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

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Background: CccDNA serves as a template for the production of 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 chronic hepatitis B(CHB) patients during different phases of undergoing antiviral therapy by a sensitive and specific quantitative real-time polymerase chain reaction(PCR) assay.

Methods & Materials: All the patients had undetectable serum HBV-DNA at the time of biopsy. Each patient underwent a liver biopsy, and a small piece of the biopsy specimens at least 2 mm in length was sent to labaratory in 0.9% NaCl solution. DNA was extracted from tissue using Alkaline phenol-chloroform- izoamyl alcohol process. PCR amplification was performed by TaqMan real-time PCR method. 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.

Results: First patient was recieving lamivudine(LAM) for 5 years. Second and third patient recieving LAM for 38 and 42 months had elevated transaminase for the last 14 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. Sixth patient was recieving TDV for 3 months following one year of pegylated-IFN. Intrahepatic total\cccDNA levels in patients were $5{,}6x10^6\backslash 6{,}4x10^4$, $8{,}8x10^7\backslash 4x10^4$, $4{,}9x10^6\backslash 9{,}5x10^4$, $5{,}5x10^6\backslash 2{,}7x10^4$, $5{,}1x10^3\backslash$-, $9{,}2x10^7\backslash 1{,}1x10^4$ coppies\mg.tissue, respectively. Although all the patients except first and second had normal levels of serum transaminase 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.

Conclusion: 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.

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Frequent Detection of HBV and HCV infection in HIV positive cancer patients undergoing chemotherapy or radiotherapy at Dr George Mukhari Academic hospital, Pretoria, South Africa

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Background: Chronic hepatitis, liver cirrhosis and hepatocellular carcinoma by HBV and/or HCV are among the leading causes of death, especially in HIV endemic regions. Though distinct viruses, HBV, HCV and HIV share common routes of transmission, albeit at different efficiency. Chemotherapy and radiotherapy for cancer have been associated with reactivation of viral hepatitis, leading to accelerated hepatitis disease and termination of cancer therapy.

Methods & Materials: The study population comprised 34 black (3 males and 31 females) HIV positive cancer patients due to undergo chemotherapy or radiotherapy at Dr George Mukhari Academic hospital, north-west of Pretoria. Routine blood tests, HIV viral load, CD4+ cell counts and liver function tests (ALT and AST) were conducted. HBV serological makers were tested with Elecsys assays (Roche). The Monolisa HCV antigen-antibody assay was used to screen for HCV. In-house PCR assays were performed to detect HCV and HBV active infection.

Results: Of the 34 patients, 75% (25) were exposed to HBV and 64% (22) were HBV DNA positive; 47% (16) had exposure to HCV and 38% (13) were HCV RNA positive; and 29% (10) were simultaneously positive for HBV DNA, HCV RNA and HIV RNA. Lower CD4+ cell count was a risk factor for active HBV or HCV infection. HBV DNA positivity was 44.1% (15/34) and 20.6% (7/34) for CD4+ cell count < 350 cells/ml and > 350 cells/ml, respectively. Similarly, HCV RNA positivity was 23.5% (8/34) and 14.7% (5/34) for CD4+ cell count < 350 cells/ml and > 350 cells/ml, respectively. Surprisingly, ALT and AST levels were normal for all but 2 patients. Most of the patients with a detectable HIV viral load and/or CD4+ cell count < 350 cells/ml at baseline were initiated on HAART before cancer therapy.

Conclusion: To our knowledge, this is the first study to report relatively high simultaneous detection of HBV, HCV and HIV active infection in cancer patients in this region. The study advocates for intensified screening of HBV and HCV in cancer patients before initiation of HAART and/or cancer therapy, and where indicated, anti-viral treatment should be extended to HBV and/or HCV, to minimize the risk of liver disease.

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