Appendix 1. The Impacts of Repeated Freezing and Thawing on Serum HBV RNA Detection. The dotted line represented LoD of serum HBV RNA detection (66.7 IU/ml, 1.8 log IU/ml). CV, coefficient of variation; HBV, hepatitis B virus; LoD, lower limit of detection; SD, standard deviation.
Appendix 2. Patients and Methods

Measurement of Serum HBV RNA Level

The procedure included serum HBV nucleic acid extraction, DNA digestion, reverse transcription of RNA to complementary DNA and real time polymerase chain reaction.

HBV nucleic acid from 200 μL serum was extracted by EasyPure Viral DNA/RNA kit (TransGen Biotech, Beijing, China) and dissolved in 50 μL RNase-free water.

The DNA digestion mixture contained 7.5 μL nucleic acid solution and other substances from the DNase I Kit (1 μL DNase I, 0.5 μL RNase inhibitor and 1 μL 10×DNase I buffer) (Thermo Fisher Scientific, Waltham, MA, USA). The reaction was run at 37 °C for 30 min and then terminated by adding 1 μL EDTA (Thermo Fisher Scientific) and incubated at 65 °C for 10 minutes.

The remaining 11 μL HBV RNA was reverse transcribed by RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) with the specific 3’ race long primer 5’-ACCACGCTATCGCTACTCAC(dT)17GWAGCTC-3’ corresponding to nt 1935-1929 of the HBV genome. The total 20 μL reaction mixture was consisted of 11 μL RNA sample, 1 μL reverse transcriptase, 1 μL RNase inhibitor, 2 μL dNTP (2.5 mM), 1 μL primer and 4 μL buffer.

The obtained cDNA was quantitatively detected by the Hepatitis B Viral DNA Quantitative Fluorescence Diagnostic Kit (PCR-Fluorescence Probing) (Sansure Biotech, Changsha, China) using the ABI StepOne Plus Sequence Detection System (Applied Biosystems, Foster City, CA) according to the manufacturers’ instructions. The lower limit of detection (LoD) of this Kit was 100 IU/mL in reaction mix corresponding to 66.7 IU/mL (1.8 log IU/mL) HBV RNA in serum. Testing raw value as lower than LoD or undetermined was defined as HBV RNA undetectable, otherwise it was recorded as detectable in this study.
Appendix 3. Detection of Serum HBV RNA in Genotype C-infected Patients at HBeAg-positive and -negative Status. A and C: HBV RNA levels in C genotype and C2 subgenotype; B and D: log HBV DNA/RNA in C genotype and C2 subgenotype. The dotted line represented LoD of serum HBV RNA detection (66.7 IU/mL, 1.8 log IU/mL). The statistics here included samples with testing values reported as lower than LoD or undetermined; and LoD values were used for these samples. HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; LoD, lower limit of detection.
Appendix 4. Phylogenetic Trees of HBV Genotype B Sequences. MEGA 7.0 software was used for the analysis. The neighbor-joining method and Kimura 2-parameter algorithm with bootstrapping 1000 replicates were applied. The subgenotype reference sequences were as follows: AF121243, AF121251, AB073823 and AB073830 (B2); EU330994 and EU330997 (B6); AP011091 and AP011092 (B7); AP011095 and AP011096 (B9).
Appendix 5. Phylogenetic Trees of HBV Genotype C Sequences. MEGA 7.0 software was used for the analysis. The neighbor-joining method and Kimura 2-parameter algorithm with bootstrapping 1000 replicates were applied. The subgenotype reference sequences were as follows: AB031262, AB111946 and AY217378 (C1); AB042284, AF182804, AY040627, AY050574 and M38636 (C2); X75658 and X75665 (C3); AB048704 and AB048705 (C4); AP011100 and EU410080 (C5); AB493841 and AB493842 (C6).
Appendix 6. Click Here

Appendix 7. Click Here