Prevalence of transmitted HIV drug resistance since the availability of highly active antiretroviral therapy

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Abstract

We investigated the prevalence of HIV-1-associated transmitted drug resistance (TDR) in Victoria from the time of first availability of highly active antiretroviral therapy. Drug resistance genotyping was performed on virus present in blood samples collected from individuals with serologically confirmed primary infection, between 1996 and 2007. The significance of any mutations detected was interpreted according to a standardised list of drug resistance mutations. The main outcomes measured were the prevalence by year of TDR to any antiretroviral drug class, the numbers of infected individuals with TDR involving multiple drug classes, and the resistance mutations implicated in all cases. There was an average annual prevalence of TDR of 16%, predominantly associated with nucleoside and non-nucleoside reverse transcriptase (RT) inhibitors and most commonly occurring at codons 41, 103 and 215 in the RT. The prevalence of thymidine-associated mutations remained high throughout the period of study. While mutations known to cause resistance to protease inhibitors were uncommon, they were present in several individuals infected with virus resistant to multiple drug classes. The prevalence of TDR in Victoria is similar to geographical locations outside Australia where HIV-specific drug treatment is widely available. Primary infection with drug resistant HIV is a future treatment issue for the individual patient and for the wider population at risk of infection. At this time TDR shows no sign of waning and our data support recent treatment guidelines recommending baseline testing for TDR before therapy is initiated. *Commun Dis Intell* 2009;33:216–220.

Introduction

The availability of highly active antiretroviral therapy (HAART) has produced significant decreases in the morbidity and mortality of patients infected with HIV. However, drug resistance is generated in a proportion of treated patients and may be directly transmitted from them to treatment-naïve individuals at the time of their primary infection. This process and its outcome is referred to as transmitted drug resistance (TDR).

Reports on TDR published during the last 10 years show average prevalences in developed countries ranging from 10 to 20% where use of HAART is widespread, with some variation from year-to-year in certain locations.[1–5] In regions where HAART-usage is less common, for example some areas of Asia and Africa, TDR has also been documented.[6,7] TDR has been regularly demonstrated for 3 antiretroviral drug classes, the nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs, respectively) and protease inhibitors (PIs).[1,8] A single case of transmitted resistance to the fusion inhibitor enfuvirtide has been reported.[9] Several cases of acquired resistance to the first available HIV integrase inhibitor (raltegravir) have been described[10] but there are no reported cases of transmitted resistance to this drug.

The presence of resistance mutations in HIV strains transmitted at the time of infection theoretically diminishes the efficacy of individual drugs to which the mutations apply and may lower the genetic barrier to other drugs in the same class. While a large clinical study has shown the clinical impact of TDR to be subtle during first-line therapy,[11] a number of reports suggest it limits treatment options and clinical response is improved by genotype-directed therapy.[2,12] The impact of TDR on second-line and subsequent treatments is largely unknown. Drug resistance testing prior to commencement of antiretroviral (ARV) drug therapy is now incorporated in the HIV treatment guidelines of many countries including Australia, where it is recommended as a baseline test even if treatment is not being considered immediately.[13]

Evidence for TDR in the above studies has been obtained by genotyping. This is also the recommended method in Australia because of its availability in State HIV reference laboratories. The interpretation applied to specific mutations detected by genotyping may influence the reported prevalence, and a standardised mutation list has been proposed that will enable TDR surveillance programs, which are increasing in number and location, to produce comparable estimates of TDR rates.[14] It is important, therefore, that laboratory testing reliably distinguishes recently infected cases from newly diagnosed cases.

This report presents the results of 12 years of surveillance of TDR in Victorian patients, commencing in 1996 when HAART became widely used for the first time, and concluding with cases to the end of 2007. The results have important implications for first-line therapy in many patients and emphasise the continuing need for baseline resistance testing as part of HIV clinical practice.

Methods

Genotyping for TDR was performed on available plasma samples from individuals infected with HIV according to one of the following test results: western blot evolving from negative or indeterminate profile to full profile within 12 months, full western blot profile following a negative enzyme immunoassay (EIA) within 12 months, or (only for patients infected in 1999 and 2000) a detuned EIA test result suggesting recent infection.[15] A total of 466 patient samples were tested by the Victorian Infectious Diseases Reference Laboratory (VIDRL), representing approximately 15% of likely new cases of infection over the study period. Because the VIDRL is the reference laboratory for HIV diagnosis in Victoria, this cohort is not thought to be subject to referral bias. The testing period included the years 1996 to 2007 inclusive. Statistical analysis was undertaken using the Pearson chi squared test. Formal ethical approval for the study was not sought. Testing was performed as a result of doctors' requests for genotyping or, prior to the widespread availability of genotyping, as part of the laboratory's surveillance role.

Genotyping of the reverse transcriptase and protease regions was performed on all samples as previously described.[16] Genotyping of the HIV gp41 region for enfuvirtide susceptibility was performed only on samples from the year 2000 onwards as previously described.[17] Testing for evidence of TDR associated with raltegravir therapy was not undertaken. Inclusion of mutations as being associated with TDR was made according to a proposed standardised list of drug-resistance mutations.[14] To enable comparison of the prevalence of transmitted mutations with that of acquired mutations, the results of genotyping performed since 1996 on samples from more than 1,500 patients with acquired drug resistance, were extracted from our electronic laboratory reporting system to generate a database providing the frequency of all recognised resistance mutations. These results were then compared to those obtained on patients with known recent infections according to the above criteria.

Results

Genotypic evidence for TDR was present in 75 of 466 (16%) recently infected individuals tested between 1996 and 2007, inclusive (Table 1). Of the cohort investigated 449 were males and 17 were females. The median age was 35. The majority of individuals were infected via homosexual transmission. Of the 75 patients in whom resistant virus was detected, 72 (96%) were male. All cases involved subtype B HIV strains except for 1 female infected with subtype C virus and a male with a subtype CRF01-AE infection.

Although some fluctuation in the peak annual prevalence of TDR occurred (33% in 1996 versus 9% in 2007, Table 1), in the intervening years the prevalence was broadly stable and overall there was no statistically significant difference from year to year ($P=0.75$). Resistance was mainly associated with NRTIs and involved 50 cases (67%). Resistance to NNRTIs was present in 27 cases (36%), while resistance to PIs was relatively uncommon (10 cases, 13%). Ten patients were infected with HIV strains resistant to more than 1 drug class and two of these were infected with virus resistant to 3 classes. There were no cases of resistance to enfuvirtide.

Codons in RT and protease associated with TDR are shown in Table 2. Mutations associated with resistance to PIs were rare. The 2 most common individual mutations, M41L and K103N, were present in 25% and 28% of TDR cases, respectively, compared with 29% and 19%, respectively, in cases of acquired resistance. Thymidine-associated mutations (TAMS) 41L, 67N, 70R, 210W, 215Y (plus any 215-revertant) and K219Q were also very common, with no obvious evidence for a decline in their prevalence over time (Table 1). Although T215Y mutations were less common in the TDR population than in patients with acquired resistance (4% versus 23%, respectively), the inclusion of 215-revertants raised the incidence of any mutation at codon 215 to 45% in the TDR cohort.

Five of the 75 (7%) cases involved a methionine (M) mutation at codon 184, a prevalence considerably lower than observed in patients in our database with acquired resistance (35%).

Discussion

Over the 12 years of this investigation the overall prevalence of TDR in Victoria was 16% and this was mainly associated with treatment involving NRTIs, zidovudine in particular. More than half the TDR cases we identified were infected with virus containing TAMs, although in 1996 and 2007 none were detected. However, in each subsequent year including 2008 (results not shown), these mutations were once again present in some cases. Therefore it appears that many of the cases in this and other studies occur as a result of the long-term use of zidovudine, including monotherapy and dual therapy prior to the advent of HAART.

Table 1: Number and percentage of cases of transmitted drug resistance, the drug classes implicated according to the mutations detected and the number of cases infected with viruses containing thymidine-associated mutations, 1996 to 2007

Year	Number of tests performed	TDR patients		Drug classes						TAMs	
		n	%	PI	NRTI	NNRTI	PI/NRTI	NRTI/ NNRTI	All classes	n	%
1996	9	3	33		3					3	100
1997	12	1	8	1						0	0
1998	14	3	21		2			1		2	67
1999	27	7	26		6			1		7	100
2000	63	12	17	1	8	1	1		1	10	83
2001	21	3	14		2	1				2	67
2002	48	7	15	2	3	1		1		3	43
2003	38	6	16		1	4	1			2	33
2004	43	7	16		5			2		6	86
2005	55	8	15	1	4	2			1	5	63
2006	72	12	17		6	5		1		5	42
2007	64	6	9	1		5				0	0
Total	466	75	16	6	40	19	2	6	2	45	60

TDR Transmitted drug resistance.
TAMs Thymidine-associated mutations.
PI Protease inhibitor.
NRTI Nucleoside reverse transcriptase inhibitor.
NNRTI Non-nucleoside reverse transcriptase inhibitor.

Table 2: Resistance mutations associated with transmitted drug resistance cases

NRTI		NNRTI		PI	
Mutation	n	Mutation	n	Mutation	n
T215S/D/C/E/I	31	K103N	21	L90M	4
M41L	19	Y181C	4	M46I	3
K219Q	7	L100I	1	I84V	2
M184V	5	V106A	1	L24I	1
L210W	5	Y188H	1	G48V	1
D67N	4	Y188L	1	I54L	1
K70R	4	L190S	1	I54T	1
T215Y	3			I54V	1
M184I	2			V82A	1
K65R	1				
L74V	1				
V75A	1				
V75M	1				

Mutations were included according to a published list.[14] The numbers indicated refer to the total number of individuals with this mutation during the study period. Some individuals had more than one mutation.

PI Protease inhibitor.
NRTI Nucleoside reverse transcriptase inhibitor.
NNRTI Non-nucleoside reverse transcriptase inhibitor.

As more potent drugs have become available for use in clinical practice and the once-widespread use of zidovudine has declined, a concomitant decrease in TDR prevalence might have been expected. However, despite increasing use of abacavir/lamivudine and tenofovir/emtricitabine in first-line therapy in recent years, we identified only 1 case involving the K65R mutation associated with tenofovir resistance[18] and the M184V resistance mutation remained uncommon. In our acquired resistance database, M184V is the single most common mutation, present in nearly one third of all patients with resistance. However it was only detected in 7% of TDR cases, which is consistent with previous reports. This observation is likely to be related to a combination of reduced transmission efficiency associated with low viral titre and poor replicative fitness of these mutants in the transmitter, as well as impaired fitness in the absence of a selective drug pressure in the TDR cases.[19]

It is unclear why the prevalence of K103N mutations was higher in TDR cases than cases of acquired resistance and it is possible that the apparent difference between the 2 populations is coincidental. Nevertheless, we have previously reported the common occurrence of both mutations in patients with

untreated primary HIV infection in Melbourne,[19] suggesting that viruses with a K103N mutation may be preferentially transmitted.

In contrast to the many reports from Europe and the United States of America, only 1 study on TDR in Australia has been described.[21] It showed a low and stable rate of resistance to PIs (consistent with this and many other studies in a variety of geographical locations) and a decrease in resistance to inhibitors of the HIV RT between 1992 and 2001, a period overlapping introduction of HAART to Australia in 1996. This decline is likely to reflect the addition of NNRTIs and PIs to ARV treatment that until that time comprised a choice of zidovudine, didanosine or zalcitabine. The increased antiviral potency associated with HAART is likely to have reduced resistance rates associated with monotherapy and, as a consequence, the incidence of TDR. From 1997, the rates of TDR in Sydney and Melbourne have been similar.[21] Nevertheless there are differences between the data gathered in these cities when the study times overlapped. In particular, several patients living in Melbourne were infected with virus resistant to multiple drug classes whereas none were seen in Sydney during the study period examined. Whether this difference has been sustained will require further studies on patients with primary infection in Sydney.

There are some drawbacks to investigations of the type we describe. In particular we studied only a proportion of the total number of newly infected patients detected on an annual basis in Victoria. In addition, the time post-infection of blood samples for genotyping varied from patient to patient, possibly biasing the proportions of individual mutations observed. This limits any generalisation that can be made between mutations considered to be transmitted versus those likely to be acquired as a result of failure of drug therapy. Finally the genotyping method employed, while standard in most reference laboratories undertaking such studies, has limited sensitivity. As such the overall TDR prevalence is possibly underestimated.

With the exception of a small number of published studies,[5,21,22] investigations on the prevalence of TDR have involved relatively short time frames post the availability of HAART. This approach highlights the importance of TDR but does not show changes in either its prevalence or the ARV drug classes implicated, both of which would be predicted to evolve as new drugs become available clinically and the use of some drugs declines. Our study is one of the longest reported to date on the prevalence of TDR. As such, it reveals a stable prevalence in Victoria over 12 years on a background of a high incidence of TAMs. Despite the relatively stable TDR rate in this location, the overall prevalence of 16% highlights the need for baseline resistance testing prior to commencing HAART.

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