SUPPLEMENT ARTICLE

Prevalence of Transmitted HIV Drug Resistance Among Newly Diagnosed Antiretroviral Therapy–Naive Pregnant Women in Lilongwe and Blantyre, Malawi

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In 2006, a survey of transmitted human immunodeficiency virus (HIV) drug resistance (TDR) was conducted in Lilongwe, Malawi. The survey followed the World Health Organization method to classify TDR to nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) among primigravid women aged <25 years. Results of the 2006 survey showed <5% TDR in all drug classes. In 2009, TDR surveys using the same method were repeated in Lilongwe and expanded to Blantyre. Findings show that in Lilongwe TDR to NRTIs and PIs was <5%, whereas TDR to NNRTIs was 5%–15%. In Blantyre, TDR was <5% to all drug classes. Observed moderate TDR in Lilongwe is cause for concern and signals the need for closer monitoring of Malawi's antiretroviral therapy program.

Malawi is one of the countries most affected by the human immunodeficiency virus (HIV) epidemic in Africa, with an estimated prevalence of 12% among adults aged 15–49 years [1]. The epidemic is predominantly spread via heterosexual contact, and prevalence estimates indicate that urban areas are more heavily affected than are rural areas [1].

Malawi provides antiretroviral therapy (ART) free of charge using a public health approach where tasks are shifted from more specialized to less specialized healthcare workers and where ART is provided at peripheral health facilities. Patients are predominantly prescribed standardized first-line ART regimens and monitored by nonphysicians using World Health Organization (WHO) clinical staging guidelines with limited laboratory monitoring. The first-line ART regimen and 3 alternate first-line regimens consist of 2 nucleoside reverse transcriptase inhibitors (NRTIs) combined with a nonnucleoside reverse transcriptase inhibitor (NNRTI). Since 2004, about 383 000 patients have initiated ART, of whom 277 000 (72%) remained alive and on therapy by June 2011 [2].

Because ART is provided on a massive scale without the benefit of individual viral load or drug resistance testing, the emergence and transmission of HIV drug resistance (HIVDR) remains a fundamental concern.

The lack of individual HIVDR testing has not limited Malawi's ability to optimize patient care, and the country has developed a comprehensive HIVDR prevention and assessment strategy based on WHO guidance consisting of routine monitoring of HIVDR early warning indicators and routine surveillance of transmitted and acquired HIVDR [3].

Prior to 2007, most African countries reported transmitted HIV drug resistance (TDR) prevalence estimates of <5% [4–9] attributed mostly to low ART coverage; Malawi was no exception, with <5% TDR reported to all

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drug classes in a 2006 survey conducted in Lilongwe [10]. However, recently, a number of cities in East Africa, including Entebbe, Uganda, and Kigali, Rwanda, have reported TDR prevalence of >5% [11]. Southern Africa also has not been spared, and TDR prevalence of >5% was recently reported in Cape Town, South Africa, and in Lusaka, Zambia [11, 12]. However, it is noteworthy that studies that have previously reported TDR prevalence estimates of >5% in this region have not used the WHO method, making comparison of results difficult. Despite these methodological differences, a trend toward increasing TDR prevalence estimates is supported by a recent meta-analysis by Gupta et al [13], which reports more countries with moderate (5%–15%) TDR prevalence after 2007 compared with pre-2007.

The presence of HIVDR prior to ART initiation is an important predictor of virological response to antiretroviral regimens [14, 15]. Therefore, the existence of significant population-level TDR may undermine the success of the national ART scale-up initiative. Surveys to classify TDR provide important programmatic information regarding likely efficacy of future first-line ART, current pre- and postexposure prophylactic regimens, and HIV prevention programs. The expected prevalence of TDR partly depends on the extent of ART scale-up and the duration that antiretroviral drugs (ARVs) have been in use within a defined geographical area [11, 12].

Blantyre and Lilongwe are 2 major urban areas where ART was first provided in Malawi and currently have the largest number of patients on ART; thus, if TDR were to be observed, it is likely to be seen in these locations. In 2009, TDR surveys were performed in Lilongwe City and Blantyre City. Results are reported here.

METHODS

Healthcare facilities within the designated 2 catchment areas of Lilongwe and Blantyre City were selected if they provided antenatal care and prevention of mother-to-child transmission of HIV services and were seeing adequate numbers of pregnant women in order to contribute to the minimum sample size ($N \leq 47$) within a reasonable period. Additionally, sites were selected based on capacity to implement the survey, including ability to perform routine CD4 counts for all newly diagnosed HIV-positive antenatal attendees. Three health facilities (Kawale, Area 25, and Bwaila) were selected in Lilongwe, and 2 health facilities (Ndirande and Limbe) were selected in the Blantyre urban area.

As a proxy for recently HIV-infected individuals, only primigravid women <25 years without clinical evidence of advanced HIV infection (only WHO clinical stage 1, as determined by healthcare providers), who self-reported naiveté to ARVs and who tested HIV positive for the first time, were eligible to participate in the survey. After routine CD4 cell count testing, remnant blood was used to prepare dried blood spots (DBSs) from consecutively eligible women.

HIVDR genotyping was performed by the WHO accredited National HIV and Retrovirology Laboratory, Public Health Agency, Ottawa, Canada. Complete HIV protease and part of reverse transcriptase were sequenced using previously described methods [16].

The WHO TDR survey method permits classification of TDR as low (<5%), moderate (5%–15%), or high (>15%) in a specific population in a defined geographic region using ≤47 specimens.

For each geographic region, 60 HIV-positive specimens based on HIV rapid tests performed at the sites were consecutively collected between January 2009 and April 2009 to generate at least 47 specimens for genotyping (taking into account a proportion of specimens that would fail to amplify). Due to the concern for preserving the routine nature of the health systems at the participating facilities, no behavioral questionnaires were used. All specimens were prepared, processed, and stored in Malawi following WHO DBS guidance and were shipped to the genotyping laboratory for testing [17].

Neighbor-joining distance method was used to assess for possible contamination, and subtyping was performed using the REGA subtyping tool [18, 19]. TDR for each drug class was determined using the 2009 WHO TDR mutations list available through the Stanford HIVDR Calibrated Population Resistance tool [20–22].

RESULTS

Blantyre Geographical Area

A total of 61 DBS specimens were collected; 54 (88.5%) were successfully amplified. All sequences were HIV subtype C. Among the first 47 specimens, 3 had TDR mutations. One specimen had both NNRTI (G190E) and NRTI (M184V) mutations. The second specimen had 1 NRTI TDR mutation (D67DN), and the third specimen had 1 NNRTI TDR mutation (Y188CY). No PI resistance was detected. When these data were analyzed by drug class as per WHO TDR guidance, the classification of transmitted resistance was <5% for NNRTI, NRTI, and PI drug classes.

Lilongwe Geographical Area

A total of 68 DBS specimens were collected, and 55 (80%) were successfully amplified. All sequences were HIV subtype C. Among the first 47 specimens, 3 had 1 detected NNRTI TDR mutation: K103S, K103N, or V106A. No NRTI or PI mutations were detected. Following WHO guidance, NNRTI TDR was classified as moderate (5%–15%), and NRTI and PI TDR was classified as low (<5%).

DISCUSSION

ART scale-up began rapidly in Malawi in 2004. Some degree of HIVDR is inevitable even when appropriate regimens are prescribed and optimal adherence to treatment is supported. Routine surveillance of TDR provides important public health information regarding the efficacy of current pre- and postexposure prophylaxis and may predict population level efficacy of first-line regimens when recently infected populations require future ART. As part of its ART program, Malawi has adopted WHO guidance for the routine monitoring of site factors associated with the emergence of HIVDR (HIVDR Early Warning Indicators) and the routine surveillance of transmitted and acquired HIVDR.

In 2006, in Lilongwe TDR was classified as low (<5%) for all drug classes. We observed moderate (5%–15%) NNRTI TDR in Lilongwe and low TDR (<5%) in Blantyre for all drug classes. Although specimens were obtained only from women who were naive to ARVs, it is possible that some did not disclose previous ART exposures. Nonetheless, the prevalence classification of 5%–15% NNRTI TDR in Lilongwe is troubling and merits attention.

Malawi is one of the first countries in sub-Saharan Africa to report moderate TDR using the WHO TDR survey method, although studies that have used other methodologies have previously suggested similar prevalence estimates in South Africa and Zambia [11, 12]. In the current survey, new infections were based on self-reports of drug naiveté, age <25 years, testing positive for the first time, and primigravidity. This definition of recent infection may not have high specificity because some women may have falsely reported their characteristics. However, given recent evidence suggesting that TDR may be increasing in sub-Saharan Africa [13, 23, 24], these results may truly reflect increasing TDR in Malawi.

In order to confirm the findings of this survey, surveillance of TDR was repeated in Lilongwe by integrating the WHO TDR survey method into routine antenatal serosurveillance in 2010. Specimens from this follow-up survey await genotyping. Additionally, in 2011, Malawi plans to monitor WHO HIVDR early warning indicators in several regions of the country including Lilongwe and Blantyre in order to assess clinic function in minimizing the emergence of possible HIVDR by containing rates of losses to follow-up, ensuring drug supply continuity, and maximizing patient adherence. In addition, as high rates of loss to follow-up have previously been identified as problematic in Malawi, a special survey to characterize reasons for loss to follow-up and assess strategies to link patients back into care is planned for 2012.

CONCLUSIONS

In conclusion, although findings of moderate TDR merit attention and warrant concern, these results must be treated cautiously. Survey results showing moderate TDR to the NNRTI drug class are insufficient to support changing current ART guidelines in Malawi but do signal the importance of robust programmatic monitoring of factors known to be associated with the emergence of HIVDR at all ART sites in Malawi and the need for routine surveillance of transmitted and acquired HIVDR. Additionally, results highlight the need to evaluate and possibly strengthen prevention messages geared to populations receiving ART and to populations recently diagnosed with HIV who are not yet eligible to initiate ART in order to both minimize HIV and HIVDR transmission.

Notes

Disclaimer. The conclusions and opinions expressed in this article are those of the authors and do not reflect those of the World Health Organization.

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