Journal of Clinical Virology 60 (2014) 347–353

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Virological characteristics of occult hepatitis B virus in a North American cohort of human immunodeficiency virus type 1-positive patients on dual active anti-HBV/HIV therapy

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ARTICLE INFO

Article history:
Received 21 November 2013
Received in revised form 10 March 2014
Accepted 25 April 2014

Keywords:
Occult HBV infection
HIV
Lymphoid cells
Genotype
Lamivudine/3TC
Canada

ABSTRACT

Background: Occult hepatitis B virus infection (OBI) is defined as low-level HBV DNA presence in serum, liver and/or peripheral blood mononuclear cells (PBMC) in individuals that lack serum hepatitis B virus surface antigen (HBsAg). HIV+ patients with OBI may be at risk for HBV reactivation, and often receive dual active anti-HBV/HIV therapy, such as lamivudine (LMV).
Objectives: To determine the presence of OBI in a North American cohort of HIV-1-positive patients.
Study design/methods: 45 HIV-1-positive, serum HBsAg-negative patients, reactive for antibodies to HBV core antigen (anti-HBc), were tested for HBV DNA in plasma and for HBV DNA and covalently closed circular DNA (cccDNA) in PBMC. Ten patients were re-tested after ~5–10 years, including genotyping and clonal sequence analysis of the HBV polymerase (P) gene and overlapping HBV surface (S) gene from 8 PBMC samples.
Results: Overall, 42% (19/45) tested HBV DNA positive, especially in PBMC (18/45), including 3/18 that were reactive for HBV cccDNA, compared to 17% (8/45) that were HBV DNA reactive in plasma. In 8 patients on LMV, sequence analysis in PBMC showed that all were HBV genotype C or D. Several carried HBV P region variants at residues associated with anti-HBV drug resistance and overlapping S gene region within the major HBsAg "a determinant".
Conclusion: OBI is common in HIV-positive, anti-HBc reactive patients on anti-HBV/HIV therapy, particularly in PBMC. HBV sequence analysis revealed that all had HBV genotype C or D and often had P/overlapping S gene variants possibly associated with dual-active anti-HIV/HBV therapy.

1. Background

Due to shared modes of transmission, hepatitis B virus (HBV) infection is common in human immunodeficiency virus type 1 (HIV-1)-positive individuals [1]. Occult hepatitis B virus infection (OBI) is defined by the presence of low-level HBV DNA in plasma, liver and/or peripheral blood mononuclear cells (PBMC) in individuals without detectable serum HBV surface antigen (HBsAg) [2]. Patients who recover from acute HBV may establish OBI with antibodies to HBV core antigen (anti-HBc) [3,4] along with HBV-specific memory T-cell responses [5,6]. In HIV-negative patients, OBI has been linked to adverse clinical sequelae such as hepatocellular carcinoma development (HCC), accelerated progression of hepatitis C virus (HCV) infection-related liver fibrosis, and cryptogenic liver disease [7–9]. There is variation in the reported prevalence of OBI/HIV co-infection, which may be attributed to the assay sensitivity for HBV DNA and HBsAg detection, the prevalence of HBV in different regions, and the history of antiretroviral therapy (ART) [10–14]. The proposed clinical impact of OBI/HIV co-infection

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http://dx.doi.org/10.1016/j.jcv.2014.04.021
1386-6532/

include: (i) higher rates of hepatotoxicity induced by ART [15]; (ii) hepatic transaminase flares [16,17], and (iii) OBI reactivation after HIV-related immunosuppression [18]. Other studies [19,20] found that OBI was associated with lower $CD4^+$ T cell counts before ART initiation. The presence of anti-HBc has been associated with more rapid HIV progression to the acquired immunodeficiency syndrome than in those without anti-HBc [21,22].

HBV and HIV-1 can cause persistent infections of lymphoid cells [23,24]. Both HIV and HBV utilize an error-prone reverse transcription step during replication leading to emergence of quasispecies [25,26]. Many of the anti-HIV nucleos/tide analog reverse transcriptase inhibitors are active against HBV and can lead to anti-HBV drug resistance [27]. Moreover, the HBV genome is arranged into overlapping reading frames such that antiviral drug-resistant mutations in the HBV polymerase (P) gene can lead to changes in the envelope/surface (S) gene [25,28]. HBV isolates with altered S antigens may exhibit reduced neutralizing antibody binding, which could affect HBV clearance, HBsAg assay detection and be even responsible for immune escape [27,29,30].

There is limited information on the prevalence and molecular virology of OBI in HIV+ patients in Canada.

1.1. Objectives

(1) To determine if HBV can be identified in plasma and lymphoid cells of serum HBsAg-negative, anti-HBc/HIV-1-positive patients despite ART.
(2) To recognize the occurrence of HBV P gene variants (i.e., anti-HBV drug resistant) or HBV S gene (i.e., immune escape mutants) in PBMC.

2. Study design/methods

2.1. Patients and samples

This study received institutional review board approval from the University of Calgary Conjoint Health Research Review Board in accordance with the ethical standards of the Declaration of Helsinki. The Southern Alberta HIV Clinic (SAC) has seen 2750 patients with HIV-1 infection over 20 years. In consented patients, matched serial plasma was stored at −80 °C and PBMC, isolated using Ficoll-Hypaque gradient centrifugation, was stored in liquid nitrogen. We identified that 17% (483/2750) carried anti-HBc and 45 randomly selected (∼10%) were tested for HBV DNA in plasma and PBMC. Ten patients were re-tested in plasma and/or in PBMC samples collected in subsequent follow-up (Table 1). Serologic markers of HBV infection, including HBsAg, total anti-HBc, and antibodies to HBsAg (anti-HBs), were assayed by commercial chemiluminescent microparticle immunoassays (i.e., ARCHITECT anti-HBc II and anti-HBs; Abbott Diagnostics, Mississauga, Ontario, Canada) All HIV-1-positive patients were on ART, yet most had detectable HIV RNA according to a clinical reverse-transcription polymerase chain reaction (RT-PCR) assay (Table 1). The HIV RNA RT-PCR sensitivity varied according to the assay used at the time; i.e., Nuclisens Easy Q® HIV-1 V 2.0 (10–10^7 virus copies/ml, Biomerieux, St. Laurent, Quebec, Canada), Nuclisens® HIV-1 QT (176–3.47 × 10^6 virus copies/ml, Organon Teknika, Durham, NC), Abbott Real Time HIV-1 assay *m*2000 (40 or 75–10^7 virus copies/ml, Abbott Diagnostics).

2.2. Detection of HBV genomes in plasma and PBMC

HBV DNA was detected in plasma and/or PBMC, as described [31,32]. Total DNA was extracted from 200 μL or 500 μL of plasma, and 4–8 × 10^6 PBMC using proteinase-K and phenol-chloroform procedure. HBV DNA was detected by nested PCR using HBV P gene-specific primers followed by detection of the amplicons by nucleic acid hybridization (NAH) to a radiolabelled recombinant HBV DNA to verify virus sequence specificity and validity of controls. For detection of HBV cccDNA in PBMC, total DNA was digested with S1 nuclease (Invitrogen Canada Inc., Burlington, Ontario, Canada) to eliminate single stranded DNA and then amplified using primers spanning the HBV genome nick region [31,33]. The archived samples from 1996/1997 were tested first, and the samples in 2004/2007 were tested ∼6–12 months later. Strict precautions were undertaken to avoid contamination. The DNA isolation and PCR step were performed in two different laboratories on two separate occasions. All experiments included parallel mock nucleic acid isolations and PCR water negative controls. Positive controls included DNA from serum and PBMC of a serum HBsAg-positive patient with chronic hepatitis B (CHB) or recombinant HBV DNA [31]. The PCR detection sensitivities were <10-virus genome equivalents (vge) per ml or <10 vge per μg total DNA for HBV DNA and <10^2 copies per μg for HBV-cccDNA.

2.3. Clonal sequence analysis of HBV polymerase/surface gene overlapping region

Amplified HBV P gene fragments from PBMC from 8 patients were cloned using the TOPO TA system (Invitrogen, Burlington, Ontario, Canada). Cloned samples were sequenced bidirectionally by universal priming using 3730 XL sequencing (Applied Biosystems, Foster City, CA). For all clones, amino acids 61–250 of the P/RT region and amino acids 100–160 of the S protein antigenic determinant region were analyzed. Cloning and sequencing was performed in two laboratories. Sequences were translated for S and P protein sequence and aligned with clonal sequences of the same genotype to determine putative non-genotypic substitutions. The Genbank reference sequences for Genotype C were AB033551, AF182804, AJ748098, AY217376, and for Genotype D were AY721606, DQ315776, AJ132335, AJ131956. Sequence analysis of the HBV P gene and the overlapping HBV S gene sequences was done with Sequencher software V4.7 (Gene Codes Co., Ann Arbor, MI) and using the phylogenetic tools of Lasergene V10 software (DNASTAR, Madison, WI) and MEGA 5.

3. Results

3.1. Clinical features of patients with OBI and HIV-1 co-infection (Table 2)

We found that 42% (19/45) of anti-HBc reactive, HIV-positive patients tested were positive for HBV DNA. In these 19 patients, the median age was 35 (range 27–60), 95% (18/19) male and 95% (18/19) Caucasian. Most were receiving ART for a minimum of 6 months in 1996–1997 and were on ART in 2004–2007. 61% (11/18, 1 unknown) of patients were on anti-HBV agents. Most tested were on Lamivudine (LMV), and 1 patient was on Tenofovir (TDF) and Emitricitabine (FTC) during follow-up. Most OBI/HIV-1-positive patients had positive anti-HBs (87%, 14/16), although titers were not recorded (>10 mIU/ml denoted as protective immunity). The median $CD4^+$ T cell count was 203/mm^3 (23–762; $n = 19$) initially and 416 (189–701, $n = 8$) at last follow-up. In 14/19 with alanine aminotransferase (ALT) testing, the median ALT was 26 (range 12–452. Overall, 36% (7/19) had co-morbid liver disease due to either HCV co-infection ($n = 3$), non-alcoholic fatty liver disease (NAFLD; $n = 3$) and alcohol abuse ($n = 1$).