Afr. J. Med. med. Sci. (2012) **41**, *Suppl.* 61-63

HIV-1 drug resistant mutations in chronically infected treatment naive individuals in the pre-ARV era in Nigeria

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Abstract

In Nigeria the Federal Government rolled out antiretroviral drugs for the management of HIV infection in year 2002. This study was carried out to determine the circulating antiviral drug mutations among ARV naïve patients with chronic HIV infection during the pre-ARV roll out era in the country. DNA was extracted from stored whole blood samples collected from 75 HIV positive patients attending the Medical outpatient clinic between December 1996 and November 2001. The Reverse transcriptase (RT) and the protease (PR) regions of the viral genome were amplified by nested PCR and then sequenced by cycle sequencing and analyzed using the ABI 3100 DNA sequencer to determine the mutations associated with protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI). Ten of the 64 (15.6%) samples with positive PCR had mutations for PR inhibitors (PI) including R8D, I 15V, G16E, M36I, M46L, L63P and H69K, while 5 of 63 harbored RT inhibitor (NRTI/NNRTI); V179I, A98T, V179E and A98S. Detection of ARV drug resistant mutations when ARV was not known to be in use in Nigeria calls for caution in the interpretation of drug resistance profile of HIV-1 from infected persons on treatment ARVs in the country.

Keywords: *Antiretroviral, HIV infection; pre-ARV, treatment, naïve.*

Résumé

Le Gouvernement Fédéral au Nigeria a lancé les médicaments antirétroviraux pour la gestion de l'infection du VIH en 2002. Cette étude a visé donc à déterminer les mutations des médicaments antiviraux en circulation parmi les malades ARV naïfs qui ont une infection VIH chronique pendant la période précédente au lancement d'ARV dans le pays. On a extrait de l'ADN des échantillons de sang conservés, recueilli de 75 malades du VIH qui fréquentent l'hôpital de jour entre Décembre 1996 et Novembre 2001. Les endroits de la transcriptase inverse (TI) et de la protéase (PR) du génome viral ont été amplifiés par PCR imbriqué et puis séquencés par cycle de séquence et analysés en utilisant le séquenceur d'ADN ABI 3100 afin de déterminer les mutations qui sont associées aux inhibiteurs protéases (IP), aux inhibiteurs nucléosides de transcriptase inverse (INTI) et à l'inhibiteur non-nucléoside de transcriptase inverse (INNTI). Dix sur les 64 (15,6%) échantillons à PCR positif avaient des mutations pour les inhibiteurs PR (PI) y compris R8D, I 15V, G16E, M36I, M46L, L63P et H69K, tandis que 5 sur 63 abritent l'inhibiteur RT (INTI/INNTI); V179I, A98T, V179E et A98S. Le dépistage des mutations résistantes au médicament ARV lorsque ARV n'était pas connue au Nigeria appelle à la prudence dans l'interprétation du profile de résistance du médicament du VIH-1 des personnes infectées qui suivent un traitement ARV dans le pays.

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Introduction

Widespread use of highly active antiretroviral (ARV) agents has tremendously reduced the morbidity and mortality among patients infected with HIV [1,5,13], particularly in sub-Saharan Africa that has the highest global burden of the infection [13]. However, a serious challenge to the use of ARV is emergence of resistance to the available ARVs [2,3,11]. According to the WHO [13], the extent to which ARV resistant HIV will be transmitted depends on a number of factors, one of the most important being ARV use including coverage in an area, duration of its use as well as proportion and numbers of ART patients whose regimens are failing [2,3]. In Nigeria, highly subsidized trial generic ARV drugs became available to limited number of HIV infected persons in year 2002 and subsequently the free branded triple combination first line regimen under the US government funded PEPFAR and Global Fund programmes in year 2004. Hitherto, very few patients that could afford the relatively expensive branded ARV drugs were treated in the country, initially with zidovudine alone and later with zidovudine and lamivudine combination (Combivir). In view of the rapid scaling up of ART in Nigeria, it

is important to define the baseline drug resistance mutation patterns in the circulating strains of HIV in Nigeria. Herein, we report patterns of ARV drug mutations among treatment naïve patients with advanced stage of HIV infection during the pre-ARV roll out in Nigeria.

Materials and methods

Aliquots of stored whole blood samples collected from 75 HIV positive patients attending the Medical Outpatient clinic of the University College hospital, Ibadan from December 1996 to November 2001 were used for this study. The samples have been previously confirmed to be HIV-1 positive by ELISA (Genescreen, BioRad®) and Western blot assays (BioRad®). Genomic DNA was extracted from each of the samples and analyzed for ARV resistance mutation as follows; Fragments of the Reverse transcriptase (RT) and the protease (PR) regions of the viral genome were amplified by nested PCR and then sequenced by cycle sequencing and analyzed using the ABI 3100 DNA sequencer to determine the mutations associated with protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI). The analysis included the RT and PR mutations that have been associated with reduced susceptibility to PI, NRTI and NNRTI as described by the International AIDS Society-USA in June 2002 [7].

Results

Target fragments from the three HIV gene sequences of 64 of the 75 genomic DNA of samples were amplifiable by the PCR technique. Ten (15.6%) of the 64 samples with positive PCR had mutations for PR inhibitors (PI) including R8D (n=1), 15V (n=1), G16E (n=2), M36I (n=1), M46L (n=1), L63P(n=2) and H69K (n=2) while 5 (7.9%) of 63 samples harbored RT inhibitor (NRTI/NNRTI); V179I (n=1), A98T(n=1), V179E(n=2) and A98S (n=1).

Discussion

The use of highly active antiretroviral therapy (ARV) has dramatically reduced morbidity and mortality among patients infected with HIV. The success of ARV is however limited by emergence of HIV drug resistance. In most developed countries, HIV drug resistance profile of infected individuals is routinely determined prior to initiation of ARV [6,8,12]. On the contrary, this is not yet possible in most developing countries because of limitation of resources and trained manpower required for the laboratory tests. Although available data show that use of ART in developing countries is almost as effective as in the developed settings, very little is known about the ARV drug resistance pattern of the predominant HIV strains in sub-Saharan Africa, which are predominantly non-B subtypes.

Detection of ARV drug resistant mutations found in this study when ARV was not known to be in use in Nigeria calls for caution in the interpretation of genotypic data of non-B HIV-1 sequences from Africa. Are these true drug induced mutations or polymorphisms that may be associated with circulating viral strains in the region? These could be true mutations or polymorphisms in the predominantly circulating non-B HIV-1 viral strains in sub-Saharan Africa. Interestingly, 10 (15.6%) of 64 HIV-1 samples analyzed showed mutation to PI drugs that only became widely available for treatment in Nigeria between year 2004. Using a different set of samples collected from ART naive HIV-1 infected individuals also from a community-based study in southwestern part of Nigeria but during a later period, Ojesina *et al.* [10] found 6 (17%) of 35 samples analyzed harboring primary mutations in the RT region but less in the PR. It was postulated that "the type specific codon usage and polymorphisms suggest the involvement of differential pathways for drug resistance and host-driven evolution" in the non-B HIV-1 subtype identified in that study compared to subtypes B.

In a similar study in Ouagadougou, analysis of HIV-1 from blood samples collected from patients shortly before and after introduction of large scale ART in Burkina Faso showed subtype specific secondary polymorphisms in the PR gene of the virus [4]. The report also indicated some subtype specific polymorphisms within important HLA epitopes. According to kozai *et al.* [9], these naturally occurring mutations in HIV-1 infected patients have important implications for therapy and the outcome of clinical studies. These mutations may also induce cross resistance to the currently available and future ARVs. There is therefore the need to carefully define the implications of these mutations for response to ART in settings with predominance of non-B HIV-1 subtype.

Acknowledgements

Support for GNO to carry out analysis of these samples in UD laboratory in Germany was provided by the University of Ibadan MacArthur Foundation short term staff development grant.

Author Contribution

GNO and DOO conceived and designed the study, GNO collected the samples, carried out the laboratory and data analyses, and wrote the

initial manuscript; SOO recruited and enrolled the patients while UD and ML supervised the bench work and data analysis in Germany. All authors reviewed the final manuscript and approved the final version. DOO and UD are guarantors of the paper.

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