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HIV drug resistance in infants increases with changing PMTCT regimens

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Abstract

Objectives—The objectives of this study were to determine HIV drug resistance prevalence in Zambian infants upon diagnosis, and to determine how changing prevention of mother-to-child transmission (PMTCT) regimens affect drug resistance.

Design—Dried blood spot (DBS) samples from infants in the Lusaka District of Zambia, obtained during routine diagnostic screening, were collected during four different years representing three different PMTCT treatment regimens.

Methods—DNA extracted from DBS samples was used to sequence a 1493 bp region of the *RT* gene. Sequences were analyzed via the Stanford HIV Drug Resistance (HIVDR) Database (http://hivdb.standford.edu) to screen for resistance mutations.

Results—HIVDR in infants increased from 21.5% in 2007/2009 to 40.2% in 2014. Non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance increased steadily over the sampling period, while nucleoside reverse transcriptase inhibitor (NRTI) resistance and dual class resistance both increased more than threefold in 2014. Analysis of drug resistance scores in each group revealed increasing strength of resistance over time. In 2014, children with reported PMTCT exposure, defined as infant prophylaxis and/or maternal treatment, showed a higher prevalence and strength of resistance compared to those with no reported exposure.

Conclusions—HIVDR is on the rise in Zambia and presents a serious problem for successful lifelong treatment of HIV infected children. PMTCT affects both the prevalence and strength of resistance and further research is needed to determine how to mitigate its role leading to resistance.

Keywords

HIV drug resistance; PMTCT; Zambia; Antiretroviral therapy; Africa

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INTRODUCTION

Antiretrovirals (ARVs), specifically prevention of mother to child transmission (PMTCT), have reduced perinatal HIV transmission rate from 15–45 percent to <5% [1, 2]. Even In resource-limited countries like Zambia, where limited access to ARVs and suboptimal adherence increase the risk of mother to child transmission, transmission dropped from 24% in 2009 to 13% in 2014 [3]. Despite these successes, when PMTCT fails, exposure of the mother or child to ARVs can select for drug resistance HIV transmitted from the mother or arise *de novo* after transmission [4, 5]. Young children infected with HIV are at an increased risk of developing drug resistance because of their immature immune responses. In addition, maternal lack of adherence, dosing difficulties due to variable infant pharmacokinetics, changing body weight and poorly formulated pediatric formulations can also result in reduced ARV efficacy [6–9].

The WHO PMTCT guidelines have undergone changes in accord with the availability of more potent drugs, and PMTCT drug regimens in Zambia have evolved. In 2006, mothers and infants were given a single dose of the non-nucleoside reverse transcriptase inhibitor (NNRTI) Nevirapine (NVP)[10]. In 2010 a version of WHO Option A [11] was adopted. The maternal regimen consisted of the nucleoside reverse transcriptase inhibitor (NRTI) Zidovudine (AZT) were given at 28 weeks gestation; NVP + Combivir (AZT+3TC) at delivery and Combivir was continued for one week postpartum. Infants received NVP throughout breastfeeding. In 2013, Option B+ was implemented, where all women are placed on a three-drug ARV regimen regardless of $CD4^+$ T cell count [10, 12].

While other studies have sought to define the impact of drug resistance on treatment failure in children [8, 9], limited information is available about the role PMTCT drug exposure played in the occurrence of resistance in infected infants. Given PMTCT regimen changes in Zambia, it is important to define how these changes were influencing drug resistance. In order to clarify correlations between PMTCT regimens and resistance in infants, we sequenced the HIV-1 *RT* region from Zambian infants, stratified over 3 distinct PMTCT regimens. Both the prevalence and the magnitude of drug resistance increased in Zambian infants from 2007 to 2014. Additionally, infants with reported PMTCT participation showed stronger and more frequent resistance than those without ARV exposure.

MATERIALS AND METHODS

Discarded dried blood spots (DBS) of infants used for routine HIV-1 screening were obtained from the University Teaching Hospital in Lusaka, Zambia. DNA was extracted from DBS using a detergent lysis with proteinase K digestion method, followed by phenol-chloroform extraction. The reverse transcriptase (*RT*) region was amplified by nested PCR as described [13]. Amplification of 1493 bp products was confirmed by gel electrophoresis and purified using E.Z.N.A Gel Extraction Kit (Omega Bio-Tek, Norcross, GA) according to the manufacturer's protocol. The purified DNA was sequenced in 5 overlapping reactions to produce a contiguous *RT* gene sequence using BigDye Terminator v3.1 cycle Sequencing

Kit (Applied Biosystems, Carlsbad, CA) according to the manufacturer's instructions and chromatograms were produced by Eurofins MWG Operon (Eurofin MWG Operon LLC).

Sequence data from all five primers were trimmed and a contig was generated in BioEdit v7.2.5 for each subject. Patient contigs were analyzed using the Stanford Drug Resistance Database (http://hivdb.standford.edu), which also provided an overall drug resistance score. A two-sided t-test was used to test for significance in mean drug resistance score using GraphPad Prism 5.

RESULTS

Cohort Characteristics

Samples analyzed were from the Lusaka District and were selected randomly. In 2007 and 2009, PMTCT consisted of a single dose NVP administered to the mother during delivery and to the infant within 72 hours of birth. No alternative regimens were available. In 2011, pregnant women recieved a) AZT at 14–28 weeks gestation; b) NVP and Combivir (AZT + 3TC) during delivery, and c) Combivir for 1 week following delivery. Infants received NVP syrup while breastfeeding. In 2014, the regimen was altered to provide women Atripla (Tenofovir [TDF], Emtricitabine [FTC], and Efavirenz [EFV]) or another multidrug combination during pregnancy and for life. Infants received NVP for 6 weeks. In 2011 and 2014, approximately 60% of patients reported receiving PMTCT, approaching WHO goals that two-thirds of HIV-1 infected sub-Saharan African pregnant women receive PMTCT in 2014 [1]. PMTCT participation data was unavailable for samples from 2007 and 2009. Infant ages, where available, were <18 months with a median age of 5 months (range: 1 day to 18 months). Most Zambian newborns are breastfed up to two years of age. At the time of sampling, two-thirds (67.4%) of infants were currently breastfeeding. Gender distribution was equal (47.4% male) for the 114 infants with data. No additional information, including any prior use or exposure to ARVs for the mothers, was available.

Drug Resistance

Amplifiable DNA was extracted from 60% of the DBS. Of 228 amplifiable samples, 219 were sequenced. These 219 samples were separated into three groups containing 79, 58, and 82 infants based on the PMTCT regimen and identified by year collected, 2007/2009, 2011, and 2014, respectively. All but seven *RT* sequences (clades A, A1, B, G, D, K) were clade C.

Patient sequences were compared using the BLAST algorithm through Los Alamos National Laboratory's HIV sequence database to rule out PCR contamination. The Stanford Drug Resistance Database was used to identify specific resistance mutations and report overall drug resistance scores for each infant. Prevalence and strength of drug resistance for each group is shown in Figure 1. Sequences from 2007 and 2009 were combined since they represent the same PMTCT regimen and had similar resistance parameters either when grouped or analyzed separately (data not shown). The percentage of infants with any major drug resistance mutation (total DR) was 21.5%, 25.9%, and 40.2% for samples from 2007/2009, 2011, and 2014, respectively (Figure 1A). The type of resistance was stratified according to the specific class of ARV. NNRTI resistance steadily increased across the

sampling intervals with 16.5% of infants showing resistance in 2007/2009, 22.4% in 2011, and 39% in 2014 samples. NRTI resistance was low in both 2007/2009 and 2011 (~5%), but doubled (12%) in 2014 (Figure 1A). The prevalence of dual class resistance, (at least one mutation to both an NRTI and NNRTI) increased from less than 2% of infants in both 2007/2009 and 2011 to over 10% in 2014 (Figure 1A).

To investigate the strength of the resistance conferred, drug resistance scores as provided by the database were compared between groups. A higher drug resistance score signifies stronger resistance. Figure 1B shows the drug resistance score for each infant by group, along with the mean and 95% confidence interval. Children with no measurable resistance (a score of 0) were not included in the analysis. Average drug resistance scores were 64.7, 129.2, 183.4 in 2007/2009, 2011, and 2014, respectively. Both 2011 (p=0.0064) and 2014 (p=0.0004) had significantly higher scores than 2007/2009 (Figure 1B). There was no significant difference between the average scores in 2011 and 2014 (p=0.2). Together, our findings suggest that prevalence of drug resistance and the strength of resistance conferred both increased temporally.

PMTCT and Drug Resistance

Because the dataset from 2014 was comprised from the largest number of samples, exhibited the highest resistance, and contained the best descriptive data, it was used to investigate the potential role of PMTCT in drug resistance. There was a significant increase in both the prevalence and strength of resistance in infants with PMTCT exposure (Figure 2A and B). Infants were considered PMTCT exposed if either the mother or child received PMTCT, while those where neither the mother nor the child had reported PMTCT were considered unexposed. Of the 82 samples in the 2014 group, 48 were exposed and 30 were unexposed; PMTCT information was unavailable for the remaining four. A higher percentage of infants involved in PMTCT had drug resistance, regardless of class, compared to those without PTMCT, with overall resistance rates of 54% and 20% respectively (Figure 2A). No unexposed infants had dual-class resistance, but all exposed infants with NRTI resistance also had NNRTI resistance. PMTCT exposed infants had a significantly higher mean drug resistance score of 128.3 as compared to unexposed at 30.3 (p=0.004, Figure 2B). Importantly, 20% of infants with no reported PMTCT exposure still showed drug resistance (Figure 2A) with several displaying modest drug resistance scores (Figure 2B). No correlation was observed between drug resistance and the age of the infant at time of sampling (1 day–15 months, median 4 months), gender (45.7% male), or current breastfeeding status (69.6% currently breastfeeding).

DISCUSSION

From 2007 to 2014, total HIV drug resistance (HIVDR) increased from 21% to 40% in Zambian infants. Strength of drug resistance also increased. Every infant with NNRTI resistance showed resistance to NVP. The most prevalent mutation in 2014, Y181C, confers high-level resistance to NVP, consistent with its prevalence in studies from South Africa [14] and Tanzania [15]. Since over one-third of infants in this study possessed high NVP

resistance, our data support the current practice of using protease inhibitor-based drug regimens as first line treatment in children previously exposed to NVP.

Our results showed that 10-fold more infants in the 2014 group displayed dual-class resistance, and twice as many had NRTI resistance than in other groups. Interestingly, these increases correlate with changes in the PMTCT regimen. The prolonged NRTI use during breastfeeding in 2014 compared to 2011 likely contributed to the increased resistance, since it has been shown that NRTIs were detectable in breast milk and selected for drug resistance in the child [4, 16]. However, the limited information available for these samples did not allow the establishment of a correlation between duration of breastfeeding and NRTI resistance.

Among 2014 samples with available clinical information, infants with PMTCT exposure had a higher prevalence and significantly stronger drug resistance than those without, which is consistent with other studies from the region [14, 17]. However, we did not find a correlation between age and prevalence of drug resistance [14]. As in prior studies, some children with no reported PMTCT exposure still had resistance, indicating a direct transmission of resistant viruses from mother to child had occurred [14, 17]. We were unable to determine if resistance originated in the mother or arose *de novo* in the infant.

Our data suggest increases in both prevalence and strength of HIVDR in Zambia, where 40% infants sampled in 2014 showed some resistance, have the potential to present serious complications for HIV-1 treatment. The increased resistance corresponds to changes in treatment regimens; however, resistance was detected in infected children with no previous ARV exposure through PMTCT, suggesting the circulation of ARV-resistant strains in the general Zambian population. Further studies are needed to determine whether mutations are transmitted from the mother or develop in the child, as well as how long they persist. Knowledge gained from such future studies will allow for the development of strategies to further reduce HIV transmission and the development of drug resistance.

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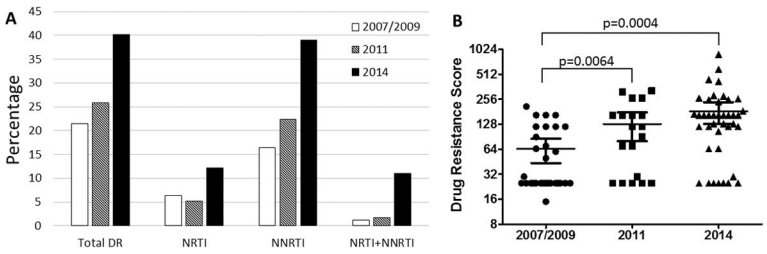

Figure 1.

Drug resistance by year. A) Percentage of infants with drug resistance by year according to class of drug resistance: any resistance, NRTI, NNRTI, or dual class (NRTI+NNRTI). B) Average drug resistance score for infants by year. A two-sided t-test was used to test for significance between means of each group. Significant differences with p-values are labeled.

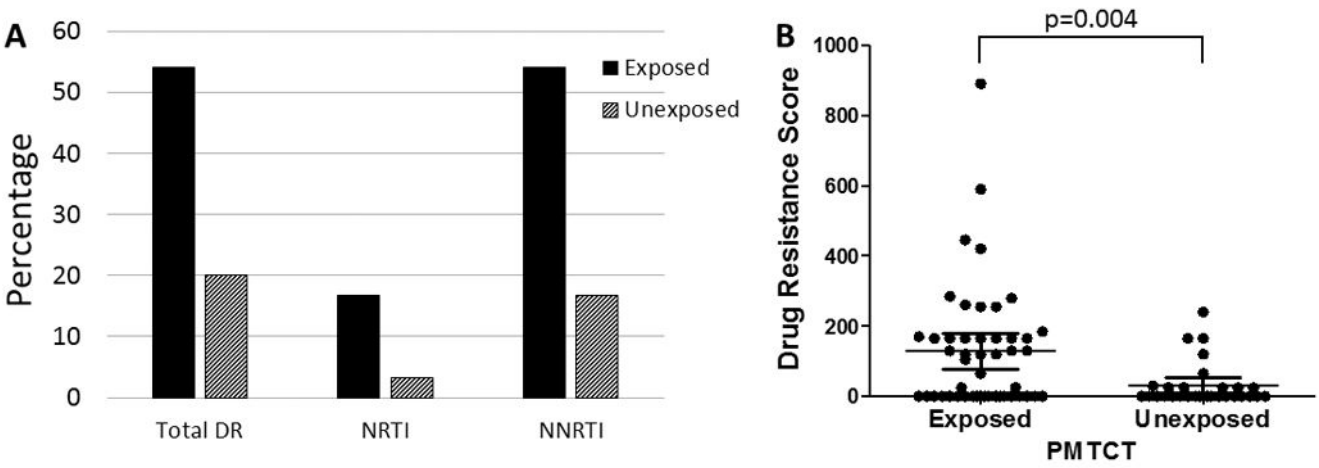

Figure 2.

Drug resistance as a factor of PMTCT participation. A) Percentage of infants with different classes of drug resistance by PMTCT status. B) Average drug resistance score for infants by PMTCT status. Infants where either the mother or child reported receiving PMTCT were classified as exposed, while those in which neither the mother nor the child reported receiving PMTCT were considered unexposed.