Supplementary

Materials and Methods

Local Ethic Committee approved additional examinations of the subjects beyond standards of care. Special examination included detection HBV infection markers (HBsAg, anti-HBs, anti-HBc, HBV DNA) in blood and in resected pancreatic tissues (HBV DNA, pregenomic HBV RNA and cccDNA), and immunohistochemistry staining of pancreatic tissues for HBxAg and cell proliferation marker Ki-67.

Blood samples were obtained after overnight fasting and analyzed immediately in the local laboratory. Immunologic tests were performed using the Sunrise analyzer (Tecan GmbH, Austria) and specific immunoassays kits (Vector-Best Co., Russia).

Plasma HBV DNA was isolated using commercial AmpliSens Riboprep kit (AmpliSens Biotechnologies, Russia) according to manufacturer’s instructions and quantified using polymerase chain reaction (PCR) assay AmpliSens HBV-FL (AmpliSens Biotechnologies, Russia) kit (lower limit of detection of 10 IU/mL). To isolate nucleic acids from biopsies, samples were first homogenized in the MagNA Lyser (Roche Diagnostics, Switzerland). HBV DNA was isolated by AmpliSens Riboprep kit (AmpliSens Biotechnologies, Russia) and quantified by AmpliSens HBV-FL (AmpliSens Biotechnologies, Russia) kit.

To quantify covalently closed circular DNA HBV (cccDNA), nucleic acids were first treated with T5 exonuclease (New England Biolabs, UK) at 37°C for 60 min and inactivation at 70°C for 20 min[19]. HBV cccDNA was quantified with specific sets of primers and probes and normalized to genomic β-globin.

To analyze pregenomic HBV RNA (pgRNA HBV) analysis, nucleic acids were treated with RNase-free DNase I (New England Biolabs, UK) for 30 min at 37°C, purified by using AmpliSens Riboprep kit (AmpliSens Biotechnologies, Russia), reverse transcribed by AmpliSens Reverta-FL (AmpliSens Biotechnologies, Russia), and quantified by AmpliSens HBV-FL (AmpliSens Biotechnologies, Russia) kit. CFX96 Real-Time System (Bio-Rad, USA) PCR machine was used for the analysis of plasma and pancreatic tissue samples. Gel-electrophoresis of PCR-amplified HBV DNA is shown in supplementary figure 1.

Immunohistochemistry of pancreatic tissues was performed after deparaffinization. Slides were fixed in 4% paraformaldehyde, washed 3 times in Tris-HCl (50 mM, pH 8.0)
followed by incubation with a blocking buffer (0.02% of Triton X-100, 10% horse serum, and 150 mmol/L NaCl in Tris-HCl, 50 mmol/L, pH 8.0) for 30 min and 1 h staining with primary rabbit anti-HBx (ab39716) (Abcam, UK). Then, slides were washed 3 times for 5 minutes in a washing buffer (0.02% of Triton X-100 and 200 mmol/L NaCl in Tris-HCl, 50 mmol/L, pH 8.0), incubated for 1 h with secondary Alexa Fluor 594 goat anti-rabbit antibodies (ab150080) (Abcam, UK). After that, the slides were treated with primary tagged Alexa Fluor® 488 rabbit anti-Ki-67 (ab197234) and Hoechst 33342 (ab228551) for 1 h, washed 3 times for 5 min in washing buffer and finally mounted with a Fluoroshield reagent (Abcam, UK). Images were captured using Thunder imaging systems (Leica Microsystems, Germany) with 10× objectives. Ki-67 and HBxAg staining was analyzed using LAS X (Leica Microsystems, Germany). Ki-67 index was counted as the percentage of Ki-67-positive cells [20].