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Detection of intrahepatic total and covalently closed circular (CCC) DNA by a sensitive and specific quantitative PCR assay, and its clinical significance

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Objectives
CccDNA serves as a template for the production of Hepatitis B virus (HBV) pregenomic-RNA and shows viral replication activity. It is responsible from the persistence of HBV during antiviral therapy. We report total-DNA and cccDNA levels in the liver biopsy samples of 6 CHB patients during different phases of undergoing antiviral therapy by a sensitive and specific quantitative real-time polymerase chain reaction (PCR) assay.

Methods
All the patients had undetectable serum HBV-DNA at the time biopsy. Each patient underwent a liver biopsy after checking abdominal ultrasonography, bleeding profile and having a signed informed consent. A small piece of the biopsy specimens at least 2 mm in length was sent to Virology Labaratory in 0.9% sodium chloride solution. DNA was extracted from tissue using 'Alkaline phenol-chloroform-izoamyl alcohol process'. PCR amplification was performed by TaqMan real-time PCR method for the each study virus. Primers and probes for intrahepatic total-DNA, cccDNA and lamivudine resistance were designed using 'Oligoware 1.0 software program'. 'ABI PRISM 7700 Sequence Detection System' was used for all the analysis. TOPO-TA cloning kit and the plasmid including cloned HBV genome was used for the quantification standards. The quantification was arranged to be per mg.tissue. The dynamic-range of the quantification was decided by serial dilutions of the created plasmid standards in distribution of 10^2-10^9.

Results
First patient was recieving lamivudine (LAM) monotheraphy for 5 years. Second and third patient recieving LAM for 38 months and 42 months had elevated transaminases for the last 14 months and 18 months despite undetectable serum HBV-DNA. Fourth patient was recieving LAM add-on adefovir dipivoxil for 63 months following 5 years of LAM. Fifth patient was recieving LAM add-on tenofovire disoproxil fumarat (TDV) for 3 months following 9 years of LAM monotheraphy. Sixth patient was recieving TDV for 3 months following one year of pegylated-IFN. Intrahepatic total\cccDNA levels in patients were $5{,}6x10^6$\$6{,}4x10^4$, $8{,}8x10^7$\$4x10^4$, $4{,}9x10^6$\$9{,}5x10^4$, $5{,}5x10^6$\$2{,}7x10^4$, $5{,}1x10^3$\-, $9{,}2x10^7$\$1{,}1x10^4$ coppies\mg.tissue, respectively. Although all the patients except first and second had normal levels of serum transaminases as well as undetectable serum HBV-DNA at the time of biopsy, YMDD mutations were identified in all except fourth and sixth one, then treatment regimens were reconstructed.

Conclusions
Our results show that cccDNA persists in hepatocyte nucleus, even in with serological evidence of viral clearance under the long-term antiviral theraphy and its detection provides valuable information for the emergence of drug-resistance to manage treatment early. Investigating the intrahepatic forms of HBV-DNA is a good implementation for evidence-based medicine as an alternative in clinical practice.

Therefore it is important to develop standardized assays to be used on clinical samples.