

Surveillance of transmitted HIV drug resistance with the World Health Organization threshold survey method in Lilongwe, Malawi

Kelita Kamoto¹ and John Aberle-Grasse^{2*} on behalf of Members of the Malawi HIV Drug Resistance Task Force

¹HIV Unit, Ministry of Health, Lilongwe, Malawi (Chair; Malawi HIV Drug Resistance Task Force)

²Centers for Disease Control and Prevention, Global AIDS Program, Atlanta, GA, USA

*Corresponding author: E-mail: joa7@cdc.gov

Background: Malawi started rapid scale-up of antiretroviral therapy (ART) in 2004 and by December 2006 had initiated 81,821 patients on treatment in the public sector. Owing to capacity constraints, standard patient care, treatment initiation and follow up are based on World Health Organization (WHO) clinical staging and do not provide laboratory monitoring to assess treatment failure.

Methods: To monitor possible transmission of HIV drug resistance (HIVDR) an HIVDR threshold surveillance based on the WHO guidelines was implemented in Malawi. Anonymous dried blood specimens were collected from routine blood samples of HIV-positive women attending primigravida antenatal care and aged <25 years.

Results: Of 59 samples tested, 54 were successfully amplified indicating good specimen quality and processing. The WHO protocol algorithm to classify the prevalence of transmitted drug resistance in the site sample only required the genotyping of 34 of the samples. None of the major drug resistance mutations on the WHO surveillance list were found in these 34 specimens.

Conclusions: Malawi HIVDR transmission can be classified as <5% for all relevant drugs and drug classes in this population. On the basis of the very positive experience of this survey, an expanded HIVDR surveillance system will be implemented to inform the ART program as it continues to scale-up.

Introduction

Malawi is a geographically small, severely impoverished country in Southern Africa with an estimated population of 12.5 million (National Statistics Office [NSO] 1998 population projections), facing one of the world's most severe generalized HIV epidemics. With an adult (ages 15–49) HIV prevalence of 14.0%, there are an estimated 930,000 adults and children living with HIV. There are ~87,000 AIDS-related deaths annually and up to 190,000 people are currently eligible for antiretroviral therapy (ART) [1]. As a major component of their response, the Malawi government began rapidly scaling up a national ART programme in June 2004.

Details and rationale of ART delivery in Malawi have previously been described [2]. A standardized ART approach is used which includes several factors (see Box 1).

This approach has provided quality treatment and care, and encourages therapy adherence in the context of severely limited resources. Alternative first-line therapy, including zidovudine or efavirenz, is available for patients with severe side effects. Second-line therapy is also available for patients in

case of failure to first-line drugs. For adults, this second-line therapy comprises zidovudine plus lamivudine, tenofovir and lopinavir/ritonavir, whereas for children the regimen comprises didanosine plus abacavir and lopinavir/ritonavir [3].

At the beginning of 2004, only 4,000 patients in mostly urban public facilities and about 500 patients in the private sector were accessing antiretroviral (ARV) drugs [2]. By the end of December 2006, there were 81,821 patients who had ever started on free ART at 103 nationally distributed public sector sites, which in Malawi include both government and most mission hospitals [4]. Of these ART patients, 54% were started in 2006, 37% were started in 2005 with the remaining 16% starting prior to 2005. There were also an additional 3,347 ART patients started in the private sector at 38 sites [4]. The private sector provides the standardized ARVs at a heavily subsidised rate of about US\$3.5 per patient per month. In 2004, >25% of ART patients were in Lilongwe, the capital city; currently, 14% of patients access ART in Lilongwe. The national ART scale-up plan is to have started 110,000 patients

Box 1. Factors incorporated in the standardized antiretroviral therapy approach employed in Malawi

- A focus on one generic, fixed-dose combination therapy of stavudine plus lamivudine and nevirapine, 'Triomune' (manufactured by Cipla, India) [3].
- Antiretroviral therapy (ART) is delivered free of charge to HIV-positive eligible patients.
- The initiation of treatment at antiretroviral therapy (ART) clinics includes patient referral from HIV testing sites, World Health Organization (WHO) clinical staging for eligibility, group counselling, individual counselling and treatment. This process takes about 2 weeks from referral to start of ART.
- Monthly patient follow-up is performed using general WHO clinical criteria only.
- A standardized system of registration, monitoring and reporting of cases and outcomes.
- Quarterly supervision and evaluation of all ART sites.

on ART by the end of 2007, and to add an additional 45,000 each year thereafter until 2010.

The quarterly comprehensive national supervision system, which includes monitoring drug supplies, has been previously described [2] and to date there have been no stock outages of ARVs in either the government or private sector ART programme [4]. These successful results stem from Malawi's centralized ARV drug forecasting, procurement and distribution system.

Public sector ART patient outcomes by the end of December 2006 have been reported: 70% were alive and on ART at the site of registration, 11% had died, 9% were lost to follow-up, 9% had transferred out to another facility (and were presumably alive) and ~1% had stopped treatment. Of 57,356 patients alive and on ART 97% were on the standard first-line regimen, 3% were on an alternative first-line regimen and 148 were on the second-line regimen. Of those alive and on ART, 5% had side effects and 93% of patients showed 95% or more adherence to therapy based on pill counts [4].

With limited human resources in the country, paramedical clinical officers and nurses are trained in addition to doctors to manage and deliver ART. Although clinical staging and follow-up of patients are the standard of care, the testing of CD4⁺ T-cell counts to assess eligibility was available and carried out for only 15% of patients [4]. The monitoring of HIV drug resistance (HIVDR) on an individual patient basis is not feasible given the limited resources and current infrastructure available in Malawi. However, in the Malawi ART Treatment Guidelines annual surveillance for HIVDR at selected sites is recognized as a vital component of successful national ART delivery and this activity is currently being institutionalized. Furthermore, knowledge about the resistance pattern in patients failing the first-line ART regimen is important given the current limited choice of cheap and simple fixed-dose generic combinations.

A previous study was conducted in 2005 where HIV DNA recovered from 21 ART-naïve Malawian adults was sequenced and analysed to determine the prevalence of mutations associated with drug resistance [5]. No major mutations associated with resistance to protease inhibitors, nucleoside reverse transcriptase inhibitors or

non-nucleoside reverse transcriptase inhibitors were found in this group of patients.

In April 2005, an HIVDR Task Force was formed to draft a methodology to implement nationally informative monitoring of HIVDR among patients receiving highly active antiretroviral therapy (HAART) and to carry out HIVDR surveillance among newly infected individuals. The core task force is led by the Ministry of Health (MOH) and includes the National AIDS Commission (NAC), the World Health Organization (WHO), and the Centers for Disease Control and Prevention (CDC). Additional co-opted members of the working group include representatives from survey sites and testing laboratories.

Methods

The Malawi HIVDR threshold protocol is based on the WHO 'Guidelines for HIV drug resistance threshold survey using specimens from antenatal (ANC)-based HIV sentinel serosurveillance in resource-limited settings' [6]. A detailed discussion of the WHO guidance is provided in an article on the threshold survey strategy published in this supplement [7].

Geographic site and patient eligibility

Residual blood samples from antenatal clinic attendees receiving prevention of mother-to-child transmission (PMTCT) services in Lilongwe city were sequentially and anonymously enrolled in the survey at three sites, Bwaila Hospital, and the Kawale and Area 25 Health Centres. The PMTCT sites at these clinics, operated by the University of North Carolina Project, test 99.2% of all new antenatal mothers using Determine and Unigold rapid HIV test kits in parallel algorithm. All HIV-positive mothers receive routine CD4⁺ T-cell count testing at the time of HIV diagnosis. As per the WHO guidelines, HIV-positive primigravida women, <25 years of age with no evidence of HIV disease and who are greater than WHO stage 1 were identified by site staff and their CD4⁺ T-cell count sample was marked for dried blood spot (DBS) preparation using the residual blood. These sites were selected because ART started in the capital city early in the national programme and, owing to the

large size of the PMTCT programme (~20,000 first antenatal care visits annually), the survey could be completed within a short time frame.

Specimen collection and processing

In brief, 67 HIV-positive specimens were collected between 2 October and 14 December 2006. A minimum set of routine patient data (eligibility information and date of blood draw) was also abstracted, recorded anonymously and entered into a survey database. DBS specimens were made at the UNC Project laboratory according to standard operating procedures. The national HIV reference laboratory transferred the DBS specimens weekly for quality assurance, eligibility and processing, and transported them to CDC Atlanta for genotype testing.

HIV antibody testing and genotype analysis

Specimens were confirmed as positive for anti-HIV antibodies by the Serology and Incidence Team, International Laboratory Branch, GAP, CDC, Atlanta using Genetic Systems HIV1/HIV-2 PLUS O EIA (Bio-Rad, Redmond, WA, USA). Specimens were of good quality for amplification. Total nucleic acids were extracted from DBS using a modified NucliSens[®] silica-based extraction method (BioMerieux Inc., Durham, NC, USA). The *pol* gene region comprising the protease and reverse transcriptase sequences was amplified with an in-house one-step reverse transcriptase PCR (RT-PCR) procedure followed by the nested PCR method. Sequence analysis was performed with an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The protease and reverse transcriptase genotypes were generated using the Stanford University HIV drug resistance database [8]. Drug resistance mutations were identified on the basis of the WHO surveillance list for transmitted drug resistance [9].

Drug resistance mutation analysis

The HIVDR prevalence for each relevant drug and drug class was categorized separately according to standard procedures recommended by the WHO/CDC and described in detail elsewhere in this supplement [10]. The preliminary HIV-1 group M subtypes were determined by using the National Center for Biotechnology Information (NCBI) genotyping tool [11].

A classification of HIVDR prevalence was made when, among the sample number genotyped, the running total of specimens found with HIVDR was less than a determined lower limit (LL) or greater than a determined upper limit (UL). When one of these conditions occurred, HIVDR prevalence was classified either as <5% (if the total number of specimens is less than the LL) or >15% (if the total number of specimens is greater than the UL). If neither of these conditions

occurred after the 47th specimen had been genotyped, prevalence was classified as 5–15%. A separate sampling plan was used for each drug or drug class of interest. For further details of the statistical methodology on determining levels of HIV drug resistance in the survey sample, refer to the article on the threshold survey strategy in this supplement [10].

Results

Of the 67 HIV-positive DBS samples, 59 were found to be eligible and included in this survey. Eight women were subsequently determined not to be eligible: one was aged 25, two were in second pregnancy, and five had symptomatic HIV-related disease. It was possible to successfully amplify 54 (92%) of the samples. All 54 of these samples were determined to be HIV-1 group M subtype C viruses.

Of these 54 specimens, genotyping of only 34 was required according to the WHO protocol algorithm to classify the prevalence of transmitted drug resistance in the site sample. There were no major drug-resistant mutations on the WHO surveillance list found in these 34 specimens. However, two specimens did have minor polymorphisms at resistance-related positions in the *pol* gene, based on the Stanford drug resistance database analysis. One specimen had the protease A71T, which is not known to be associated with drug resistance; the other specimen had the reverse transcriptase mutation V118I, which is associated with drug resistance but only in the presence of other mutations and it is polymorphic among nearly all subtypes.

Discussion

Malawi HIVDR transmission can be classified as <5% for all relevant drugs and drug classes in this population on the basis of the WHO surveillance list for transmitted resistance. It is likely that, despite rapid ART scale-up, transmission of HIVDR is still relatively uncommon. On the basis of these results, the current first-line ART regimen should continue to be used with confidence. The HIVDR threshold survey will be repeated next year to gain further experience and to validate the survey findings and methodology. Furthermore, it will be extended to additional geographic areas as ART scale-up continues. Interpretation of the current results is of course limited to antenatal care clients in Lilongwe, although the samples are considered a good representation of the population at risk for transmitted HIVDR within the past three to five years in this area, where transmitted HIVDR would be likely to be seen first.

In addition to surveillance of transmitted resistance, the Malawi HIVDR working group recognizes the

importance of assessing the potential for HIVDR emergence in treatment and the functioning of ART programs as part of its HIVDR prevention strategy. The low level of transmitted resistance in Lilongwe is likely to be a function of the success of the ART programmes in minimizing the emergence of resistance and the relatively small proportion of HIV-infected individuals who have received treatment. HIVDR early warning indicators described elsewhere in this supplement [12] provide evidence of successful ART programme functioning. Briefly, the Malawi ART programme performs very well on drug supply and prescribing practices at the national and site level, and most sites successfully reach the adherence and loss to follow-up targets. Although many ART sites do not achieve the targets for patient retention, owing to the large number of patients starting treatment severely ill and dying in the initial months, mortality rates do not vary from findings of ART programmes in other southern African countries [13].

Malawi is also currently implementing HIVDR monitoring in ART patients on treatment for 1 year to assess HIVDR emergence and potentially related ART programme factors at representative ART sites, based on the WHO protocol. HIVDR monitoring is crucial for resource-poor countries to assess the prevalence of HIVDR mutations and individual and programmatic associations for complete ART programme evaluation and public health action. This is particularly true in Malawi where decisions on ART regimens are made on a population basis.

Minimal transmitted HIVDR has been seen in Africa to date. In Western countries, the HIVDR transmission levels were low in the early years of ART scale-up, rose in the mid-to-late 1990s with the widescale use of monotherapy and dual therapy, and now appear to be levelling off or decreasing with the use of highly active three- and four-drug regimens [14]. Although transmitted HIVDR was not seen at this time in Malawi, it is important to obtain a baseline result and to routinely implement the threshold survey. Based on the experience of this survey, an expanded HIVDR surveillance system can be implemented to inform the ART program as it continues to scale up.

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Disclosure statement

The authors declare that they have no competing interests.

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