obtained from GenBank (NCBI). A phylogenetic tree based on HBV S gene sequences constructed by Mega 7.0 with the neighbor-joining method was used to determine HBV genotypes, and the phylogenetic tree is shown in Fig. 5. Viral quasispecies diversity of the HBV BCP/preC gene was evaluated using two parameters (Shannon entropy and genetic distance) in each sample as previously described (Yang *et al*., 2017). Shannon entropy was calculated as -∑(pi*ln pi)/ln N, where pi represents the frequency of

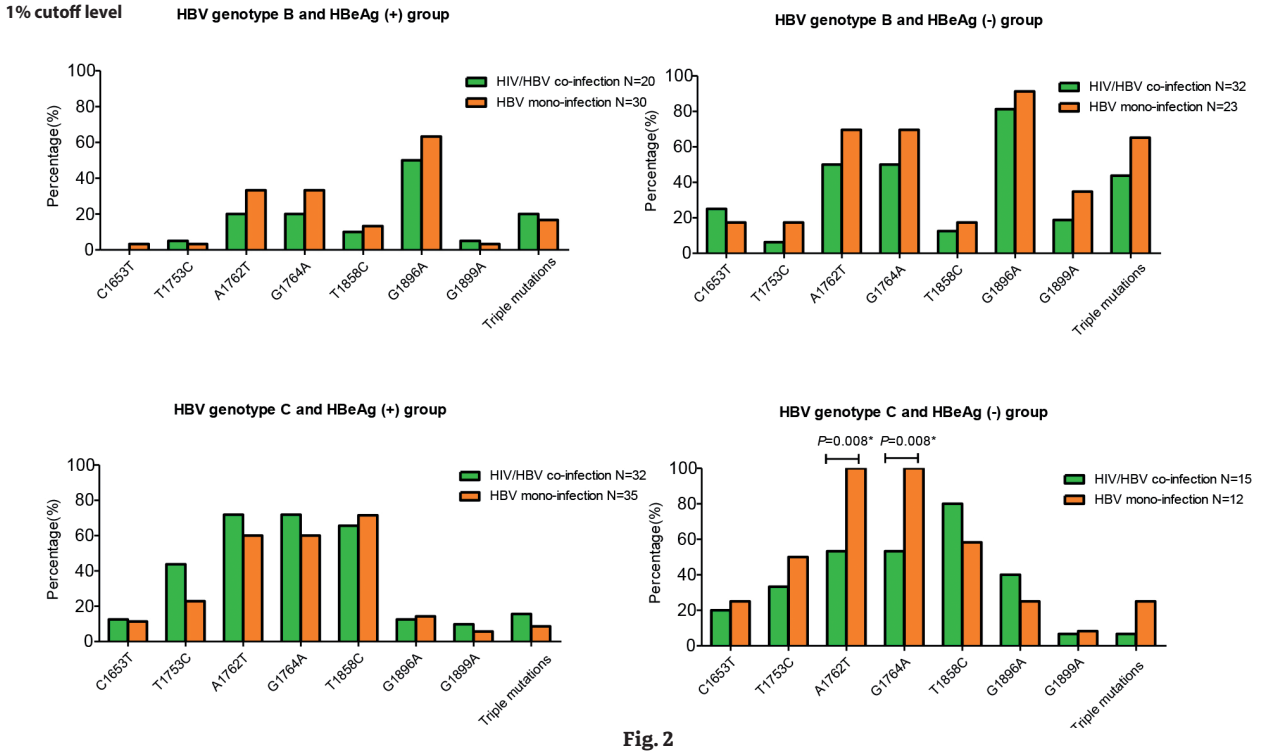

Fig. 2

Comparison of HBV BCP/preC gene mutations with 1% cutoff value

Comparison of HBV BCP/preC gene mutations in HIV/HBV co-infected patients and HBV mono-infected patients with different HBV genotypes and HBeAg statuses with 1% cutoff value. Triple mutations: A1762T/G1764A and G1896A mutations.

the unique read (frequency of repeated sequences in NGS data >0.001 defined a unique read, based on the NGS error rate of the HBV plasmid), and N represents the total number of unique reads, calculated with the Vegan package in R software (V2.15.3). Genetic distance was calculated with Mega 7.0 software as the overall mean distance for each sample.

Statistical analysis. Demographic, clinical and viral mutations and quasispecies diversity data were analyzed for all study subjects. Continuous variables were represented as the mean ± standard deviation or median (interquartile range, IQR); categorical variables were represented as percentages. Student's *t*-test was used for normally distributed continuous variables; the Mann-Whitney *U* test was used for nonparametric variables; and the X^2 test and Fisher's exact test were used for categorical variables. SPSS 13.0 was used for all analyses. A *P*-value less than 0.05 was considered statistically significant.

Results

Study population characteristics

A total of 199 patients were enrolled in this study, including 99 HIV/HBV co-infected patients and 100 HBV mono-infected patients. All subjects' characteristics are shown in Table 1. Among the patients enrolled in this study, no significant differences were observed in sex, age, serum ALT level, HBV DNA level, or HBeAg status between HIV/HBV co-infected and HBV mono-infected patients (P >0.05). Heterosexuality (71.7%) was the most dominant risk factor in HIV/HBV co-infected patients, with $CD4^+$ T cell counts of 63.0 (18.0–260.0) cell/mm^3 in HIV/HBV co-infected patients.

Sequencing depth and HBV genotypes in co-infected and mono-infected patients

The average sequence data generated for the HBV BCP/preC gene were 15653 ± 6825 and 14252 ± 8556 reads in HIV/HBV co-infected patients and HBV mono-infected patients, respectively (t = 1.274, P = 0.204). Two HBV genotypes, genotypes B (subtype B2) and C (subtypes C1 and C2), were isolated from HIV/HBV co-infected and HBV mono-infected patients. Because the sample sizes of subtypes C2 were relatively small, in both HIV/HBV co-infected and HBV mono-infected patients, subtypes C1 and C2 were combined as genotype C and analyzed together in this study. The frequencies of HBV genotype

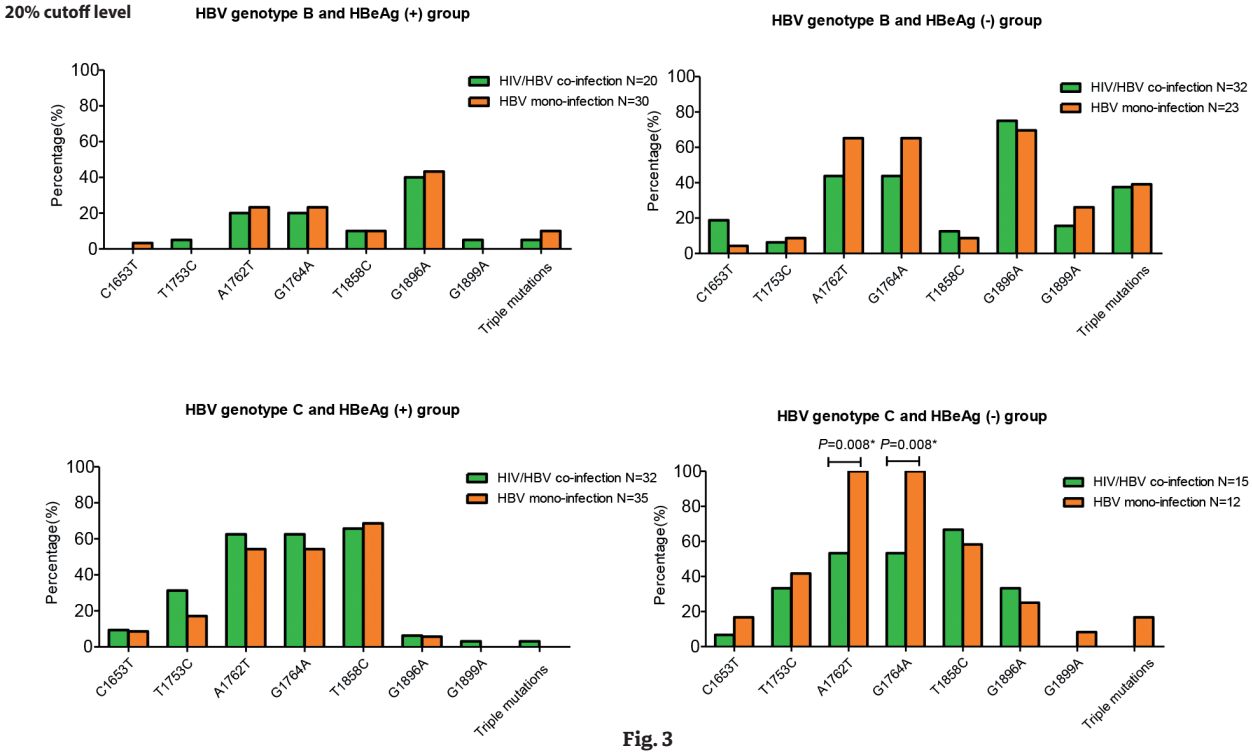

Fig. 3

Comparison of HBV BCP/preC gene mutations with 20% cutoff value

Comparison of HBV BCP/preC gene mutations in HIV/HBV co-infected patients and HBV mono-infected patients with different HBV genotypes and HBeAg statuses with 20% cutoff value. Triple mutations: A1762T/G1764A and G1896A mutations.

B (52.5% vs. 53.0%) and C (47.5% vs. 47.0%) did not differ in HIV/HBV co-infected and HBV mono-infected patients (X^2 = 0.004, P = 0.947).

Comparative analysis of the clinically significant mutations in the HBV BCP/preC gene

In this study, C1653T, T1753C, A1762T, G1764A, T1858C, G1896A and G1899A mutations in the HBV BCP/preC gene were analyzed. HBV BCP/preC mutations were firstly compared in HIV/HBV co-infected and HBV mono-infected patients at 1% cutoff level. The results showed that in the HBV genotype C and HBeAg-negative group, the frequency of A1762T/G1764A double mutations was significantly higher in HBV mono-infected patients than in HIV/HBV co-infected patients (100.0% vs. 53.3%, P = 0.008). However, in the other groups, A1762T/G1764A double mutations and other mutations did not differ between HBV mono-infected and HIV/HBV co-infected patients (P >0.05), as detailed in Fig. 2 and Supplementary Table 2.

To compare our data to previous studies (some studies using the Sanger sequencing method), the cutoff value was elevated to 20%, and the NGS data were reanalyzed. The results showed that at the 20% cutoff level, the BCP/preC gene mutation frequencies were similar to those at the 1% cutoff level. In the HBV genotype C and HBeAg-negative group, A1762T/G1764A double mutations were also significantly less frequent in HIV/HBV co-infected patients than in HBV mono-infected patients (53.3% vs 100.0%, P = 0.008). Also, in the other groups, A1762T/G1764A double mutations and other mutations did not differ between HBV mono-infected and HIV/HBV co-infected patients (P >0.05), as detailed in Fig. 3 and Supplementary Table 3.

The clinical samples containing HBV A1762T/G1764A double and G1896A triple mutations were also analyzed in this study. The results showed that A1762T/G1764A and G1896A triple mutations did not differ between HIV/HBV co-infected and HBV mono-infected patients (P >0.05), regardless of different HBV genotypes, HBeAg status and cutoff level, as detailed in Fig. 2–3 and Supplementary Table 2–3.

Comparative analysis of HBV quasispecies diversity

In this study, viral quasispecies diversity of the HBV BCP/preC gene was compared between HIV/HBV co-infected and HBV mono-infected patients, and Shannon

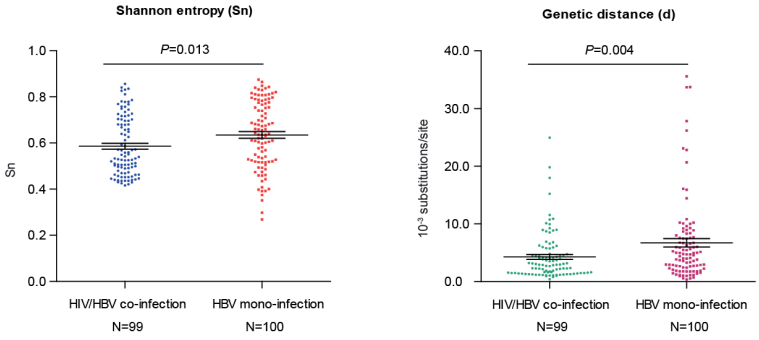

Fig. 4

The differences in viral quasispecies diversity between HIV/HBV co-infected and HBV mono-infected patients
Shannon entropy and genetic distance were used as parameters to compare the viral quasispecies diversity.

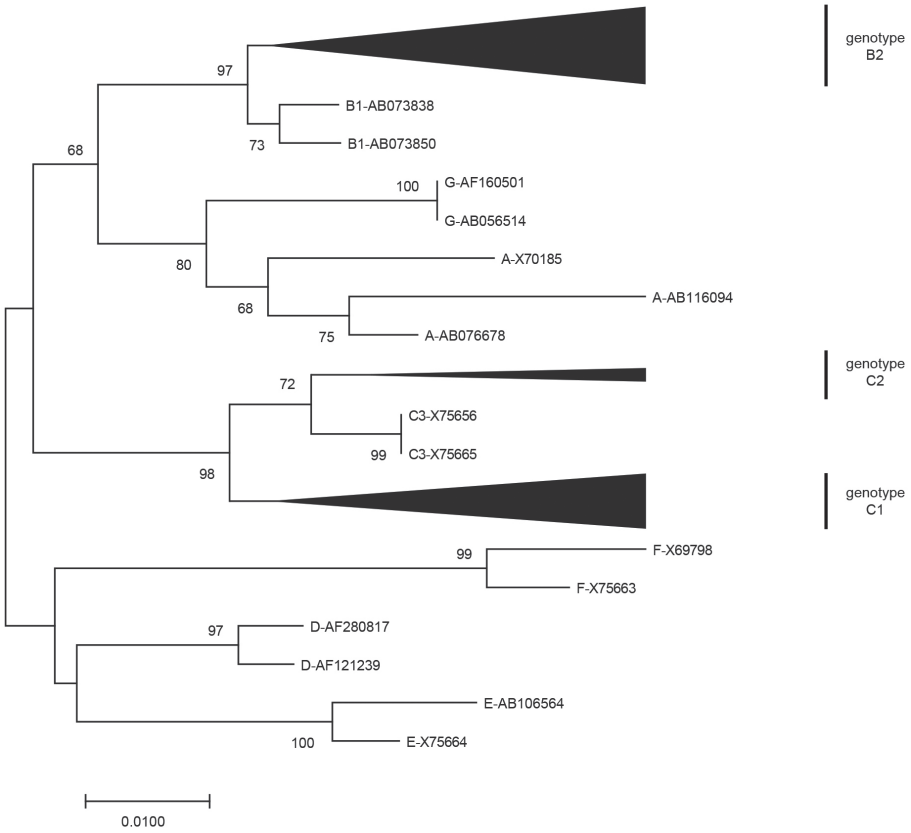

Fig. 5

Phylogenetic tree of HBV genotypes
Phylogenetic tree constructed by Mega 7.0 with the neighbor-joining method was used to determine HBV genotypes; the substitution model was the Tamura-Nei model, and the number of bootstrap replications was set to 1000. HBV S gene sequence data were used to construct the phylogenetic tree.

entropy and genetic distance were used as parameters of viral quasispecies diversity. The results showed that Shannon entropy was significantly lower in HIV/HBV co-infected patients than in HBV mono-infected patients (t = -2.519, P = 0.013) and that genetic distance was also significantly lower in HIV/HBV co-infected patients than in HBV mono-infected patients (t = -2.902, P = 0.004), as detailed in Fig. 4.

Discussion

In China, approximately 8.7% of HIV-positive patients are co-infected with HBV (Zhang *et al.*, 2014). Previous studies have shown that HIV/HBV co-infection accelerates the progression of HBV-related end-stage liver disease (Hoffmann and Thio, 2007; Kim, 2020), but the reasons are still unknown. HBV mutations may be one of the most important factors, especially mutations in the BCP/preC gene. In the current study, we enrolled 99 HIV/HBV co-infected and 100 HBV mono-infected patients. In HBV genotype C and HBeAg-negative patients, the frequency of HBV A1762T/G1764A double mutations was lower in HIV/HBV co-infected patients than in HBV mono-infected patients, whereas A1762T/G1764A double mutations did not differ in the other groups. In addition, HBV quasispecies diversity was lower in HIV/HBV co-infected patients than in HBV mono-infected patients.

The difference in A1762T/G1764A double mutations between HIV/HBV co-infected and HBV mono-infected patients has been reported in some studies, but the results vary greatly. A study that included 20 treatment-naïve HIV/HBV co-infected and 19 HBV mono-infected patients from Argentina whose HBV genotypes were A2 and F1. The results showed that A1762T/G1764A double mutations in HIV/HBV co-infected patients were less frequent than those in HBV mono-infected patients (Cassino *et al.*, 2009). Another study from Australia that included 88 HIV/HBV co-infected and 63 HBV mono-infected patients or study with HIV/HBV co-infected patients recruited from the USA, Australia and Thailand, and HBV mono-infected patients that were recruited from Australia and Hong Kong with HBV genotypes A, B and C revealed that A1762T/G1764A double mutations were less frequent in HIV/HBV co-infected patients than in HBV mono-infected patients only in patients with HBV genotype C (Audsley *et al.*, 2010). However, the results of a study in Thailand showed that A1762T/G1764A double mutations did not differ between HIV/HBV co-infected and HBV mono-infected patients; this study included 24 HIV/HBV co-infected patients and 31 mono-infected patients, and all the patients were infected with HBV genotypes B2 and C1 (Tangkijvanich *et al.*, 2013). Recently, a study from Guangxi Province in southern China included 61 HIV/HBV co-infected and 61 HBV mono-infected patients, the HBV genotypes of which were B, C and I. This research showed that the HBV A1762T/G1764A double mutations in HBV genotype C or I were significantly more frequent in HIV/HBV co-infected patients than in HBV mono-infected patients, whereas there were no differences in those in HBV genotype B (Li *et al.*, 2017). The studies mentioned above revealed that the difference in A1762T/G1764A double mutations between HIV/HBV co-infected and HBV mono-infected patients is still unknown.

In the current study, we enrolled a large sample of HIV/HBV co-infected and HBV mono-infected patients and compared the BCP/preC gene mutations in HIV/HBV co-infected and HBV mono-infected patients with different HBV genotypes and HBeAg statuses. The results of this study were similar to those of studies in Argentina and Australia; however, the results were different from those of the studies in Thailand and Guangxi of China. The different results may be due to the following reasons. (i) The geographical distribution of HBV genotypes is not the same, so the patients enrolled in different studies were infected with different HBV genotypes. (ii) Some of these studies enrolled relatively small sample sizes. (iii) Neither the study in Thailand nor that in Guangxi compared the different HBeAg statuses within the same HBV genotypes, which could lead to different conclusions because BCP A1762T/G1764A double mutations were strongly associated with different HBeAg statuses (Turyadi *et al.*, 2013). NGS used in this study did not impact the results because we adjusted the cutoff value to 20%, and the results were nearly the same as those with the 1% cutoff value.

HIV infection mainly targets CD4-positive T cells (Maartens *et al.*, 2014), and HBV-infected patients co-infected with HIV exhibit a negative influence on the natural history of HBV infection. A study from Argentina showed that the HBV evolutionary rate, Shannon entropy and genetic distances were higher in HBV mono-infected than in HIV/HBV co-infected patients (Cassino *et al.*, 2012). In the current study, the results were consistent with those of the study in Argentina, which showed that HIV co-infection reduced the quasispecies diversity of HBV. The reasons may be that HIV infection impairs the frequency and functionality of HBV-specific CD4-positive T cells (Chang *et al.*, 2009). Viral mutations and quasispecies diversity are strongly related to host immunity, and reduced CD4-positive T cell levels lead to lower selection pressure on HBV, so HBV mutations and quasispecies diversity were decreased in HIV/HBV co-infected patients.

In conclusion, in this study, we investigated the HBV BCP/preC gene mutations in patients with different HBV genotypes and HBeAg statuses and compared the viral quasispecies diversity in HIV/HBV co-infected and HBV mono-infected patients in China, revealing that BCP/preC mutations are not more frequent and that HBV viral quasispecies diversity is lower in HIV/HBV co-infected patients than in HBV mono-infected patients. Based on the results of this study, BCP/preC mutations may not be the reason for accelerated progression of HBV-related end-stage liver disease in HIV/HBV co-infected patients. Further studies are needed to focus on the mechanism by which HIV/HBV co-infection accelerates the progression of HBV-related end-stage liver disease.

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Supplementary information is available in the online version of paper.

References

Alidjinou EK, Bocket L, Pigot V, Lambert V, Hallaert C, Canva V, Hober D (2018): Sanger sequencing versus INNO-LiPA® HBV PreCore assay for routine detection of precore and basal core promoter mutations in hepatitis virus B chronically infected patients. Diagn. Microbiol. Infect. Dis. 90(4), 277–279. https://doi.org/10.1016/j.diagmicrobio.2017.12.006

Audsley J, Littlejohn M, Yuen L, Sasadeusz J, Ayres A, Desmond C, Spelman T, Lau G, Matthews GV, Avihingsanon A, Seaberg E, Philp F, Saulynas M, Ruxrungtham K, Dore GJ, Locarnini SA, Thio CL, Lewin SR, Revill PA (2010): HBV mutations in untreated HIV-HBV co-infection using genomic length sequencing. Virology 405(2), 539–547. https://doi.org/10.1016/j.virol.2010.06.038

Cassino L, Laufer N, Salomon H, Campos R, Quarleri J (2009): Hepatitis B precore/core promoter mutations in isolates from HBV-monoinfected and HBV-HIV coinfected patients: a 3-yr prospective study. J. Clin. Virol. 46(4), 354–359. https://doi.org/10.1016/j.jcv.2009.09.015

Cassino L, Torres C, Mbayed V, Laufer N, Campos RH, Quarleri J (2012): Comparative analysis of hepatitis B virus genotype a molecular evolution in patients infected with HBV and in patients co-infected with HBV and HIV. J. Med. Virol. 84(4), 562–569. https://doi.org/10.1002/jmv.23233

Chang JJ, Sirivichayakul S, Avihingsanon A, Thompson AJ, Revill P, Iser D, Slavin J, Buranapraditkun S, Marks P, Matthews G, Cooper DA, Kent SJ, Cameron PU, Sasadeusz J, Desmond P, Locarnini S, Dore GJ, Ruxrungtham K, Lewin SR (2009): Impaired quality of the hepatitis B virus (HBV)-specific T-cell response in human immunodeficiency virus type 1-HBV coinfection. J. Virol. 83(15), 7649–7658. https://doi.org/10.1128/JVI.00183-09

Deng H, Liang S, Xu M, Zhuo L, Gao H, Chen K, Shi Y, Li H, Jiao Q, Lin L, Lei Y, Liu H (2020): Clinical efficacy and safety in telbivudine- or tenofovir-treated hepatitis B e antigen-positive pregnant women. Antivir. Ther. 25(1), 33–41. https://doi.org/10.3851/IMP3345

Deng H, Guo F, Yu W, Li L, Xia Y, Guan Y, Li J (2021): Dynamic changes of HCV genomes under selective pressure from DAAs therapy in relapsed patients. Virus. Res. 302, 198453. https://doi.org/10.1016/j.virusres.2021.198453

Easterbrook P, Sands A, Harmanci H (2012): Challenges and priorities in the management of HIV/HBV and HIV/HCV coinfection in resource-limited settings. Semin. Liver Dis. 32(2), 147–157. https://doi.org/10.1055/s-0032-1316476

Hoffmann CJ, Thio CL (2007): Clinical implications of HIV and hepatitis B co-infection in Asia and Africa. Lancet. Infect. Dis 7(6), 402–409. https://doi.org/10.1016/S1473-3099(07)70135-4

Kim, HN (2020): Chronic Hepatitis B and HIV Coinfection: A continuing challenge in the era of antiretroviral therapy. Curr. Hepatol. Rep. 19(4), 345–353. https://doi.org/10.1007/s11901-020-00541-x

Kim JH, Psevdos G, Suh J, Sharp VL (2008): Co-infection of hepatitis B and hepatitis C virus in human immunodeficiency virus-infected patients in New York City, United States. World. J. Gastroenterol. 14(43), 6689–6693. https://doi.org/10.3748/wjg.14.6689

Kourtis AP, Bulterys M, Hu DJ, Jamieson D (2012): HIV-HBV coinfection--a global challenge. N. Engl. J. Med. 366(19), 1749–1752. https://doi.org/10.1056/NEJMp1201796

Li KW, Kramvis A, Liang S, He X, Chen QY, Wang C, Yang QL, Hu LP, Jia HH, Fang ZL (2017): Higher prevalence of cancer related mutations 1762T/1764A and PreS deletions in hepatitis B virus (HBV) isolated from HBV/HIV co-infected compared to HBV-mono-infected Chinese adults. Virus. Res. 227, 88–95. https://doi.org/10.1016/j.virusres.2016.10.002

Liu S, Zhang H, Gu C, Yin J, He Y, Xie J, Cao G (2009): Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. J. Natl. Cancer. Inst. 101(15), 1066–1082. https://doi.org/10.1093/jnci/djp180

Lowe CF, Merrick L, Harrigan PR, Mazzulli T, Sherlock CH, Ritchie G (2016): Implementation of Next-generation sequencing for hepatitis B virus resistance testing and genotyping in a clinical microbiology laboratory. J. Clin. Microbiol. 54(1), 127–133. https://doi.org/10.1128/JCM.02229-15

Maartens G, Celum C, Lewin SR (2014): HIV infection: epidemiology, pathogenesis, treatment, and prevention. Lancet 384(9939), 258–271. https://doi.org/10.1016/S0140-6736(14)60164-1

Mak D, Kramvis A (2020): Molecular characterization of hepatitis B virus isolated from Black South African cancer patients, with and without hepatocellular carcinoma. Arch. Virol. 165(8), 1815–1825. https://doi.org/10.1007/s00705-020-04686-4

Rajput MK (2020): Mutations and methods of analysis of mutations in Hepatitis B virus. AIMS. Microbiol. 6(4), 401–421. https://doi.org/10.3934/microbiol.2020024

Rybicka M, Stalke P, Dreczewski M, Smiatacz T, Bielawski KP (2014): High-throughput matrix-assisted laser desorption ionization-time of flight mass spectrometry as an alternative approach to monitoring drug resistance of hepatitis B virus. J. Clin. Microbiol. 52(1), 9–14. https://doi.org/10.1128/JCM.01891-13

Tangkijvanich P, Sa-Nguanmoo P, Avihingsanon A, Ruxrungtham K, Poovorawan K, Poovorawan Y (2013): Characterization of hepatitis B virus mutations in untreated patients co-infected with HIV and HBV based on complete genome sequencing. J. Med. Virol. 85(1), 16–25. https://doi.org/10.1002/jmv.23430

Thio CL (2009): Hepatitis B and human immunodeficiency virus coinfection. Hepatology 49(5 Suppl), S138-145. https://doi.org/10.1002/hep.22883

Tiollais P, Pourcel C, Dejean A (1985): The hepatitis B virus. Nature 317(6037), 489-495. https://doi.org/10.1038/317489a0

Turyadi, Thedja MD, Ie SI, Harahap AR, El-Khobar KE, Roni M, Muljono DH (2013): HBsAg, HBeAg and HBV DNA level changes and precore/basal core promoter mutations in the natural history of chronic hepatitis B in Indonesian patients. Hepatol Int 7(4), 969–980. https://doi.org/10.1007/s12072-013-9438-z

Wang W, Shu Y, Bao H, Zhao W, Wang W, Wang Q, Lei X, Cui D, Yan Z (2019): Genotypes and hot spot mutations of hepatitis B virus in northwest Chinese population and its correlation with diseases progression. BioMed research international 2019, 3890962. https://doi.org/10.1155/2019/3890962

Yang G, Liu Z, Yang J, Luo K, Xu Y, He H, Fu Q, Yu S, Wang Z (2017): Quasispecies characteristics in mother-to-child transmission of hepatitis B virus by next-generation sequencing. The Journal of infection 75(1), 48–58. https://doi.org/10.1016/j.jinf.2017.04.012

Zhang F, Zhu H, Wu Y, Dou Z, Zhang Y, Kleinman N, Bulterys M, Wu Z, Ma Y, Zhao D, Liu X, Fang H, Liu J, Cai WP, Shang H (2014): HIV, hepatitis B virus, and hepatitis C virus co-infection in patients in the China National Free Antiretroviral Treatment Program, 2010-12: a retrospective observational cohort study. Lancet. Infect. Dis. 14(11), 1065–1072. https://doi.org/10.1016/S1473-3099(14)70946-6