Virological Response and HIV Drug Resistance
12 Months After Antiretroviral Therapy Initiation at 2 Clinics in Nigeria

Richard Ugbede,1 John Aberle-Grasse,2 Karidia Diallo,3 Orji Bassey,1 Tapdiyel Jelpe,1 Erin Rottinghaus,3
Aderemi Azeez,4 Raphael Akpan,1 Mukhtar Muhammad,1 Vedapuri Shanmugam,3 Satvinder Singh,1 and Chunfu Yang3

1CDC-Nigeria, Centers for Disease Control and Prevention, Abuja; 2Strategic Information and Epidemiology Branch and 3International Laboratory Branch, Division of Global HIV/AIDS, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia; and 4Ministry of Federal Health Nigeria, Abuja

This report describes a pilot study, conducted in Nigeria, of the World Health Organization protocol for monitoring human immunodeficiency virus (HIV) drug resistance (HIVDR) and associated program factors among patients receiving first-line antiretroviral therapy (ART). In 2008, 283 HIV-infected patients starting ART were consecutively enrolled at 2 ART clinics in Abuja. Twelve months after ART initiation, 62% were alive and on first-line ART, 3% had died, 1% had transferred out of the program, and 34% were lost to follow-up. Among patients on first-line ART at 12 months, 90% had viral suppression. However, in view of the high loss to follow-up rate (34%), strategies for patient retention and tracking are critical to minimize possible HIVDR and optimize treatment outcomes.

Nigeria is the most populous country in Africa (156 million persons in 2009) with an estimated human immunodeficiency virus (HIV) prevalence of 3.6% [1]. Nigeria ranks second among the 3 countries with the highest HIV burdens worldwide: South Africa, Nigeria, and India [2]. In Nigeria, approximately 3.3 million people are HIV infected, of whom 1.4 million require antiretroviral therapy (ART) per 2010 World Health Organization (WHO) adult and adolescent HIV treatment guidelines [3].

Since 2001, the Nigerian government, in collaboration with the Global Fund to Fight AIDS, Tuberculosis, and Malaria and the US President’s Emergency Plan for AIDS Relief (PEPFAR), has scaled up its ART program to >300 clinics. By the end of 2009, >300 000 patients in Nigeria had started ART. In Nigeria, ART scale-up has been a significant achievement given the challenges with the country’s minimal health infrastructure; lack of trained personnel; complexity of antiretroviral drug (ARV) procurement, delivery, and storage; and inadequate laboratory capacity.

The national ART committee recently adapted the 2010 WHO adult and adolescent treatment guidelines to the Nigerian context, selecting the following standard first-line regimens: zidovudine (AZT)/tenofovir (TDF) plus lamivudine (3TC)/emtricitabine (FTC) plus nevirapine (NVP)/efavirenz (EFV). Currently in the public sector, the only available second-line option is lopinavir/ritonavir (LPV/r) with AZT/TDF plus 3TC/FTC. A switch to the second-line treatment may be indicated by clinical, immunologic, or virologic criteria. Because of limited options for treatment after first-line failure and the high cost of second-line therapy [4], high rates of retention on first-line ART—with associated high rates of virological suppression—coupled with low rates of loss to follow-up are critical to successful long-term treatment of HIV. Because of the error-prone nature of HIV replication, its high mutation rate, and the need for lifelong treatment, some HIV drug resistance (HIVDR) will inevitably occur among patients on ART even when
appropriate first-line drugs are provided and optimal adherence to therapy is supported [5].

The WHO has developed a global HIVDR prevention and assessment strategy that includes surveillance of acquired HIVDR in populations receiving ART [6, 7]. This report documents Nigeria’s pilot study of a WHO survey to assess acquired HIVDR at sentinel ART clinics [6]. The survey objectives were to assess rates of population-level viral load (VL) suppression and to characterize HIVDR and associated program factors among patients failing to achieve VL suppression 12 months after the start of first-line ART.

METHODS

We conducted our survey following the generic WHO protocol [6] to monitor acquired HIVDR and used both routinely collected program data and specimens from patients on ART. However, we modified the generic WHO survey inclusion criteria and excluded from our survey individuals with a history of previous triple ARV exposure. We selected 2 clinics in Abuja, a tertiary-level and a secondary-level facility, for convenience according to the minimum data required for the survey were routinely collected. The Institutional Review Board of the Nigeria Federal Ministry of Health and the US Centers for Disease Control and Prevention (CDC) approved the protocol.

Enrollment of Participants

A total of 283 consenting patients were consecutively enrolled at the 2 clinics between January and July 2008. Included were patients newly initiating ART (women who had received single-dose NVP for prevention of mother-to-child transmission of HIV during a pregnancy were eligible), age ≥18 years, and eligible for ART based on the national treatment guidelines (CD4 count, WHO clinical stage) [2]. At enrollment (T1; baseline) and the 12-month end point (T2), we abstracted clinical and demographic information from medical records and drew blood on which to perform CD4 count, HIV RNA quantification, and genotyping. T2 end points included: on ART 12 months (11–15 months) after initiation; lost to follow-up; stop ART; transfer out (documented transfer of care to another ART clinic); or death.

Data and Specimen Collection

At T1 and T2, a trained survey nurse abstracted relevant data from patients’ medical records. At T2, data were abstracted on facility-level information (eg, ARV drug supply continuity). Following routine practice, patients who missed 2 clinic appointments were actively traced by phone call or home visit to determine their status.

Laboratory Testing Methods

The CD4 count was performed at the clinics with CyFlow (ParTech). HIV RNA quantification and genotyping were performed at the CDC (Atlanta, Georgia), using Roche Ampli-cor HIV Monitor test, version 1.5 (Roche Diagnostics) for T1 specimens and NucliSENS EasyQ HIV version 1.1 RUO test (bioMérieux) for T2 specimens with detection limits of 400 copies/mL and 150 copies/mL of plasma, respectively. HIVDR genotyping was performed with a broadly sensitive genotyping assay [8] for all specimens at T1 and for those specimens with detectable VL (≥150 copies/mL) at T2. We analyzed HIVDR mutations using the Stanford HIVDR database [9].

Data Management and Analysis

We developed a database using Epi Info version 3.5.1. Proportions, 95% confidence intervals, and tests for significance were calculated using Epi Info. We classified each patient into 1 of 3 HIVDR outcome categories at T2: (1) HIVDR prevention: on first-line ART at 12 months and VL <1000 copies/mL; (2) possible HIVDR: on first-line ART at 12 months with VL ≥1000 copies/mL and no detected HIVDR, loss to follow-up, no record, or stopped treatment; (3) detected HIVDR: on first-line ART with VL ≥1000 copies/mL and ≥1 HIVDR mutations classified as causing low, intermediate, or high level of resistance according to the Stanford HIVDR database [6, 7, 9].

RESULTS

We enrolled 283 ART-naive patients eligible to start ART from the 2 clinics. Their baseline characteristics are summarized in Table 1. The median age was 34 years (interquartile range [IQR], 28–40 years); 62% were female; and 4.6% were pregnant among female enrollees at the time of ART initiation. The median CD4 count was 149 cells/µL (IQR, 73–205 cells/µL), and 72% of participants had a CD4 count ≥200 cells/µL. All patients were classified by WHO stage: 34% were stage I, 26% stage II, 37% stage III, and 1% stage IV. Among the 283 patients enrolled at T1, 272 had plasma specimens available for VL and genotyping. VL testing was performed on 270 and genotyping on 271 specimens successfully; specimens without results either could not be amplified or had insufficient volume for testing. The median VL was 85 351 copies/mL (IQR, 20 300–223 050). Genotyping results showed that 2.6% of specimens (7 of 271) from patients prior to ART initiation had low-, intermediate-, or high-level resistance to either nonnucleoside reverse transcriptase inhibitors (NNRTIs); eg, NVP, EFV, and etravirine (ETR)) or nucleoside reverse transcriptase inhibitors (NRTIs); eg, AZT, stavudine (d4T), and didanosine (DDI)].

Table 2 summarizes primary T2 end points. Among 283 patients enrolled at T1, 174 (61.5%) were alive and on first-line ART 12 months after initiation, 9 had died, 3 had transferred out, 97 were lost to follow-up or had missing records, and none had stopped ART or switched to second-line therapy. Of the 174 patients alive and on first-line ART at T2, 173 (99.4%) had VL
data and 120 (69.0%) had a CD4 count recorded at T2; the median increase was 168 cells/µL from T1 to T2.

At T2, the HIVDR prevention rate (VL suppression <1000 copies/mL) was 60.9% at clinic 1 and 53.6% at clinic 2. Possible HIVDR rates were 35.3% at clinic 1 and 39.9% at clinic 2. The HIVDR prevention rates were substantially lower than the WHO recommended target of 70% primarily because of high rates of loss to follow-up at both clinics [6]. Of those patients with VL data at T2, 90.0% (81 of 90) at clinic 1 and 89.2% (74 of 83) at clinic 2 had VL <1000 copies/mL, resulting in a combined viral suppression rate of 89.6% (155 of 173).

HIV isolates from 100% patients with VL <1000 copies/mL at T2 were genotyped (Supplementary Table 2). Overall, detected HIVDR was low: 3.8% at clinic 1 and 6.5% at clinic 2. Among the 8 isolates genotyped, 7 (88%) were successfully genotyped (Table 2 and Supplementary Table 2). Among these 7 isolates, 5 had mutants resistant to both NRTIs and NNRTIs, 1 had intermediate-level resistance to the protease inhibitor nelfinavir (NFV), and the remaining isolate was wild type. All 5 isolates with dual resistance to NRTIs and NNRTIs had high-level resistance to 3TC, FTC, and NVP. In addition, 1 isolate had intermediate-level resistance to TDF.

Overall, only 5 (31.3%) of the 16 isolates with NRTI and NNRTI resistance had thymidine analogue mutations (TAMs) resulting in low- or intermediate-level resistance to AZT and d4T. Among these 5 isolates, only 1 (6.3%), from a patient treated with the combination of TDF/FTC/NVP, had intermediate-level resistance to TDF. The most prevalent NRTI and NNRTI mutations were M184V (n = 15), K103N (n = 7), and Y181C (n = 8), respectively. Of the 7 patients treated with TDF-based regimens at clinic 2, 3 developed the K65R mutation, resulting in intermediate-level TDF resistance.

Whereas both clinic 1 and clinic 2 had 9 patients with VL <1000 copies/mL, the proportion of isolates with detected HIVDR at clinic 2 (6.5%) was greater than that at clinic 1 (3.8%).
Factors that may contribute to treatment outcomes and HIVDR emergence were similar between the 2 clinics, including on-time drug pickup, clinic attendance, and ARV drug supply continuity. Neither clinic had any days of drug stockout during the survey period (Supplementary Table 1). However, clinic 2 did have more patients lost to follow-up than clinic 1 (37.8% vs 30.7%, Table 2). Possible HIVDR at both sites was high, 35.3% at clinic 1 and 39.9% at clinic 2, largely because of high loss to follow-up rates. No significant association between virologic failure and on-time drug pickup was observed.

DISCUSSION

The availability and widespread use of ART in both high-income and resource-limited countries has substantially reduced AIDS mortality and morbidity [10, 11, 12]. In this survey, baseline genotypes showed that 2.6% (7 of 271) of the participants had HIVDR to NNRTIs or NRTIs before initiation of ART, although most resistance levels were either low or intermediate. Possible explanations are that some patients were infected with drug-resistant HIV [13] or were previously exposed to ARVs. Before the national ART program started in 2002 [1], many individuals who could afford ART were treated with nonstandard regimens, and some of these patients may have entered the government ART program but may not have volunteered previous ARV-exposure information.

Among 7 patients with resistance mutations to NRTIs or NNRTIs at T1, 2 patients (one with K103N and the other with M41L) suppressed viral replication to <1000 copies/mL. A third with K101E and E138A mutations selected for additional HIVDR, resulting in high-level resistance to NRTIs (3TC and FTC) and increased resistance to NNRTIs from low and intermediate at T1 to intermediate and high at T2 (Supplementary Table 2).

Results from T2 demonstrated HIVDR mutations conferring resistance to all ARVs in first-line regimens. The 3 isolates harboring the K65R mutation and resulting in intermediate-level resistance to TDF were from patients on TDF-based first-line regimens. K65R is selected by TDF, DDI, ABC, and possibly d4T usage and causes a variable loss of susceptibility, depending on the presence of TAMs [14, 15]. This has been described in populations with HIV subtype B infection [16]. More recently, the K65R mutation has been reported in patients infected with non–subtype B receiving non–TDF-based first-line ART [15, 17, 18]. These findings may have implications for the choice of second-line ART regimen in resource-limited settings, where TDF is often removed from use because of its relatively higher cost and resultant sustainability considerations [3].

Overall, patients started ART late, with only 28% having CD4 count ≥200 cells/μL. At the time of initiation, 39% had WHO stage III or IV disease. Late ART initiation is associated with high mortality [19, 20]. Although only 3% of participants were reported as having died during the follow-up period,
some loss to follow-up may have been due to unreported deaths.

Although 18 patients experience virologic failure (VL ≥1000 copies/mL) and 14 of them developed major HIVDR mutations to either NRTIs or NNRTIs or both at T2, none had been identified and switched to second-line therapy by the routine ART program–monitoring criteria in place at these 2 clinics. This survey supports findings from other studies that patients may continue on failing first-line regimens despite no change in clinical or immunological criteria, which may lead to additional selection of HIVDR, thus jeopardizing the efficacy of second-line ART [17, 21].

Site factors influencing selection of HIVDR at T2 were similar at both clinics. All patients were started on standard first-line ART and there was no interruption of drug-supply continuity at either clinic. Whereas the on-time clinic appointment attendance was greater than the 80% WHO target, the on-time drug pickup fell short of the WHO recommended target of >90% (Supplementary Table 1), suggesting that some patients failed to pick up their drugs on time, which could have resulted in missed doses and selection of drug-resistant HIV [22]. Notably, loss to follow-up rates were much higher than the WHO-recommended target (<20%) [6, 7] at both clinics (Supplementary Table 1). Patients lost to follow-up may include individuals who had died but were not reported, self-transferred to another facility, or stopped therapy. The active tracking of patients, although routine at both clinics, was challenging because of denial by some patients when contacted by phone or at home visits that they had been started on ART. Discussions with clinic staff revealed that tracking difficulties were associated with patient concerns regarding HIV-associated stigma. Additionally, erroneous contact information and lack of human and financial resources hampered patient tracking. This survey underscores the need for strengthened tracking mechanisms at these 2 clinics and possible investigation of loss to follow-up at ART clinics across Nigeria.

Several challenges were noted during the survey. (1) Human resources: With the staff already challenged by routine work, additional data abstraction affected staff commitment and job quality; the future success of HIVDR surveillance activities rests on clinic engagement and the integration of activities into the expectations of routine ART program functions. Additionally, frequent staff rotation led to the need for training of new staff and may have affected data quality. (2) Infrastructure: The lack of regular electricity supply and the frequent breakdown of CD4 count machines delayed enrollment and end-point specimen collection, and resulted in <50% of the patients having CD4 counts at T2 even though CD4 count testing is theoretically routinely practiced. Lack of ~80°C freezers for specimen storage at facilities necessitated frequent transportation of specimens to locations with appropriate freezers. (3) Poor coordination caused eligible patients to be missed for recruitment, which subsequently led to an extended enrollment period. Furthermore, poor routine documentation in patient medical records made data abstraction difficult.

**CONCLUSIONS**

This pilot survey demonstrated that a survey of acquired HIVDR using a standard WHO method was feasible in Nigeria but required substantial effort. Although 90% of patients remaining on ART at the 1-year follow-up end point achieved viral suppression, indicating effectiveness of first-line ART, HIVDR prevention fell short of WHO targets because of high loss to follow-up rates. Innovative strategies for patient retention suitable for Nigeria are urgently needed to minimize loss to follow-up and to reduce the emergence of possible HIVDR, to ensure the long-term efficacy of current and future first-line ART regimens.

**Supplementary Data**

Supplementary materials are available at Clinical Infectious Diseases online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

**Acknowledgments.** The authors acknowledge the patients who participated in this survey, the staff of Wuse General Hospital and Abuja University Teaching Hospital, Institute of Human Virology Nigeria/University of Maryland, Family Health International/Global HIV/AIDS Initiative in Nigeria, HIV/AIDS Division, Federal Ministry of Health, Nigeria for their leadership role, WHO Nigeria, International Laboratory Branch and HIVDR monitoring Team, Division of Global HIV/AIDS, Center for Global Health, US Centers for Disease Control and Prevention (CDC Atlanta), CDC Nigeria, and Drs Diane Bennett (CDC Atlanta) and Don Sutherland (Public Health Agency of Canada) for their guidance. We also thank Dr Michael R. Jordan (WHO Geneva) for his support in data analysis and writing the manuscript. Dr Erin Rottinghaus was a recipient of 2009–2011 Emerging Infectious Disease (EID) Fellowship Programs sponsored by American Public Health Laboratory and the CDC.

**Disclaimer.** The conclusions and opinions expressed in this article are those of the authors and do not reflect those of the CDC.

**Financial support.** This work was supported by the US President’s Emergency Plan for AIDS Relief.

**Supplement sponsorship.** This article was published as part of a supplement entitled “The World Health Organization HIV Drug Resistance Prevention and Assessment Strategy: Global, Regional, and Country Progress,” sponsored by The Bill & Melinda Gates Foundation (38180).

**Potential conflicts of interest.** All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.
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