

Prevalence of drug-resistant mutations in newly diagnosed drug-naïve HIV-1-infected individuals in a treatment site in the Waterberg district, Limpopo province

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Aim. We studied the prevalence of resistance mutations in drug-naïve HIV-infected individuals at the Bela-Bela treatment site to gather information on the presence of antiretroviral (ARV) drug-resistant viruses in drug-naïve populations, so as to improve treatment guidance.

Subjects and methods. Drug-naïve HIV-1-infected individuals were sequentially recruited between February 2008 and December 2008 from individuals visiting the voluntary counselling and testing (VCT) services of the Bela-Bela HIV/AIDS Wellness Clinic. Viral subtyping was done by phylogenetic analysis; drug-resistant mutations were determined according to the Stanford HIV Drug Resistance Interpretation and the International AIDS Society-USA Guidelines.

Results. A drug-resistant mutation prevalence of 3.5% (95% confidence interval 0.019796 - 0.119077) comprising Y181C and L33F was observed; 98% of the viruses were HIV-1 subtype C on the protease (PR) and reverse transcriptase (RT) gene regions.

Conclusion. The prevalence of drug-resistant mutations in drug-naïve persons may be low in Bela-Bela after 8 years of access to antiretroviral treatment (ART), and resistance testing before initiating treatment may not be needed.

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Highly active antiretroviral therapy (HAART) has dramatically reduced morbidity and mortality among HIV/AIDS patients. However, a major drawback of HAART is the development of drug resistance.^{1,2} South Africa has a high HIV prevalence, and access to treatment is expanding. A regimen based on the genetic resistance profiles of patients' viruses before initiating therapy generally correlates with a better outcome, compared with the absence of such resistance data.³ The emergence of drug resistance in drug-naïve populations should be monitored, particularly in regions such as Limpopo Province, where such data are scarce. We aimed to determine the prevalence of ARV drug-resistance among drug-naïve HIV-infected individuals in a community where treatment has been provided for a considerable time.

Methods

Study subjects were recruited from the HIV/AIDS Wellness Clinic in Bela-Bela, Waterberg District, Limpopo Province. Treatment at the clinic began in 2001. HIV-positive individuals without prior exposure to ARV were recruited sequentially between February 2008

and December 2008. Five ml of venous blood was collected into EDTA vacutainer tubes from each consenting subject. Demographic data were obtained by questionnaire administration. Viral RNA was isolated using the viral mini RNA kit (Qiagen). Viral DNA was synthesised by a one-tube reverse transcription-polymerase chain reaction (RT-PCR), followed by a nested PCR.³ The PCR products were verified for expected size by electrophoresis of 1% agarose gel. PCR amplicons were purified with a QIAquick PCR purification kit (Qiagen), and direct population-based sequencing was performed on both strands. HIV subtyping was done by phylogenetic analysis. Predicted amino acids for the PR and RT genes were aligned using the BioEdit programme (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Owing to the broad scope of viral mutations, drug-resistant mutations were identified using the Stanford HIV Drug Resistance Interpretation Algorithm (<http://hivdb.stanford.edu/>) and the International AIDS Society (IAS)-USA Guidelines.⁴ Two algorithms were used to describe as broadly as possible the significance of observed amino acid substitutions. The Bayesian analysis was used to calculate the 95% confidence interval (CI), to estimate the confidence limits around the prevalence estimate. The PR and RT gene sequences reported here have been submitted to GenBank with the following respective accession numbers: PR [GU139577-GU139633] and RT [GU139634-GU139688]. Approval of the study protocol was obtained from the Health, Safety, and Research Ethics Committee of the University of Venda, Thohoyandou.

Results

A total of 79 HIV-infected individuals were sequentially recruited. The mean age was 43.5 (18 - 69) years. The most important risk factor for HIV transmission was sexual intercourse (88.6%), but the questionnaire was not designed to determine whether this was heterosexual or homosexual. In 90% of the cases the most probable place of HIV infection was South Africa. Viral DNA was obtained for 62 out of 79 (78%) individuals. Reliable nucleotide sequences were obtained for 57 PR genes and 55 RT genes. Using the Stanford and IAS guidelines, major drug-resistant mutations were detected in 2 out of 57 subjects (3.5%) (95% CI 0.019796 - 0.119077). One primary NNRTI mutation (Y181C) and one major PI mutation (L33F) were

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Table 1. Drug resistance-associated mutations, frequency, coding nucleotides and HIV-1 subtypes from HIV-infected patients in the Waterberg District

Protease gene (N=57)	Coding nucleotides			Reverse transcriptase (N=55)	Frequency	Coding nucleotides			HIV-1 subtype	Comment
	Wild type	Mutant	HIV-1 subtype			Wild type	Mutant	HIV-1 subtype		
L101/F	2 (3.5)	CTC	ATT/CTT	C	1 (1.8)	ACT	AAT	C	Secondary mutation (Stanford); primary mutation (IAS)	
K20R	19 (33.3)	AAG	AGG	C	1 (1.8)	TTA	GTA	C	Secondary mutation (Stanford); undocumented (IAS)	
L23F	2 (3.5)	CTA	TTT	C	1 (1.8)	AAA	CAG	C	Secondary mutation (Stanford); undocumented (IAS)	
D30G	1 (1.7)	GAT	CGT	C	3 (5.5)	GTT	ATT	B & C	Secondary mutation (Stanford); undocumented (IAS)	
L33F	1 (1.7)	TTA	AGA	C	1 (1.8)	GAG	GCA	C	Polymorphism (Stanford); but a secondary mutation to etravirine (IAS)	
M36I	46 (80.7)	ATG	ATA	C	1 (1.8)	GTT	GAT	C	Minor mutation according to Stanford and IAS	
L63S	7 (12.3)	CTC	TCT/AGT	C	1 (1.8)	TAT	GTC	C	Primary mutation to efavirenz/etravirine/nevirapine (Stanford and IAS)	
A71L/T	2 (3.5)	GCT	TGT/AAA	C	1 (1.8)	TTG	AAA	C	Secondary mutation (Stanford); undocumented (IAS)	
T74S	4 (7.0)	ACA	TCA	C						
L89M	46 (80.7)	CTG	ATG	C						
I93L	54 (94.7)	ATT	CTT	C						

detected in two different patients. No primary NRTI mutation was recorded. The detected mutations, nucleotide substitution, and their potential significance are shown in Table I.

Phylogenetic analysis of the PR and RT genes showed that 56/57 (98.2%) of the isolates were HIV-1 subtype C on both gene regions. One isolate (08BBVCT31ZA) was HIV-1 subtype B on the PR and RT genes (phylogenetic trees not shown). Mean genetic distance for the PR sequences ranged from 0.0101 to 0.2035 and 0.0331 to 0.1377 for the RT sequences. Considering the first 300 amino acids in the RT gene, sequence alignment showed that the consensus sequences of the test viruses were identical to the global subtype C consensus, except at 2 positions (V60I and Q174K). The consensus sequence differed from the global subtype B consensus at 17 positions (V35T, E36A, T39E, S48T, V60I, K122E, D123G, K173A, Q174K, D177E, T200A, Q207E, R211K, V245Q, A272P, K277R, and T286A). Amino acid alignment of the PR gene showed that the test consensus was identical to the global subtype C consensus, except at position I13T. It differed from the global subtype B consensus at 8 positions (T12S, I15V, L19I, M36I, R41K, H69K, L89M, and I93L).

Discussion

The development of drug resistance is an important attribute of HIV biology. An inevitable drawback of HAART is the emergence of drug-resistant variants in patients under treatment, which complicates treatment options and hampers good prognosis. Antiretroviral therapy was started at the Bela-Bela Wellness Clinic about 8 years ago, within which time resistance could have possibly emerged. The low rate (3.5%) of drug-resistant mutations detected in this study is similar to reports in other parts of South Africa. Drug-resistance studies among naïve patients reported a prevalence in Gauteng Province in 2002 and 2004 of 4.2%;⁵ in Cape Town of 2.5%;⁶ and 3.6% in Free State Province.⁷ Low levels (<5%), or the absence of drug-resistant mutations, have been reported in Zambia⁸ and Malawi.⁹ Higher rates have been reported in several developed countries with more than a decade of ART history.^{10,11}

Low rates of ARV drug resistance are expected in developing countries because most patients start therapy on highly potent regimens, unlike in the developed world where ART scale-up began with resistance-associated monotherapy and one-class dual therapy. In this study, one primary NNRTI mutation (Y181C) was observed (Y181C mutation causes high-level resistance to nevirapine and delavirdine and low-level resistance to efavirenz), while no primary NRTI mutation was noted. This mutation occurred in a 40-year-old married woman. At the time of the investigation, the South African national drug regimen guideline stipulated the use of stavudine, lamivudine and efavirenz as first-line therapy for those who had never been exposed to ARVs, with nevirapine replacing efavirenz for women of child-bearing age.¹² It is not clear whether the patient who harboured the Y181C mutation had been enrolled in a mother-to-child transmission prevention programme in which nevirapine was used.

One major PI mutation (L33F) was detected. This mutation is classified as a major mutation by the Stanford Drug Resistance

Interpretation Algorithm, and as a minor mutation by the IAS-USA guidelines (L33F mutation is selected by fosamprenavir/ritonavir, duranavir/ritonavir, lopinavir/ritonavir, atazanavir/ritonavir, and tipranavir/ritonavir, and contributes to resistance to these drugs).

Conclusion

This study indicates that the prevalence of drug-resistant HIV among the drug-naïve population in Bela-Bela is low after 8 years of free ART. We used population-based sequencing in our study, which might have contributed to an underestimation of drug-resistance prevalence. Patients may harbour populations of minor-resistance viruses that are usually not detected by population sequencing. This prevalence study also used chronically infected patients, which could mean that resistance acquired might have disappeared over time, which might have also led to an underestimation of the results.

Apparently, the testing of patients for drug-resistant viruses before initiation of therapy may not be required. However, subsequent periodic studies are required in Bela-Bela and other treatment sites to monitor the emergence and spread of drug-resistant viruses. Such data are important for guiding policy on sentinel surveillance and treatment algorithms. A high prevalence could indicate the need for regular sentinel surveillance or for baseline genotypic drug-resistance testing before treatment.

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