East African Medical Journal Vol. 88 No. 1 January 2011
REVERSE TRANSCRIPTASE INHIBITORS DRUG RESISTANCE MUTATIONS IN DRUG-NAIVE HIV TYPE 1 POSITIVE KENYAN INDIVIDUALS

A. K. Nyamache, BSc, MSc, PhD, Lecturer, Department of Plant and Microbial Sciences, Kenyatta University, P. O. Box 43844-00100, Nairobi, Kenya, R. Waihenya, BSc, MSc, PhD, Senior Lecturer, Z. W. Ng'ang'a, BSc, MSc, PhD, Associate Professor, Director Institute of Tropical Medicine, A. W. Muigai, BSc, MSc, PhD, Associate Professor, Department of Botany and Microbiology, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000-00200, Nairobi and S. A. Khamadi, BSc, MSc, PhD, Senior Research Scientist, Centre for Virus Research, Kenya Medical Research Institute, P. O. Box 54840-00200, Nairobi, Kenya

Request for reprints to: A. K. Nyamache, Lecturer, Department of Plant and Microbial Sciences, School of Pure and Applied Sciences, Kenyatta University, P. O. Box 43844-00100, Nairobi, Kenya

REVERSE TRANSCRIPTASE INHIBITORS DRUG RESISTANCE MUTATIONS IN DRUG-NAIVE HIV TYPE 1 POSITIVE KENYANS

A. K. NYAMACHE, R. WAIHENYA, Z. W. NG'ANG'A , A. W. MUIGAI and S. A. KHAMADI

ABSTRACT

***Objective*: To evaluate the extent of HIV-1 drug resistance among drug naïve Kenyan individuals.**
***Design*: Cross-sectional study.**
***Setting*: Kenya Medical Research Institute HIV laboratory Nairobi, Kenya.**
***Subjects*: A total of seventy eight HIV-1 positive drug naïve subjects randomised from five Kenyan provincial hospitals between April and June 2004.**
***Results*: A major non-nucleoside reverse transcriptase (NNRTI) an associated mutation was found in one patient (1.3%). NNRTI associated resistance mutations were present at amino acid codon sites G98A (2.56%); K103E (1.3%) and L100F (3.57%) prevalences. Baseline resistance may compromise the response to standard NNRTI-based first-line ART in 1.3 % of the study subjects.**
***Conclusion*: This indicates in general, that drug resistance among HIV-1 positive drug naïve individual is at low thresholds (1.3%) but the problem could be more serious than reported here. Continuous resistance monitoring is therefore warranted to maintain individual and population-level ART effectiveness.**

INTRODUCTION

HAART therapies have dramatically reduced the mortality rate from human immunodeficiency virus (HIV) in the developed world (1). Unfortunately, current therapies are not curative, and many treated patients develop resistance to one or more drugs, which is costly and may lead to complete treatment failure and death. The drug resistant HIV-1 variants emergent in HAART treatment individuals are the major obstacle to anti-retroviral therapy and these drug resistant variants may be transmitted in newly infected individuals (2-3).

By the end of 2009, it was estimated that there were between1.3 and 1.6 million people living with HIV/AIDS in Kenya (4). Most of these patients are being treated by anti-retroviral treatment regimens (5). The provision of treatment has continued to be expanded through interventional programs such as the World Health Organization's (WHO's) 3 by 5 plan to treat three million people by the end of 2005 and the President's Emergency Plan for AIDS Relief has significantly promoted access to ART in low-income and middle-income countries (6-7). As access to ART rapidly increases in these resource-limited countries, the prevalence of HIV-1 drug-resistant strains among drug-naive patients is also expected to increase (7). In Kenya, the current standard first-line therapy consists of two NRTIs, Azidovudine (AZT) and lamivudine (3TC), plus one NNRTI, either nevirapine (NVP) or efavirenz (EFV). PI containing regimens are used as second line drugs (7).

The rapid up scaling of ART was accompanied by increased availability to those in need, increasing to 17% by the end of 2005 (8-9), 31% by 2008 and to 36% by 2009(4). WHO's ART guidelines for surveying and monitoring HIV drug resistance in resource-poor countries is being implemented but the high cost on constant drug resistance surveillance is still a challenge. In addition, the use of single dose NVP among HIV-infected antenatal clinic attendees influenced the need for laboratory monitoring (10-11). Studies have shown cases of acquisition of drug resistance mutations among HIV-1 vertically infected Kenya children (12). However, there are few reports on the magnitude of drug resistance in adults. The

current study was conducted to determine the RTI resistance-associated mutations among HIV-1-infected drug naive adults in Kenya where ART is being rapidly scaled up.

MATERIALS AND METHODS

Study population and samples: Individuals who were 18 years of age or older and who presented themselves to the clinic for treatment were considered for recruitment after giving informed consent (Table 1). Patients who reported prior exposure to ART or on a single dose NVP for prevention of mother-to-child transmission (PMTCT) of HIV, together with those who declined to consent were excluded from the study. A total of 78 HIV positive drug naive subjects randomised by computer generation to get representative sample of the five Kenyan provincial hospitals between April and June 2004 were evaluated. These were from Nairobi, Nyanza, Western, Central and North Eastern provinces. Five millilitres of blood was collected from each participant and tested for anti-HIV-1 antibodies using Unigold (Trinity Biotech, NY) and Determine (Abbott, IL). HIV-1 antibody positivity was further confirmed by enzyme-linked immunosorbent assay (ELISA) (Enzygnost, Dade-Behring, Marburg, Germany). Ethical clearance was obtained from the National Ethics Committee through the Kenya Medical Research Institute (KEMRI) National Ethical Review Committee.

PCR and sequencing: Peripheral blood mononuclear cells (PBMCs), from confirmed ELISA-positive samples, were obtained by Ficoll-Hypaque density gradient centrifugation and proviral DNA extracted by using DNAzol (GIBCO BRL, Life Technologies) lysis and ethanol precipitation (7). Briefly, blood was mixed with Ficoll-Hypaque and centrifuged with breaks to allow separations according to gradient and PBMCs separated by pipetting. Subsequently, 500μl DNAzol genomic DNA extract reagent (Gibco BRL®) was added to PBMCs pellet and mixed gently up and down by a pipette. To dissolved pellet, 1ml of cooled 4^0C absolute ethanol was added and mixed gently and centrifuged at 906xg for 15 minutes. Proviral DNA was then precipitated with 70% ethanol, air dried and extracted viral DNA resuspended in 100 μl DNAse/RNase free water. Nested polymerase chain reaction (PCR) was performed using AmpliTaq Gold (Roche Molecular Systems, Branchburg, NJ).

A segment of the HIV-1 RT gene corresponding to nucleotides 2265–3180 of HIV-1HXB2 was amplified using the primers RT18 (5′-GGAAACCAAAAAT GATAGGGGGAATTGGAGG-3′) and KS104 (5′-TGACTTGCCCAATTTAGTTTTCCCACTAA-3′) in the first round and KS101 (5′-GTAGGACCTACACC TGTTCAACATAATTGGAAG-3′) and KS102 (5′-CCC ATCCAAAGAAATGGAGGAGGTTCTTTCTGATG-3′) in the second round. Amplification was achieved using 1 cycle of 95^0C for ten minute and 35 cycles of 95^0C for 30 s, 55^0C for 30 s, and 72^0C for one minute, with a final extension of 72^oC for ten minute. The amplicons were sequenced as previously described (7, 12-14).

Genotypic drug resistance analysis: Genotypic drug resistance in the pol-RT region was defined as the presence of one or more resistance-related mutations, as specified by the consensus mutation figures of the International AIDS Society-USA (15).

RESULTS

RTI resistance-associated mutations: Minor RTI resistance–associated resistance were found in 1.3% of the seventy eight sequences (Table 2). Of these sequences one had NNRTI resistance associated mutations; at A98G, L100F and K103E codons. The detected NNRTI mutations were found from a sequence obtained from Nyanza province. In NRTIs, no primary nucleoside reverse transcriptase inhibitor mutations were detected. Nevertheless, other NRTI associated mutations were detected. These were L74F (1.3%), Y115I (1.3%), V118G (1.3%), Q151T (1.3%), Q151K (1.3%), E44G (1.3%), T69P (1.3%), K70Q (1.3%), V75R (1.3%), Y115L (1.3%) and V118G (1.3%) prevalences (Table 2). These were detected in only four (5.1%) of the subjects under study.

Table 1

Baseline Characteristics of HIV-1-Infected Kenyan Patients before Initiating Antiretroviral Therapy

Characteristic	Gender		
	All N=78	Female (n=46)	Male (n = 32)
Age (years)			
Mean (Range)	35(18-67)	31(18-67)	39(24-55)
CD4+ T cell count (Cells/mm^3)			
Mean (Range)	420(100-890)	433(44-800)	399(55-980)
Range			
<300	30	18	12
301-400	15	7	8
400-500	13	6	7
>500	20	8	12

Table 2

Prevalence of mutations-associated with drug resistance findings in HIV-1 positive drug naïve persons[a]

Drug class	N(78%)	Mutations positions detected
NNRTI major	1(1.3)	A98G, L100F, K103E
NNRTI associated	5(6.4)	S3R, E6A, V35T, E36D, K11P, L12F, V35T, K49R, S3R, E6A, K22Q, V35T, E6D, K20R, V21I, V35T, E6D, L12F, G15X, G18X
NRTI major	0(0)	None
NRTI associated	4(5.1)	L74F, Y115I, Q151T, Q151K, E44G, T69P, K70Q, V75R, Y115L, V118G

[a]NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor

DISCUSSION

In the current study, the prevalence of mutations that confer RTI resistance was found to be 1.3% among Kenyan drug-naive individuals sampled in 2004. These results assumed no differences in prevalence in provinces with small sample used being the reason. However compared to recent study conducted on samples collected in 2005, the prevalence was 7.5%, an indication of possible increase in drug resistance probably due to increased coverage for anti-retroviral (7). This finding is consistent with other findings from countries in Africa that have as well showed prevalence to be less than 5% (16-19). However, other studies conducted in the recent past have showed over 5% increase in drug resistance prevalence. Cameroon and Mozambique in 2004 showed drug resistance prevalence of 9.8 and 5.9% respectively (20-21). The difference may be due to the criteria used in the different studies. The latter studies were based on the WHO's HIV drug resistance threshold survey (21-22) but the former and our study were hospital based, that is, among patients seeking treatment.

In this study, one patient had NNRTI resistance mutations. The mutations occurred at codons A98G, L100F and K103E positions potentially implicated in drug resistance to the non-nucleoside reverse transcriptase inhibitor, Nevirapine. The detected mutation against Nevirapine meant that the person, if treated with Nevirapine might not respond to the medication. The mutations detected conferred low-level resistance to other NNRTI like Etravirine, Efavirence and Delaviridine suggesting that there could be low effectiveness of these drugs in persons taking them. In NRTIs, no primary nucleoside reverse transcriptase inhibitor mutations were detected.

The NRTI resistance mutations have a higher fitness cost for the virus and would not develop in the absence of drug pressure (24-25). Although mutations conferring NRTI resistance have previously been reported among drug naive patients (17, 26-28), the possibility that our patients had previous unreported contact with anti-retroviral drugs could not be excluded. Therefore, considering the limitations of a self-reporting system, drug resistance testing would be necessary before initiating ART in order to achieve a better clinical outcome.

The prevalence of HIV among Kenyan adults has remained relatively steady since 2003, after decreasing from a high of 14% in the late 1990s. The Kenya demographic and health survey of 2003 found a prevalence of 6.7% among individuals aged 15–49 years (4.6% in men and 8.7% in women) (7, 29), 7.1% by 2007 (30), and 6.3% by 2008(4). Access to ART in Kenya has significantly increased since the start of WHO's 3 by 5 initiative. The Kenya AIDS indicator survey of 2007 showed that of the estimated 392,000 Kenyan adults in need of ART, 138,000 (35%) had received the treatment by September 2007, which increased to 212,000 (54%) by June 2008(29). The increase in ART coverage is expected to lead to an increase in drug-resistant strains among drug-naive patients. In addition, stigma and cultural backgrounds still existing in Kenya may affect ART compliance, resulting in an accelerated appearance of drug-resistant mutants, which are a potential source of transmitted drug resistance (7). It has been shown that, not much has been reported on HIV drug resistance among drug-naive adults in Kenya, though no PI sequencing was completed to assess for primary resistance. The observed prevalence of mutations conferring drug resistance prompts the establishment of strong intervention strategies to keep the circulation of drug-resistant strains low. Despite the lack of technical capacity (23) and the high cost of resistance testing in resource-poor countries, efforts

to mitigate the impact of the pandemic through the surveillance and monitoring of HIV drug resistance have proven viable (22).

Continuous countrywide surveillance is required to determine the magnitude of transmitted drug-resistant mutants trends in Kenya. Furthermore, the presence of drug resistance mutations in drug-naive persons may represent natural polymorphisms of the virus due to adaptation mechanisms of the virus due to immune pressure and may not be suggestive of transmission of drug resistance mutations. The impact of such natural polymorphisms on the development of drug resistance in those people at commencement of ART is unknown and such individuals need to be monitored. Indeed the impact of such polymorphisms on the HIV-1 subtypes infected population and the speed of more widespread drug resistance are also unknown. Therefore it may be important to further examine some of the individuals and mutations found during this study in view of a long-term treatment strategy in the country. This study underscores the need to have readily available, high through put drug resistance testing for the increasing number of infected individuals in order to effectively manage those initiating ART.

ACKNOWLEDGEMENTS

To the patient who participated in the study, the Kenya Medical Research Institute, Centre for Virus HIV Laboratory division staff for their support. This work is published with the permission of the Director, Kenya Medical Research Institute.

REFERENCES

1. Richman DD: HIV chemotherapy. *Nature* 2001, **410**: 995-1001.
2. Madsen, T. V., Lohse, N., Jensen, E. S., *et al*. Short communication:high prevalence of drug-resistant human immunodeficiency virus type 1 in treatment-naive patients in Greenland. *AIDS Res. Hum. Retroviruses* 2008, **24**: 1073-1077.
3. Little, S. J., Frost, S. D., Wong, J. K., *et al*. Persistence of transmitted drug resistance among subjects with primary human immunodeficiency virus infection. *J.Virol.* 2008, **82**: 5510-5518.
4. National AIDS & STDs Control Council (NASCOP) Kenya AIDS Indicator Survey United Nations General Assembly, special session on HIV and AIDS, country report, 2010.
5. Jianping, S., Liying, Ma, Xiaoling, Yu., *et al*. Replication and drug resistant mutation of HIV-1 subtype B' (Thailand B) variantsisolated from HAART treatment individuals in China. *Virol. J.* 2009, **6**: 201-206.
6. del Rio, C. Updated antiretroviral treatment guidelines from DHHS and EACS. AIDS Clin Care 2008; **20**: 7.
7. Lihana, W. R., Khamadi, S. A., Lubano, K., *et al*. HIV Type 1 Subtype Diversity and Drug Resistance among HIV Type 1-Infected Kenyan Patients Initiating Antiretroviral Therapy. *AIDS Res. Hum. Retroviruses* 2009; **25**: 1211-1217.
8. World Health Organization, Joint United Nations Programme on HIV/AIDS (UNAIDS). Progress on global access to HIV antiretroviral therapy: A report on "3 by 5" and beyond, 2006. Geneva, Switzerland: World Health Organization, Joint United Nations Programme on HIV/AIDS (UNAIDS), 2006. Available at http:www.who.int hiv fullreport_en_highres.pdf.
9. National AIDS & STDs Control Council (NASCOP) Kenya AIDS Indicator Survey United Nations General Assembly, special session on HIV and AIDS, country report, 2009.
10. Chung, M. H., Kiarie, J. N., Richardson, B. A., *et al*. Independent effects of nevirapine prophylaxis and HIV-1 RNA suppression in breast milk on early perinatal HIV-1 transmission. *J. Acquir. Immune. Defic.Syndr.* 2007; **46**: 472–478.
11. Lehman, D. A., Chung, M. H., John-Stewart, G. C., *et al*. HIV-1 persists in breast milk cells despite antiretroviral treatment to prevent mother-to-child transmission. *AIDS* 2008; **22**: 1475–1485.
12. Lwembe, R., Ochieng, W., Panikulam, A., *et al*. Antiretroviral drug resistance-associated mutations among non-subtype B HIV-1-infected Kenyan children with treatment failure. *J. Med. Virol*. 2007; **79**: 865–872.
13. Ndembi, N., Abraha, A., Pilch, H., *et al*. Molecular characterization of human immunodeficiency virus type 1 (HIV-1) and HIV-2 in Yaounde, Cameroon: Evidence of major drug resistance mutations in newly diagnosed patients infected with subtypes other than subtype *B. J. Clin. Microbiol*. 2008; **46**: 177–184.
14. Songok, E. M., Lwembe, R. M., Kibaya, R, *et al*. Active generation and selection for HIV intersubtype A=D recombinant forms in a coinfected patient in Kenya. *AIDS Res. Hum. Retroviruses* 2004; **20**: 255–258.
15. Johnson, V. A., Brun-Vezinet, F., Clotet, B., *et al*. Update of the drug resistance mutations in HIV-1. *Top HIV Med.* 2008; **16**:138–145.
16. Afework, K., Masayuki, F., Masakazu, M., *et al.* Molecular Epidemiology of HIV Type 1 in Treatment-Naive Patients in North Ethiopia *J. AIDS Research and Human Retroviruses* 2007; **23**: 564-568.
17. Ndembi, N., Lyagoba, F., Nanteza, B., *et al*. Transmitted antiretroviral drug resistance surveillance among newly HIV type 1-diagnosed women attending an antenatal clinic in Entebbe, Uganda. *AIDS Res. Hum. Retroviruses* 2008; **24**: 889–895.
18. Somi, G. R., Kibuka, T., Diallo, K., *et al*. Surveillance of transmitted HIV drug resistance among women attending antenatal clinics in Dar es Salaam, Tanzania. *Antivi.r Ther*. 2008; **13**(Suppl 2): 77–82.
19. Kamoto, K., Aberle-Grasse, J., *et al*. Surveillance of transmitted HIV drug resistance with the World Health Organization threshold survey method in Lilongwe, Malawi. *Antivir. Ther*. 2008; **13**(Suppl 2): 83–87.
20. Koizumi, Y., Ndembi, N., Miyashita, M., *et al*. Emergence of antiretroviral therapy resistance-associated primary mutations among drug-naive HIV-1-infected individuals in rural western Cameroon. *J. Acquir. Immune. Defic. Syndr*. 2006; **43**: 15–22.

21. Bartolo, I, Casanovas, J, Bastos R, *et al*. HIV-1 Genetic diversity and transmitted drug resistance in health care settings in Maputo, Mozambique. *J. Acquir. Immune. Defic. Syndr.* 2009; **51**: 323–331.
22. World Health Organization. Priority interventions: HIV/AIDS prevention, treatment and care in the health sector, 2008. Available atwww.who.int-HIV-pub-priorityinterventions-en. Isolated from HAART treatment individuals in China *Virol. J.* 2009, **6**: 201.
23. Bennett, D. E., Myatt, M., Bertagnolio, S., *et al*. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. *Antivir. Ther*. 2008; **13**(Suppl 2): 25–36.
24. Wainberg, M. A. Perspectives on antiviral drug development. *Antivir. Res*. 2009; **81**: 1–5.
25. Paredes, R., Sagar, M., Marconi, V. C., *et al*. In vivo fitness cost of the M184V mutation in multidrug-resistant human immunodeficiency virus type 1 in the absence of lamivudine. *J. Virol*. 2009; **83**: 2038–2043.
26. Bussmann, H., Novitsky, V., Wester, W., *et al*. HIV-1 subtype C drug resistance background among ARV-naive adults in Botswana. *Antivir. Chem. Chemother.* 2005; **16**: 103–115.
27. Turriziani, O., Russo, G., Lichtner, M., *et al*. Study of the genotypic resistant pattern in HIV-infected women and children from rural west Cameroon. *AIDS Res. Hum. Retroviruses* 2008; **24**: 781–785.
28. de Mendoza, C., Rodriguez, C., Garcı´a, F., *et al*.: Prevalence of X4 tropic viruses in patients recently infected with HIV-1 and lack of association with transmission of drug resistance. *J. Antimicrob. Chemother*. 2007; **59**: 698–704.