Drug resistance mutations and viral load in human immunodeficiency virus type 2 and dual HIV-1/HIV-2 infected patients in Ghana

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Abstract

Antiretroviral therapy (ART) and drug resistance studies worldwide have focused almost exclusively on human immunodeficiency virus type 1 (HIV-1). As a result, there is limited information on ART and drug resistance in HIV-2 patients. In Ghana, the HIV epidemic is characterized by the domination of HIV-1, with cocirculating HIV-2. We, therefore, sought to determine viral load and drug resistance mutations in HIV-2 patients to inform the clinical management of such individuals in Ghana.

We used purposive sampling to collect blood from 16 consented patients, confirmed as HIV-2 or HIV-1/2 dual infections by serology. A 2-step real-time RT-PCR assay was used to determine plasma HIV-2 RNA viral loads. For drug resistance testing, nucleic acids were extracted from plasma and peripheral blood mononuclear cells. The reverse transcriptase and protease genes of HIV-2 were amplified, sequenced and analyzed for drug resistance mutations and HIV-2 group.

HIV-2 viral load was detected in 9 of 16 patients. Six of these had quantifiable viral loads (range: 2.62–5.45 log IU/mL) while 3 had viral loads below the limit of quantification. Sequences were generated from 7 out of 16 samples. Five of these were classified as HIV-2 group B and 2 as HIV-2 group A. HIV-2 drug resistance mutations (M184V, K65R, Y115F) were identified in 1 patient.

This study is the first to report HIV-2 viral load and drug resistance mutations in HIV-2 strains from Ghana. The results indicate the need for continuous monitoring of drug resistance among HIV-2-infected patients to improve their clinical management.

Abbreviations: 3TC = lamivudine, ABC = abacavir, ABI = applied biosystems, AIDS = acquired immunodeficiency syndrome, ART = antiretroviral therapy, AZT = zidovudine, Bp = base pair, CD4 = cluster of differentiation 4, CPN = certified protocol number, DNA = deoxyribonucleic acid, dNTPs = Deoxyribonucleotide triphosphate, EFV = efavirenz, HIV = human immunodeficiency virus, HIV-2 = human immunodeficiency virus type 2, IRB = Institutional Review Board, IU = international unit, LLOQ = lower limit of quantification, MMIR = Noguchi Memorial Institute for Medical Research, NNRTIs = non-nucleoside reverse transcriptase inhibitors, NRTIs = nucleoside reverse transcriptase inhibitors, PBMC = peripheral blood mononuclear cells, PCR = polymerase chain reaction, PR = protease inhibitor, RT = reverse transcriptase, TDF = tenofovir, UNAIDS = Joint United Nations Programme on HIV/AIDS.

Keywords: antiretroviral therapy, drug resistance, HIV-2, mutation, viral load
1. Introduction

It is estimated that 36.7 million people worldwide are living with the human immunodeficiency virus (HIV). Sub-Saharan Africa is most affected, with 25.8 million people living with HIV in 2015. (HIV type 1 (HIV-1) and type 2 (HIV-2) infections have been described with HIV-1 responsible for over 90% of the global acquired immunodeficiency syndrome (AIDS) pandemic.) Two million people are known to be infected with HIV-2, most of whom live in West Africa. HIV-2 infection has spread to other countries in Europe, Asia, and North America with a high prevalence (5.4%) reported in Portugal. HIV-1 and HIV-2 have similar routes of transmission, however, HIV-2 is associated with lower viral loads resulting in reduced transmission and slower disease progression.

The HIV epidemic in Ghana is characterized by cocirculation of both viruses with HIV-1 being predominant. In view of this, the national antiretroviral therapy (ART) guidelines for the management of HIV/AIDS patients make provisions specifically for persons with HIV-2 and HIV-1/2 dual infections. For such individuals, the guidelines recommend a protease inhibitor (PI)-based regimen instead of those based on non-nucleoside reverse transcriptase inhibitor (NNRTI). In 2017, the estimated adult national HIV prevalence in Ghana was 1.67% with 99% HIV-1, 0.2% HIV-2, and 0.8% HIV-1/2 dual infections. The emergence of drug-resistant strains during treatment is a major obstacle to the success of any ART program. Both HIV-1 and HIV-2 are known to develop resistance against antiretroviral drugs. The development of drug resistance in HIV-2 infections globally has been examined in several studies, however, information is scarce on HIV-2 drug resistance. Previous studies in Ghana have documented drug resistance mutations in treatment-naive or experienced HIV-1 patients. However, there is little drug resistance data on HIV-2 infected patients in Ghana, probably due to the lack of commercially available HIV-2 viral load assays and optimized HIV-2 genotyping protocols. This study therefore optimized available protocols and applied it to investigate viral loads and drug resistance mutations in HIV-2 and dual HIV-1/HIV-2 infected patients from Ghana.

2. Methods

2.1. Study design and population

This was a cross-sectional study that recruited patients from ART care centers at the Eastern Regional Hospital, Korlebu Teaching Hospital, Accra from November 2014 to June 2015. The participants were limited to Ghanaian men and women aged 18 years and older. There were 2 cohorts in this study; an ART-naive group and an ART-experienced group. Patients eligible for the study included patients who were seropositive for HIV-2 or dual HIV-1/2. To be able to identify such patients, we searched for patients who were diagnosed as HIV-2 or dual HIV-1/2 from the hospital folders. Pusposive sampling was therefore used to collect blood from 27 consented patients who were seropositive for HIV-2 or dual HIV-1/2 using First Response HIV-1/2-0 card test (Premier Medical Corporation Ltd, India) performed at the hospitals. The status of these patients was confirmed as HIV-2 or dual HIV-1/2 using the innogenetics line immuno assay HIV-1/II score assay (Fujirebio Europe N. V, Belgium). After confirmatory testing, 16 patients; 10 ART-naive and 6 ART-experienced, who agreed to take part in the study were enrolled after written informed consent was sought. Sample size was determined based on the national prevalence of HIV-2 (0.8%) in Ghana for 2014 using a f value of 1.96 at 95% confidence level and 0.05 confidence interval. Clinical histories were retrieved from hospital folders of study patients. The study was conducted in accordance with procedures approved by the Institutional Review Board of the Noguchi Memorial Institute for Medical Research (NMIMR-IRB CPN 063/14-15) and the Ethical and Protocol Review Committee of the University of Ghana Medical School (MS-Et/M.4-P4.3/2014-2015).

2.2. Whole blood processing into plasma and peripheral blood mononuclear cells

Blood was processed into plasma and peripheral blood mononuclear cells (PBMC) using a sucrose-gradient based protocol with Histopaque 1077 (Sigma Aldrich Company; Darmstadt, Germany). Plasma was stored at −80°C while PBMCs were stored in freezing medium, consisting of 1% dimethyl sulfoxide (DMSO) (Sigma Aldrich, Germany) in fetal bovine serum (Sigma Aldrich), at −80°C until use.

2.3. Nucleic acid extraction and purification

Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were extracted from plasma and PBMCs, respectively using the QiAamp viral RNA mini kit and the DNAeasy Blood and Tissue kit (Qiagen, Germany) according to the manufacturer’s instructions.

2.4. HIV-2 viral load and genotyping

HIV-2 viral load was performed on 0.2 ml of plasma following a published protocol. Viral load testing was completed at the Wadsworth Center, New York State Department of Health according to the approved IRB protocol (#17-039). The HIV-2 viral load assay had a lower limit of detection of 32 international units (IU)/ml (1.50 log IU/ml) and a lower limit of quantification (LLQ) of 2.25 IU/ml (2.35 log IU/ml). For genotyping, reverse transcriptase (RT) and protease (PR) genes of HIV-2 were amplified separately using specific primers. A nested polymerase chain reaction (PCR) for the RT gene from PBMC portions was carried out to amplify a genomic region of 1050 base pairs (bp) encoding the RT gene (Table 1). The reactions were carried out in a total volume of 25 μl containing 5 μl of DNA template, 12.5 μl of Supermix (Life Technologies, Invitrogen; Austin, TX) and 0.5 μl of 20 μM of primers (Table 1). The first round PCR conditions were: 94°C for 2 minutes, 40 cycles of 94°C for 30 seconds, 55°C for 1 minute and 72°C for 1 minute 30 seconds, and then an elongation step at 72°C for 7 minutes. A nested PCR was carried out using 5 μl of the first-round PCR product under the following cycle conditions: 94°C for 2 minutes, 40 cycles of 94°C for 30 seconds, 56°C for 1 minute and 72°C for 1 minute, and an extension at 72°C for 5 minutes. The entire coding region for the PR gene (297 bp) was amplified by nested PCR using primers (Table 1). The cycling conditions for PR gene PCRs were the same as that for RT gene. The RT and PR genes in plasma were amplified by nested PCR using QiAgen OneStep RT-PCR Kit (Qiagen, Germany) for the first round in a total reaction volume of 25 μl containing 5 μl of RNA, 5 μl of 5× buffer, 0.75 μl of 20 μM of primers, 1 μl of...
enzyme mix and dNTPs followed by nested PCR with AmpliTaq Gold master mix kit (Applied Biosystems, USA) in 25 μl reaction volume containing 12.5 μl AmpliTaq Gold, 0.5 μl of 20 μM of primers (Table 1). The first round PCR had reverse transcription at 50°C for 30 minutes, and enzyme degradation at 95°C for 15 minutes followed by 40 amplification cycles (94°C for 30 seconds, 55°C for 1 minute, 72°C for 1 minute 30 seconds) and then an extension at 72°C for 2 minutes. The second round PCR conditions were: 94°C for 2 minutes, followed by 40 amplification cycles (94°C for 30 seconds, 56°C for 1 minute and 72°C for 1 minute) and extension at 72°C for 5 minutes.

The PCR products for both PBMC and plasma were visualized on 1.5% agarose gel under ultraviolet trans-illumination using the KODAK Gel Logic 100 Imaging system (Eastman Kodak Company; Rochester, New York)

2.5. DNA sequencing

Nested PCR products were purified using QIAquick PCR purification system (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Cycle sequencing was performed using the Big Dye terminator v3.1 Cycle Sequencing kit (Applied Biosystems). The DNA fragments were sequenced on both strands with sense primers RT3 and RT5-HIV2 and antisense primers RT4 and RT6-HIV2 for the RT region and primers PR3 and PR2 for the PR region (Table 1). A 10 μl reaction mixture, consisting of 2 μl each of 5X Big Dye Sequencing Buffer, Big Dye Terminator Mix, 2 μM primer, nuclease-free water and purified PCR product, was taken through 94°C for 2 minutes, 25 cycles of 94°C for 30 seconds, 50°C for 15 seconds, and 60°C for 4 minutes. Cycle-sequenced products were purified using Agencourt CleanSeq kit (Agencourt Bioscience Corporation; Beverly, MA) according to manufacturer’s protocol and loaded onto an ABI 3130xl genetic analyzer (Applied Biosystems) to read out sequences.

2.6. Sequence data analysis

Sequences were edited using the Sequencher software v4.0 (Gene Codes Corporation; Ann Arbor, MI) and aligned in BioEdit.[39] The Fasta-formatted sequences were then analyzed in the HIV basic local alignment search tool programme (http://www.hiv.lanl.gov) for identification of groups. Nucleotide sequences were aligned to the consensus group A HIV-2 ROD and group B HIV-2 EHO. HIV-2 group and drug resistance mutations were obtained by submitting the sequences to an online tool used for HIV-2 drug-resistance interpretation (HIV-2 EUv2).[40] Sequences were analyzed for the presence of mutations associated with nucleoside reverse transcriptase inhibitor (NRTI), NNRTI), or PI resistance and were categorized as susceptible, intermediate resistance, or resistant.

3. Results

3.1. Demographic and clinical characteristics

Sixteen HIV-2 and HIV-1/2 dual-infected patients from 2 hospitals in Southern Ghana were enrolled into the study from December 2014 to May 2015. Eleven of these patients were HIV-2 infected and 5 were HIV-1/2 dual infected. Ten were ART-naive and 6 were ART-experienced. Demographic, clinical and viral load data of study patients are shown in Table 2. Four of the ART-experienced patients were infected with HIV-2 and the remaining 2 were infected with HIV-1/2. Treatment options differed in the 2 ART centers for both HIV-2 and HIV-1/2 patients. The details of the composition of individual regimen are shown in Figure 1.

3.2. HIV-2 viral load and CD4 cell counts

HIV-2 RNA was detected in the plasma of 9 out of the 16 study patients; 7 were ART-naive and 2 were on treatment. Three had detectable RNA below the LLOQ and 6 had quantifiable viral loads ranging from 2.62 to 5.45 log IU/ml (Table 2). The highest viral load (5.45 log IU/ml) was recorded in an ART-naive patient; the highest viral load in an ART-experienced patient was 4.62 log IU/ml.

Generally, cluster of differentiation 4 (CD4) cell counts ranged from 11 to 916 cells/μl. The least (11 cells/μl) and highest (916 cells/μl) CD4 cell count were recorded in the ART-naive group (Table 2). Patients CD4 cell count taken at baseline and enrollment are shown in Figure 1.
3.3. PCR and sequencing of the RT and PR genes from plasma and PBMC

The RT and PR genes were successfully amplified in 5 and 4 samples, respectively. For the RT gene, 4 of the samples amplified were from PBMC and 1 was from plasma. All 5 of these samples were successfully sequenced. Included in this group were sequences obtained from both plasma and PBMC of 1 HIV-2 ART-naïve patient. For the PR gene, 2 were amplified from PBMC and the other 2 from plasma, all from ART-naïve patients. Sequences were only obtained from the PBMC amplicon (Table 3).

3.4. HIV-2 grouping and drug resistance mutation analysis

Five sequences from the RT region were identified as HIV-2 group B, including those obtained from the paired plasma and PBMC portions of the blood. Two PR sequences were both HIV-2 group A. One patient (KBAN-14-01) had RT and PR sequences that were classified in different groups; RT as group B and PR as group A (Table 3). There were no drug resistance-conferring mutations in any of the sequences derived from ART-naïve patients. In the ART-experienced patient, 3 major drug resistance mutations (M184V, K65R, and Y115F) were found in both the PBMC and plasma sequences. These mutations were classified as NNRTI resistant (Table 3).
4. Discussion

Attempts to curb the HIV menace globally are almost exclusively focused on HIV-1. The need for HIV-2 viral load and genotyping protocols to monitor HIV-2 infected patients in regions where HIV-1 and HIV-2 cocirculate is imperative. In this study, we estimated HIV-2 viral loads and detected drug resistance mutations by sequencing the RT and PR genes in HIV-2 strains from HIV-2 and HIV-1/2 dual-infected patients in Ghana.

HIV-2 strains in our study were found to belong to groups A and B, with group B predominating thus supporting previous reports on the HIV-2 epidemic. In addition, we also identified 1 intergroup (A/B) recombinant HIV-2 strain. This strain was classified as group A in the PR gene and group B in the RT gene. Similar recombinant HIV-2 strains had been documented previously in Cote d'Ivoire and Cameroon. Our study identified matching HIV-2 drug resistance mutations in plasma and PBMC from the same patient. A previous study also documented matching drug resistance mutations in paired PBMC and plasma for HIV-1.

This finding is in contrast to observations in previous reports on the ART-naive patients. This ART-experienced patient indicating potential high risk of HIV-2 transmission. The lack of an HIV-2 viral load assay to monitor the progress of patients on therapy at the time of sampling is a limitation to this study. Generally, the lack of commercially available assay for HIV-2 viral load is a major limitation in monitoring HIV-2 infected patients on therapy.

Our study did not find any drug resistance mutations in ART-naive patients, implying the absence of transmitted drug resistance. This finding is in contrast to observations in previous studies in Burkina Faso, Guinea Bissau, Belgium, and Luxembourg, where transmitted drug-resistant HIV-2 strains were reported in ART-naive patients.

The presence of NRTI mutations M184V, K65R, and Y115F found in an ART-experienced person in this study confirms the emergence of ART resistance to the currently available antiretroviral drugs under drug pressure.

This is the first report on HIV-2 viral load and drug resistance mutations in patients infected with either HIV-2 or HIV-1/2 in Ghana. The study showed evidence of major mutations in HIV-2 strains of patients receiving HIV-1 targeted ART. This underscores the need for continuous monitoring of HIV-2 resistance mutations to improve clinical management of HIV-2 patients.

Author contributions

Funding acquisition: Christopher Z. Abana, William K. Ampofo.
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References


