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Undiagnosed Chronic Hepatitis B Infection and HIV Type 1 Drug Resistance Profile in AIDS Patients Receiving Tenofovir-containing Antiretroviral Regimens: Considerations for Monitoring Resistant HIV Variants during Treatment

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JNT, NO, ESH and CK designed the study. Authors MK, NO, CK and SCT supervised clinical work and revised manuscript. Authors DT, PA, VMA, SS and JNT carried out HIV-1 sequencing, HIV-1 viral load and data analyses. Authors CK, VMA and JNT wrote the first manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: During antiretroviral therapy (ART), resistant HIV-1 variants may be selected resulting in clinical failure. Co-infection rate of Hepatitis B virus in AIDS patients in Cameroon is 11.8%, but few studies have described the profile of resistance associated mutations (RAMs) in HIV in these patients on first-line ART containing antiviral drugs against hepatitis B infection. Thus, we aimed to determine the rate of HBV infection and profile of HIV-1 RAMs in AIDS patients on ART.

Methods: A cross-sectional study was carried out from November 2013 to April 2014 in two AIDS Treatment Centres in Yaoundé, Cameroon. Ninety-six adult AIDS patients on tenofovir-containing regimens were tested for HBsAg by serology. Direct sequencing of amplicons generated for the HIV-1 protease/reverse transcriptase region, was performed for 21 HIV/HBV co-infected patients. The Stanford HIV Drug Resistance Database tool was used to predict RAMs, and genotyping was determined by phylogeny.

Results: Overall, 21 patients were co-infected with HBV (21.9%). Eighteen (85.7%) of these were infected with recombinant variants, and HIV-1 CRF02_AG was most frequently identified (52.9%), and 15 (71.4%) harboured at least one RAM. Prediction of resistance to NNRTIs was reported in 14 (66.7%), and among 13 (61.9%) to NRTIs, 2 (9.5%) to PIs, and 8 (38.1%) carried Thymidine Analog Mutations (TAMs) of M184VI (61.9%), V75I (23.8%), T215F (23.8%), M41L (19.0%) and K70R (19.0%).

Conclusion: Rate of HBV infection and frequency of HIV-1 RAMs among AIDS patients on ART is high. The observed NNRTI RAMs may affect the susceptibility of efavirenz. Thus, the need to monitor HIV-1 drug resistance profile during treatment with unsuppressed viral load.

Keywords: HIV; HBV; drug resistance; mutations; variant; co-infection.

1. INTRODUCTION

Human immunodeficiency virus (HIV) and Hepatitis B virus (HBV) infections are among the ten leading causes of infectious disease deaths worldwide [1,2]. Both viruses have similar routes of transmission, show a broad genetic diversity and co-infection poses an increased risk of life-threatening complications [3]. While the tide of HIV infection is increasing in many countries in sub-Saharan Africa, Cameroon recorded a rate of 3.8% in the general population in 2016 [4]. Several studies have reported the prevalence of hepatitis B surface antigen (HBsAg) in Cameroon ranging between 5.4% and 19.9% over the past three decades [1,2,5,6,7] and a rate of overt HBV infection of 11.8% was reported in HIV-infected individuals in 2016 [8]. Meanwhile, some studies showed no impact on the progression to AIDS or on viral and immunological responses to ART in both HIV mono- and co-infected patients with HBV [9,10]. However, liver toxicity is more frequent among subjects with chronic HBV and HIV co-infection than in HIV mono-infected patients [11].

HIV genotypic resistance testing is not recommended at initiation of antiretroviral therapy (ART) in Cameroon [12]. However, the use of ART to improve the quality of life of these patients may result to the emergence of drug-resistant variants [13]. The nucleoside analogues tenofovir (TDF), emtricitabine (FTC) and lamivudine (3TC) have activity on both HIV and HBV replication, by inhibiting reverse transcription (RT) and DNA polymerase activity, respectively [14].

The 2015 National Guidelines on the Prevention and Management of HIV/AIDS for Cameroon recommend the use of TDF and 3TC (or FTC) as the NRTI backbone for the management of HIV/HBV co-infected patients [15]. The rate of transmitted drug resistance (TDR) of 8% has been reported in Cameroon [16,17,18] hence the central role of HIV genotypic resistance testing in the clinical management of patients on ART. Given the limited data on the clinical outcome of people co-infected with HIV and HBV and on ART in Cameroon, we, therefore, sought to determine the rate of co-infection, and the profile of HIV-1 resistance to ARVs in these patients in routine hospital care in Yaoundé.

2. METHODS

2.1 Study Design

A cross-sectional study was carried out from November 2013 to April 2014 in two AIDS Treatment Centres in Yaoundé. A questionnaire was administered to 96 AIDS patients in routine

care, who gave informed consent to obtain demographic data, pertinent HIV-related clinical observations, ART history and HIV follow-up para-clinical results.

2.2 Laboratory Procedures

Plasma specimens from 96 AIDS patients were tested for HBsAg by ELISA and HIV-1 RNA quantification by realtime PCR. Direct sequencing (of 21 samples of HIV/HBV co-infected patients only) was done after reverse transcription and semi-nested PCR (RT-PCR) of the HIV-1 protease and reverse transcriptase region using the DNA Analyzer 3130XL (Applied Biosystems) to obtain an 1197 base pair sequence as previously described [19]. The sequences were assembled and edited using Seqscape version 2.5., aligned in BioEdit version 5.0.6 using CLUSTAL W and compared with reference sequences for the major HIV-1 subtypes and Circular Recombinant Forms (CRFs), available in Los Alamos National Library (LANL) database. Prediction of resistance of HIV-1 to ARV drugs was analyzed using the Stanford University HIV Drug Resistance Database tool.

3. RESULTS

3.1 Demographic and Clinical Characteristics of the Study Population

Ninety-six AIDS patients on tenofovir-containing first-line antiretroviral therapy, who were unaware of their HBV infection status were involved in this study. The mean age of the 21 participants co-infected with HIV and HBV was 37.3±10.8 years, and females were predominant (66.7%). CDC AIDS Clinical Classification varied from Stage A to Stage C with the majority of patients [12/21(57.1%)] being at Stage B.

3.2 Antiretroviral Treatment and Adherence

The duration of ART of the 21 patients co-infected with HIV/HBV ranged from 9 months to 44 months with an average duration of 24.71± 9.4 months. Twenty of the co-infected patients were on 2 NRTIs and 1 NNRTI following the National AIDS Treatment Guidelines for Cameroon while one patient was on a second line regimen of 2 NRTIs and one protease inhibitor. Nineteen patients (90.5%) had received ART for more than 12 months, while 13 (61.9%), 4 (19.0%), 3 (14.3%) and 1 (4.8%) had received 1, 2, 3 and 4 different treatment regimens, respectively, since the initiation of ART (Table 1).

3.3 HIV-1 Genetic Variants and Drug Resistance-associated Mutations

3.3.1 Frequency of HIV-1 genetic variants

The average plasma HIV-1 RNA level for the 21 HIV/HBV co-infected participants was 36363.9 (SD: 66096) copies/ml (4.5 Log10 copies/ml). Eighteen participants (85.7%) were infected with recombinant variants, while the remaining were non-recombinants (Subtypes D, J and K). HIV-1 CRF02_AG was most frequently identified (52.4%) and CRF01_AE at a rate of 9.5% (Table 2).

Table 1. Clinical characteristics of study population (N = 21)

Characteristics	Number (%)
Classes of antiretroviral received	
Nucleos(t)ide reverse transcriptase inhibitors (NRTI)	21 (100)
Non-nucleoside reverse transcriptase inhibitors (NNRTI)	21 (100)
Protease inhibitor (PI)	1 (4.8)
Duration of antiretroviral therapy (months)	
< 12	2 (9.5)
12 to 24	9 (42.9)
24 to 36	7 (33.3)
≥ 36	3 (14.3)
Antiretroviral regimen switch	
None	13 (61.9)
Once	4 (19.0)
Twice	3 (14.3)
Thrice	1 (4.8)

Table 2. HIV-1 genetic variants

HIV-1 genetic variant	Number (%)
CRF02_AG	11 (52.4)
CRF01_AE	2 (9.5)
CRF02_AG/F	3 (14.3)
CRF01_AE/02_AG	1 (4.8)
K/D	1 (4.8)
D	1 (4.8)
J	1 (4.8)
K	1 (4.8)

3.3.2 Rates of HIV-1 drug resistance-associated mutations in protease-reverse transcriptase region

Of the 21 HIV/HBV co-infected participants, 14 (66.67%) were infected with an HIV-1 variant with at least one mutation associated with resistance to NRTI or NNRTI class in their treatment regimen. Eighty-one percent of the participants (17/21) had plasma viral HIV RNA load ≥ 1000 copies/ml.

3.3.2.1 Rates of drug resistance-associated mutations to nucleus (t)ide reverse transcriptase inhibitors (NRTIs)

The most frequently detected RAM to NRTI included M184V/I identified in 13 patients (61.9%), T215F and V75I found in 5 (23.8%), respectively, and M41L and K70R found in 4 (19.0%), respectively (Table 3). Among these patients, 8 (38.09%) had Thymidine Analog Mutations (TAMs), which included M184V/I, M41L, T215Y/F, L210W, D67N, K219E/Q, and K70R (Table 3).

3.3.2.2 Rates of drug resistance-associated mutations to non-nucleoside reverse transcriptase inhibitors (NNRTIs)

Thirteen patients (61.9%) harboured viruses that could modulate susceptibility of NNRTIs. There were eight major RAMs to NNRTIs, and the most frequent was K103N/S (40.9%), followed by Y181C/I/V (28.6%) (Table 3).

3.3.2.3 Rates of drug resistance-associated mutations to protease inhibitors (PIs)

Two (9.5%) participants had developed resistance-associated mutations to PI (including M46I/L, I84V, V82A/T/F/S and L76V) although only one was on second line protease inhibitor-containing therapy.

Table 3. Frequency of resistance-associated mutations of HIV-1 protease and reverse transcriptase region

Mutation code	Number (%)
Confer resistance to NRTI	
T69D	2 (9.5)
M41L	4 (19)
D67N	3 (14.3)
K70R	4 (19)
L210W	2 (9.5)
T215F	5 (23.8)
K219Q/E	3 (14.3)
K65R	1 (4.8)
L74V	1 (4.8)
V75I	5 (23.8)
M184V/I	13 (61.9)
Q151M	1 (4.8)
Confer resistance to NNRTI	
K103N/S	9 (40.9)
Y181C/I/V	6 (28.6)
Y188L/C/H	2 (9.5)
G190A/S/E	2 (9.5)
M230L	1 (4.8)
K101P/E	3 (14.3)
V106A/M	1 (4.8)
V179F	1 (4.8)
Confer resistance to PI	
M46I/L	2 (9.5)
V82A/T/F/S	1 (4.8)
I84V	2 (9.5)
L76V	1 (4.8)

4. DISCUSSION

HBsAg seroprevalence is more than double that of HIV in the general population in Cameroon, and > 10% of co-infection of these viruses has been reported. Although HBV screening is recommended in routine care of AIDS patients, HBV acquisition and reactivation can go unnoticed and therefore make management of the patients challenging. Therefore, knowledge of HBV status and HIV drug resistance pattern in HIV/HBV co-infected patients, would guide the choice of the treatment regimen and minimize the emergence of drug resistance-associated mutations that may comprise treatment options. We, therefore, sought to investigate the rate of HBV infection among AIDS patients on ART regimens containing drugs

with activity against HBV infection, in two major AIDS Treatment Centres in Yaounde, Cameroon.

We report a majority (57.1%) of the HIV/HBV co-infected patients at CDC AIDS Clinical stage B, similar to the findings of Kouanfack et al. of 2012 in Yaoundé [20], and Aghokeng et al. of 2013 [21]. However, the rate of 21.9% of HIV/HBV co-infection found in this study is higher than the reports of Chambal et al. in 2017 of 9.1% [22] and of 9.8% by Fouelifack in 2012 [2], 8.99% by Molu et al. in 2018 [23] and 11.8% reported by Salpini et al. in 2016 [8]. This could be explained by the small sample size of our study. Notwithstanding, a broad diversity of HIV-1 strains with a predominance of CRF02_AG (52.4%) was recorded. This is consistent with previous studies that have demonstrated that CRF02_AG is the most prevalent HIV-1 variant in Cameroon since the late 1990s [16,19,21]. The broad genetic diversity of HIV-1 implies that there is an increased risk of emergence of more complex recombinants with the propensity to emerge into antiretroviral drug-resistant variants in Cameroon. Similar studies showed that HIV-1 recombinants predominate the AIDS epidemic in Cameroon with over 80% of circulating and unique recombinant forms (CRFs and URFs) [16,19].

Although we could not detect when HBV infection occurred, we, however, reported that seventeen of the twenty-one patients (81%) could not maintain a sustained virologic response after an average duration on ART of 24.7+9.4 months. On the other hand, among HIV-mono-infected patients, Aghokeng et al. [21] reported 17.1% failing treatment after a median of 36 months of ART. HBV infection might reduce the response of HIV to ART, leading to HIV virologic failure, as reported in China in 2012 [24]. Another study in Nigeria reported by Idoko et al. in 2009 [25] showed a rate of 70% of HIV/HBV co-infection, in patients with an HIV-1 viral load < 400 copies/ml after a median treatment duration of 24 months. This could be explained by the fact that Aghokeng et al. and Idoko et al. described prospective studies with a better follow-up of patients and compliance to treatment, whereas most of our study subjects were followed-up in routine care services on irregular visits of their own schedule.

The emergence of drug-resistant variants of HIV-1 or HBV is evident with or without antiviral drugs. About two-thirds of our study participants were infected with an HIV-1 variant with at least one major mutation that can confer resistance to NRTIs or NNRTIs. Studies on HIV mono-infected patients reported by Ceccarelli et al. and HIV/HBV co-infected patients described by Laurent et al. [16,26] in Yaoundé, Sungkanuparph et al in Thailand [27] and Hosseinipour et al. [28] in six sub-Saharan African countries (including Cameroon), found similar rates of M184V/I in HIV/AIDS patients failing first-line ART. The M184V/I mutation is known to confer a high level of resistance to lamivudine (3TC) and emtricitabine (FTC), both known for their dual activity against HIV and HBV infection, but may increase susceptibility to tenofovir. We found that patients with greater than 1000 copies/ml of plasma RNA HIV-1 viral load, accumulated RAMs and TAMs, and RAMs that could modulate NRTI susceptibility including tenofovir and lamivudine. This could be explained by their low genetic barrier in addition to the high rate of their use in Cameroon.

Similarly, high rates of K103N/S and Y181C/I/V had been reported by Aghokeng et al. Ceccarelli et al. and Laurent et al. in Cameroon [16,20,26]. This could also be explained by the frequent use of NNRTIs and their low genetic barrier. The mutation K103N/S affects nevirapine and efavirenz susceptibility. It should be noted that the extensive use of nevirapine as monotherapy a few years ago in pregnant women for the prevention of mother-to-child transmission of HIV, could have contributed to the development of acquired resistance to this drug. Burda et al. [29] reported a similar mutation rate of K103N/S (46%).

The Cameroon National Guidelines for HIV/AIDS Management recommend tenofovir and lamivudine (or emtricitabine) for hepatitis B infected patients irrespective of mono-infection or HIV/HBV co-infection status. Hence, the use of these drugs in HIV mono-infected patients could compromise future treatment decisions if these patients become co-infected with HBV. Other common mutations T215F (23.8%), M41L (19.0%), K70R (19.0%), and D67N (14.3%) were found in higher frequencies compared to results by Kouanfack et al. of 2009 [30]. The low rate of RAMs in the latter study could be explained by the close monitoring of these patients according to “WHO Public Health Approach”. The high rate of TAMs found in our study is similar to other studies [27,31]. The main TAMs, M41L, D67N, K70R, T215Y/F, D67N and M41N detected are known to cause cross-resistance to tenofovir,

which is active on both HIV and HBV. Germanaud et al. [32] however low levels of TAMs were found in ARV-treated Malian children and associated it to the fact that combination of 3TC and AZT or d4T could minimize the appearance of TAMs.

The very low proportion of PI resistance reported in our study is similar to findings reported by Ceccarelli et al. [16]. This could be explained by the rarity of its use in Cameroon, recommended only for second-line treatment and also its high genetic barrier. The only patient (4.8%) with PI mutations had never received a PI. This could be a transmitted RAM, but baseline genotypic resistance testing was not done before treatment initiation for more clarity in this case. Other surveys in Yaoundé however, showed a low level (<5%) of transmitted PI-resistance mutations [29] similar to our findings.

5. CONCLUSIONS

From our findings and those of other investigators, we highlight the importance of screening for HBV infection in HIV/AIDS patients to determine the right treatment options for HIV/HBV co-infected patients. Several major RAMs were identified in the reverse transcriptase region which may have an overall impact on the response to first-line antiretroviral therapy of HIV/HBV co-infected patients. However, the limited number of participants in our study and lack of data from HIV-mono-infected individuals, make it difficult to draw tangible conclusions. Notwithstanding, these data point to challenges in the management of patients co-infected with HIV and HBV with unknown HBV infection status, but who are being treated with antiviral drugs with activity against both HIV-1 and HBV. These data are useful for designing other studies to understand the clinical and virologic outcomes of HIV/HBV co-infected, HIV-mono-infected and HBV-mono-infected patients. Genotypic HIV drug resistance testing should be promoted during treatment of HIV/HBV co-infected patients in Cameroon.

CONSENT

All authors declare that 'written informed consent' was obtained from the patient for publication of this case report.

ETHICAL APPROVAL

All authors at this moment declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed by the ethical standards laid down in the 1964 declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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