Incidence of Transmitted Antiretroviral Drug Resistance in Treatment-Naive HIV-1-Infected Persons in a Large South Central United States Clinic

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Abstract

Background: Transmitted drug resistance (TDR) can limit effective treatment options to antiretroviral-naive HIV-infected persons and increase the risk of treatment failure. Limited estimates of TDR have been reported from the South Central United States. Objective: To describe the incidence of TDR in Oklahoma and to examine whether TDR rates have increased with time. Methods: This was a retrospective observational study of antiretroviral-naive patients at the Infectious Diseases Institute, a large infectious diseases clinic in Oklahoma City, Oklahoma, who had received baseline antiretroviral resistance testing. Mutations were screened using the 2011 International Antiviral Society-USA Drug Resistance Mutation (DRM) update, and categorized using the 2009 World Health Organization (WHO) Surveillance Drug Resistance Mutation (SDRM) list. Results: Genotypic sequences from 428 patients revealed a 6.0% to 13.6% incidence of SDRMs between 2007 and 2011, though no progression in the frequency was apparent during the study period. Primary DRMs were detected in 12.6% of the sampled patients, most commonly involving nonnucleoside reverse transcriptase inhibitors (NNRTIs; 8.2%), followed by protease inhibitors (PIs; 3.5%) and nucleoside reverse transcriptase inhibitors (NRTIs; 3.3%). The K103N/S and E138A reverse transcriptase mutations were the most common DRMs identified, both present in 3.5% of patients. The L90M mutation was the most frequently observed PI SDRM (1.6%), while the T215C/D/I mutation was the most common NRTI SDRM identified (1.9%). This study was limited by the fact that the WHO SDRM list was last updated in 2009. Conclusions: The frequency of DRMs in central and western Oklahoma is similar to recently reported rates in the United States which lack data from this region. However, the frequency of second-generation NNRTI DRMs (4.4%) suggests the need to closely monitor epidemiologic trends for increasing resistance rates to individual classes of ARVs in order to predict the impact of TDR on therapeutic options.

Keywords

drug resistance, antiretroviral, HIV-1, transmission, Oklahoma

Introduction

Antiretroviral therapy (ART) has produced marked declines in HIV-attributable morbidity and mortality in HIV-infected persons. However, incomplete adherence to medication regimens results in the emergence of viral mutations that can confer resistance. These resistant strains can be transmitted to others and can limit the selection of effective first-line therapeutic options, increase the time to virologic suppression and the risk of treatment failure, and decrease the efficacy of pre- and postexposure prophylaxis.

Estimates of the percentage of transmitted drug resistance (TDR) among patients in developed nations vary. Reasons for these differences include geographic differences in sampled populations, genotype timing with respect to seroconversion, exposure and access to antiretrovirals in

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the population, selection bias in patient sampling, and differing risk behaviors.² A Centers for Disease Control and Prevention (CDC) epidemiologic study evaluating data from the Variant, Atypical, and Resistant HIV Surveillance (VARHS) system from 11 locations across the United States revealed a TDR percentage of 14.6% between 2006 and 2009.³ An analysis of 25 European cohorts reported 9.5% (95% confidence interval = 8.9-10.0) of patients had at least one TDR mutation between 1998 and 2008.⁴ The TDR percentage in North Carolina was reported at 17.8% between 1998 and 2007.⁵ One study of rates in New York City additionally reported an increase in TDR percentage over time from 13.2% between 1995 and 1998 to 24.1% between 2003 and 2004.⁶

The purpose of this surveillance study was to describe the incidence of TDR in Oklahoma, a state with a large rural population of patients with HIV infection, and to examine whether TDR rates have increased with time over a 6-year period, as has been described in other regions in the United States.

**Materials and Methods**

**Study Design**

This was a retrospective observational study of existing patients in the Infectious Diseases Institute, a Ryan White–funded infectious diseases clinic at the University of Oklahoma Health Sciences Center, Oklahoma City campus. Patients were screened for eligibility if a genotype assay was available in the vLink Online Patient Reporting database (Monogram Biosciences, South San Francisco, CA). This secure online database houses all genotypes, phenotypes and other resistance assays collected from HIV-infected patients receiving care at the University of Oklahoma Health Sciences Center from September 2005 to present. The first genotype collected from each patient between September 1, 2005 and August 30, 2011 was reviewed for viral point mutations. Demographic (gender, age, race, country of birth, and HIV risk category) and disease parameters (CD4 cell count and plasma HIV RNA at the time of genotype collection, and date of HIV diagnosis) were also collected to examine associations with TDR events. This study was approved by the University of Oklahoma Health Sciences Center Institutional Review Board.

**Study Population**

Antiretroviral-naïve, HIV-infected subjects aged 18 to 70 years and pregnant emancipated minors in the Infectious Diseases Institute clinic who received genotypic resistance testing prior to initiation of treatment were eligible for inclusion in the study. Patients were excluded if the genotype was unable to be performed for any reason (eg, insufficient viral load, specimen volume). Approximately 20% of HIV-infected patients who attend the clinic reside in rural settings, predominantly in Central and Western Oklahoma. The remaining population resides in urban and suburban areas.

**Resistance Analysis**

Primary (ie, initially selected) and secondary (ie, compensatory) drug resistance mutations (DRMs) were identified from genotypes using the 2011 International Antiviral Society–USA DRM (IAS) list for nucleoside reverse transcriptase inhibitors (NRTI), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs).⁷ DRMs were also categorized according to the 2009 World Health Organization (WHO) surveillance drug resistance mutations (SDRMs) list for final analysis.⁸ The SDRM list is a standardized subset of mutations on the IAS list (and 4 other expert mutation lists), which has been identified as being important to follow for surveillance purposes, and is the worldwide preferred list for surveillance reporting of this kind.

**Statistical Methods**

Descriptive and inferential statistics were conducted using Stata v13 (StataCorp LP, College Station, TX), with the a priori level of significance set at \( P < .05 \). Subjects were divided into yearly time periods based on date of genotype collection. The yearly time periods were analyzed by \( \chi^2 \) test to assess the potential association between time period and frequency of mutations. Descriptive statistics were used to describe demographic data and frequency of DRMs and SDRMs. A test of proportions was used to compare the frequency of DRMs seen in Oklahoma with national data. Bivariable analyses were performed using Pearson \( \chi^2 \) tests and independent \( t \) tests, depending on the variable measurement, to assess potential associations between baseline patient demographic variables and presence of DRMs and SDRMs. Variables identified in the previously described bivariable analyses as being significantly associated with the presence of DRMs were included in a multivariable logistic regression.

**Results**

**Demographics**

A total of 999 patients were screened for eligibility between September 2005 and December 2011; 428 (43%) met inclusion criteria. The most common reason for exclusion was prior use of antiretrovirals (37.2%). Thirty-nine patients (3.9%) were excluded because of genotype assay failure.
The majority of the study population was male (74%); 49% were white, 35% black, 10% Hispanic, and 6% Native American (Table 1). Median age was 33 years (interquartile range [IQR] = 25-44). More than half (57%) of the patients reported men having sex with men as the risk factor for HIV disease acquisition. Median CD4 count at time of genotype collection was 335 cells/mm³ (IQR = 169.75-526.00 cells/mm³), with 30% having a CD4 count < 200 cells/mm³. The median number of days from diagnosis to genotype collection was 104 (IQR = 49.0-457.8). HIV-1 subtype B was most commonly present (97%) in patients meeting the inclusion criteria. Non-B subtypes included subtypes A (1.2%), C (0.7%), G (0.2%), and recombinant viruses (1.2%).

### Drug Resistance

**International Antiviral Society–USA Drug Resistance Mutations.** Primary DRMs in the IAS DRMs list were detected in a total of 54 patients (12.6%; Table 2). This proportion is similar to the CDC’s national estimate of 14.6% (P = .08, one sample test of proportions). Primary mutations to NNRTIs were observed most frequently (35 patients, 8.2%), followed by PIs (15 patients, 3.5%) and NRTIs (14 patients, 3.3%). DRMs to first-generation NNRTIs were present in 21 patients (4.9%) and to second-generation agents in 19 patients (4.4%). The K103N/S mutation was the most common first-generation NNRTI DRM observed, occurring in 3.5% of patients, followed by the K101P/E (0.5%), Y181C (0.5%), and G190A/S (0.5%) mutations (Table 3). The E138A accounted for the majority of DRMs to second-generation NNRTIs and occurred with the same frequency (3.5%) as the K103N/S mutation. The H221Y mutation was present in 2 patients (0.5%). Primary protease inhibitor mutations were detected in 3.5% of patients and included L90M (1.6%), M46L/I (1.2%), and V82A (0.7%). DRMs to NRTIs included the T215C/D/I (1.9%), M41L (1.4%), T69D (0.7%), and K219Q/N (0.7%). Mutations observed in 0.5% or less of patients included K70E/R, K65R, D67N, and...
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V75M, and L210W. Similarly, only 0.5% of patients had an M184V mutation.

World Health Organization Surveillance Drug Resistance Mutations. Surveillance drug resistance mutations were detected in 49 of 428 treatment-naive patients sampled (11.4%; Table 3). NRTI SDRMs were most commonly observed (21 patients, 4.9%), followed by NNRTIs (18 patients, 4.2%) and PIs (16 patients, 3.7%). Dual class SDRMs were detected in 6 patients (1.4%), and were also on the IAS list. No triple class resistance was found during the study period.

Time Trends

No change in the frequency of overall drug resistance over time was apparent during the study period when analyzing SDRMs detected annually between 2008 and 2011 (P = nonsignificant; Figure 1). No increase in resistance to individual antiretroviral classes was detected when comparing yearly time periods. Dual class SDRM resistance was first evident in 2009 in a single patient and continued to be detected in 2010 (2 patients) and 2011 (3 patients).

Bivariable and Logistic Regression Analyses

Bivariable analyses of virtually all patient demographic and baseline variables yielded no significant differences or associations with the presence of DRMs or SDRMs, with the exception of age, where patients with DRMs were found to be, on average, 4 years older than patients without DRMs (38.4 vs 34.3 years, respectively; P = .02). However, this significant difference disappeared when demographic and baseline variables were examined using a multivariable logistic regression. No variables were identified as being significantly associated with the presence of DRMs or SDRMs (results not shown).

Discussion

We sought to characterize the epidemiology of DRMs in antiretroviral treatment-naive patients in an outpatient clinic that serves a large rural population of HIV-infected patients in central and western Oklahoma. The estimated rate of HIV transmission in Oklahoma is 8.8 per 100,000 population—a rate almost half of the national average, and lower than many of the states with published TDR data. To our knowledge, this is the first published study to look for the presence of TDR exclusively in the South Central United States. Primary DRMs were seen in 12.6% of patients throughout the 6 years of this study, which is consistent with the average frequency of 14.6% observed by the CDC in eleven locations across the United States (P = nonsignificant, one-sample test of proportions). Similar to TDR results from North Carolina, but unlike findings from New York City and San Francisco, we observed no significant increase in DRM frequency over the 6-year evaluation period in our population. The fact that our clinic population includes a significant number of rural patients may decrease the epidemiologic clustering of TDR that has been observed in larger urban populations of HIV-infected patients.

The primary clinical implication of this finding is that, consistent with guideline recommendations, assessment of a baseline genotype prior to ART initiation is critical to minimize risk of virologic failure on first-line ART, even in a geographic location that has been underrepresented in previous TDR surveillance studies. DRMs to NNRTIs were most commonly observed in our study sample, consistent with findings from other published epidemiologic studies on drug resistance to antiretrovirals. However, a surprising observation was the frequency of DRMs to second-generation NNRTIs (4.4%), where the occurrence of mutations was nearly as common as with first-generation NNRTIs (4.9%). The most common second-generation DRM observed was E138A, which has been observed in patients experiencing virologic failure on NNRTIs and is associated with a 1.9-fold change in susceptibility to rilpivirine. The frequency of this mutation was higher (P = .04, test of proportions) in our sampled population (15/428; 3.5%) than in a recent surveillance report of wild-type viruses (150/7257; 2.1%) with matching phenotypic and genotypic data. This mutation has previously been described as more prevalent in therapy-naive HIV-1 subtype C–infected persons (8% to 12%) compared with subtype B–infected persons (2.6%). However, a post hoc analysis of our data to determine whether the higher rate of E138A mutations was because of a higher frequency of non–subtype B infections found no relationship (n = 1 vs n = 14 for subtype B). The single non–B subtype present was reported as a complex recombinant virus and did not have a primary subtype.

The E138A mutation was first observed among our data in 2 genotypes from 2007, predating the market approval of
the second-generation NNRTI, etravirine, in 2008. Other second-generation NNRTI DRMs (Y181C, H221Y) that affect drug susceptibility were also observed but were infrequent (≤0.5%). High-level resistance to etravirine and rilpirivirine typically requires accumulation of multiple DRMs; however, specific mutations (eg, Y181C, E138A/G/K/Q/R) can significantly impair the activity of these agents and limit their utility in treatment. The frequency of E138A in our population may limit the role of rilpirivirine-based ART in patients who develop resistance to first-generation NNRTIs.

Mutations to NRTIs consisted primarily of T215C/D/I (1.9%) and the thymidine analog mutation, M41L (1.4%). Fortunately, these mutations have little impact on expected virologic success with the 2 first-line NRTIs recommended for treatment-naive patients by the US Department of Health and Human Services, emtricitabine and tenofovir.7 As mutations at codons 41 and 215 were most likely selected for by older NRTIs, including zidovudine and stavudine, this information sheds light on the treatment history of source patients who transmitted their resistance, and would limit the therapeutic options for the newly infected patients if they were to develop resistance to first-line NRTIs.

Differences in the WHO SDRM and IAS DRM lists accounted for whether NRTI or NNRTI mutations were more commonly observed. For example, T215C/D/I is recognized as an SDRM but is not included as a DRM in the IAS mutation list, while E138A/G/K/Q/R is recognized as a DRM in the IAS list but not included on the WHO list. Both the M184V and K65R mutations were infrequently observed (0.5% and 0.2%, respectively); however, it is possible that some patients harbored archived M184V mutations that could not be detected because of overgrowth of wild-type virus. Fewer patients with SDRMs to protease inhibitors were observed compared with other antiretrovirals (16 vs 18 for NNRTIs and 21 for NRTIs).

Our study had several important limitations. First, the list of SDRMs that was applied in our analysis was last updated in 2009. Primary point mutations associated with rilpirivirine were thus not counted in the SDRM data, though they were accounted for in the IAS mutation list. Therefore, it is likely that our estimate of mutation frequency would have been higher with a more recently updated list of SDRMs. Resistance testing to integrase strand transfer inhibitors was not commercially available until 2009 and is not routinely performed at baseline in our clinic because it requires a separate genotype test.17 Given the low frequency of baseline integrase RAMs reported in recent clinical studies of antiretroviral-naïve patients, we do not believe the absence of integrase resistance testing results represents a significant limitation.18,19 Also, our clinic population is composed of patients from central and western Oklahoma. Data from a separate clinic in Tulsa that provides care to patients from eastern Oklahoma were not included; neither were data from patients that are seen by private physicians around the state.

It is estimated that our clinic population represents approximately 60% of the patients with HIV infection in the State of Oklahoma, so we believe our findings are likely to be representative of the frequency of DRMs in those additional populations. However, because of the small numbers of patients in certain subgroups (eg, racial/ethnic minorities, foreign birth, or HIV risk factors not involving sexual transmission), we cannot exclude the possibility that our findings regarding associations with the presence of DRMs or SDRMs would have been different had there been a more balanced representation in each of the subgroups. In addition, genotype testing was not consistently performed by all providers in treatment-naive patients in our clinic until 2007, so the smaller number of genotypes conducted in the years 2005 and 2006 may have limited our ability to detect changes in TDR rates across the 6-year evaluation period.

Conclusion

The frequency of DRMs in HIV-infected patients in central and western Oklahoma was similar to national rates across the United States, and reinforces the need for baseline genotypes prior to ART initiation regardless of location of care. Although the overall distribution of DRMs was consistent with national trends, the frequency of second-generation NNRTI DRMs in our population was unexpected and may limit future treatment options when resistance to first-generation NNRTIs develops. Close attention should be paid to epidemiologic studies to detect trends signifying increasing rates of resistance to individual classes which may impact first-line recommendations and subsequent therapy options.

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Authors' Note

At the time of data collection and article preparation, Dr Kleyn was a PGY2 HIV Pharmacotherapy Resident at the University of Oklahoma College of Pharmacy.

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