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≈Review paper≈

HIV-1 drug resistance among untreated patients in Kenya: Current status

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ABSTRACT

High active antiretroviral therapy (HAART) has dramatically reduced mortality and morbidity among people living with HIV and AIDS globally. However, drug resistant mutations of HIV are a great challenge to the benefits of HAART. Development of drug resistance to antiretroviral medication can occur in persons on antiretroviral therapy, by acquisition of an already resistant strain in persons who have never taken medication or by natural polymorphism of the virus in vivo. This leads to treatment failure hence complicating management of HIV patients. With the introduction of generic HAART, there has been a steep increase in the number of patients put on HAART in Kenya but this has not reached anticipated 100% coverage. Therefore, most patients either pay for medications out of their own pockets or fail to access completely. Interruptions in therapy due to monetary constraints are not uncommon. Little is known on HIV drug resistance in resource constrained settings like Kenya where the predominant circulating HIV-1 sub-type is A1. The transmissibility of drug-resistant forms of the virus is also a major concern especially when formulating treatment guidelines. This article reviews published data available on the patterns of HIV-1 drug resistance among drug naive population in Kenya.

Keywords: Antiretroviral drugs, HIV drug resistance, non-B subtypes, primary drug resistance

INTRODUCTION

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) (Lihana *et al.,* 2009; NASCOP, 2009), 7.1% by 2007 (NASCOP, 2008), and 6.3% by 2008 (NASCOP, 2008). Access to antiretroviral therapy (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 (NASCOP, 2009). 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 (Lihana *et al.,* 2009; NASCOP, 2009).

It has been shown that, not much has been reported on HIV drug resistance among drug-naive

adults in Kenya, as well as complete protease inhibitors (PI) sequencing 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 (Bennett *et al.,* 2008) 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 (WHO, 2008).

Since the introduction of generic antiretroviral (ARVs), there has been a steep increase in the number of individuals initiating antiretroviral therapy (ART) primarily due to the government initiative in accessing free ARVs through the government run HIV comprehensive HIV clinics. Highly active antiretroviral therapy (HAART) has dramatically improved survival and quality of life in people living with HIV and AIDS (Palella *et al*., 1998). However, these benefits can be greatly compromised by the drug-resistant forms of the virus. As the first therapeutic regimen is probably the most important for virologic suppression, drug-resistant variants of HIV greatly challenge the efficacy of HAART in producing adequate viral suppression (O'Neil *et al.,* 2002). In settings of incomplete viral suppression, drug-resistant mutations can easily evolve resulting in widespread drug resistance (O'Neil *et al.,* 2002; Mbisa *et al.,* 2005).

There are numerous factors that result in the development of drug-resistant strains of the virus. The high replication capacity of HIV and its error-prone transcription is a major factor contributing to the development of resistance. It has been shown that retroviral replication is a highly error-prone process with varying estimates of roughly 7×10^{-6} to 1.4×10^{-4} base-pair substitutions occurring per nucleotide per replication cycle (Mbisa *et al.,* 2005; Tee *et al.,* 2005; Mulky *et al.,* 2005). Another significant source of genetic variation is recombination. Recombination between HIV-1 genomes has been demonstrated and probably occurs *in vivo* as a result of simultaneous infection of an individual by two distinct HIV-1 strains (Steain *et al.,* 2004; Kijak *et al.,* 2005; Geretti *et al.,* 2006). However, the observed degree of HIV-1 genetic diversity may also be influenced by selective pressure such as the host's immune response, cell tropism of the virus and the genetic makeup of the host Thomson *et al.,* 2005). Irregularity in adherence to ART is probably the most important factor contributing to the development of resistance (Balakrishnan *et al.,* 2010). Following recent post election violence in Kenya in 2008, many HIV patients had their ARVs accessibility interrupted and paying from their own pockets was not possible. In addition, with the recent low or reduced funding on accessibility of ARVs drugs by the President's Emergency Plan for AIDS Relief (PEPFAR)there may be more HIV drug resistance levels in Kenya.

Since ART has been available in the western world since 1987, there have been numerous reports of the transmission of HIV strains with resistance to single or multiple antiretroviral drugs (Balakrishnan *et al.,* 2010). Moreover, some subtypes of HIV-1 can be less susceptible to protease inhibitors or non-nucleoside reverse-transcriptase inhibitors (NNRTI) than the subtype B strains that are prevalent in the United States and Europe (Fonjungo *et al.,* 2002; Shafer *et al.,* 2005). Although the transmission of drug-resistant strains of HIV has been well-documented, a concern with testing chronically infected patients is that, drug-resistant mutations will disappear in the absence of drug selection pressures and would hence be undetectable by resistance assays. Drug-resistant mutations also become undetectable if the infecting strains revert to a wild type or become overgrown by fitter wild-type viruses, persisting as archived viruses or as minority species and that may not be detectable by current assays (Little *et al.,* ; *et al.,* 2005).

Despite widespread use of ARV agents, it has been shown that, not much has been reported on HIV drug resistance among drug-naive subjects in Kenya (Lihana *et al.,* 2009). From the little published observations on prevalence of mutations conferring drug resistance, it prompts the establishment of strong intervention strategies to keep the circulation of drug-resistant strains low

(Bennett *et al.,* 2008). As treatment programs are expanded, the prevalence of HIV-1 drug resistance among ART naive patients is of paramount importance in selecting treatment regimens and planning national policies (Balakrishnan *et al.,* 2010). Despite the lack of technical capacity (Bennett *et al.,* 2008) 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 (WHO, 2008). Continuous countrywide surveillance is therefore required to determine the magnitude of transmitted drug-resistant mutants and viral evolutionary trends in Kenya. This review underscores the need to have readily available, high throughput drug resistance testing for the increasing number of infected individuals in order to effectively manage those initiating ART.

HIV-1 PRIMARY DRUG RESISTANCE

In Kenya, the number of individuals seeking treatment for infection with HIV has increased through the government initiative of accessing ARVs to HIV patients through the President's Emergency Plan for AIDS Relief (PEPFAR) fund. The most common regimens are three drug combinations of zidovudine (ZDV) or stavudine (d4T) plus lamivudine (3TC) plus nevirapine (NVP) or efavirenz (EFV) (Kumarasamy *et al.,* 2005). Generic ART has been shown to be safe and tolerable and has also been shown to suppress viral replication in clinical trials (Kumarasamy *et al.,* 2003). However, one of the biggest issues with the management of HIV disease in Kenya is the high rate of sub-optimal adherence. This sub-optimal adherence is brought about by a combination of various factors; like disruption in supplies of antiretroviral Drugs, inappropriate use and practices of drugs , , forgetting to take drugs, changing medication frequently; administering and taking the wrong dose; interruptions in treatment due to financial constraints and stigma. Hence, the monitoring of prevalence of drug-resistant strains is a major public health concern especially now that the major financial PEPFAR has reduced its funding on ARVs, a factor that is going to contribute to drug interruptions hence an increase in HIV drug resistance.

This situation is consistent with other findings from countries in Africa that have as well showed prevalence to be less than 5%. (Afework *et al.,* 2007; Ndembi *et al.,* 2008; Somi *et al.,* 2008; Kamoto *et al.,* 2008) However, findings from recent studies by Lihana *et al.,* in 2009 had prevalence of 7.5% an indication of possible increase in drug resistance probably due to increased coverage for antiretroviral (Lihana *et al.,* 2009). Lihana *et al.,* study indicated that four isolates out of 53 (7.5%) had the M184V, K65R, D67N/K70R/K219Q mutation, indicating primary drug resistance to 3TC a NRTI. This primary resistance was detected among drug-naive patients in the early stages of HIV disease with average (CD4 + T- cell count of 475cells/mm^3), in Nairobi. In addition, Nonnucleoside RTI resistance-associated mutations K103N and Y181C were also detected in three patients (5.7%). There is still not much literature available in Kenya that has demonstrated the presence of primary drug resistance to protease inhibitors. This indicates how not much has been done on drug resistance evaluation among drug naive subjects in Kenya and consequently transmission of drug-resistant viruses remains unclear.

MUTATIONS AT PI DRUG POSITION

Mutations at more than 20 positions in human immunodeficiency virus type 1 (HIV-1) protease have been associated with resistance to present protease inhibitors (PIs). Several PI resistance mutations are of particular importance because they occur in the substrate cleft or independently reduce drug susceptibility. Six of these mutations (D30N, G48V, I50V, V82A/ F/S/T, I84V, and L90M) have been designated primary resistance mutations in recent published guidelines for

antiretroviral drug resistance testing (Hirsch *et al.,* 2000). The most frequently observed positions of amino acid substitutions (primary mutations) are 12, 13, 15, 22, 35, 36, 43, 55, 60, 62, 63, 69, 74, 79, 89 and 93. Little has been reported in literature in Kenya reason being the high cost involved in drug resistance.

MUTATIONS AT NRTI AND NNRTI DRUG POSITION

Just like the PI drugs positions, higher levels of polymorphisms have been reported in RTI drug positions in Kenyan isolates. They are 35, 39, 48, 60, 13, 177, 200, 207, 214 and 245. Other minor mutations are at the positions 20, 22, 67, 118 and 210 in NRTI region and 103, 230 and 238 in NNRTI region.

INHIBITORS OF HIV ENTRY

Fusion inhibitors are a new class of antiretroviral drugs (ARVs) for the treatment of human immunodeficiency virus infection. Enfuvirtide is the first in this class to reach market approval. Fusion inhibitors block the last step in the three-step viral entry process consisting of attachment, co-receptor binding and fusion, thereby preventing viral capsid entry into the host cell. Enfuvirtide has a unique mechanism of action and high viral target specificity, and in clinical trials has been shown to exhibit both high efficacy and low toxicity. Enfuvirtide is a peptide mimetic of an essential region within viral envelope glycoprotein gp41 that functions by blocking gp41 structural rearrangements at a transitional pre-fusion conformation. Although different clinical isolates show variation in susceptibility to enfuvirtide, primary resistance has not been observed, and thus enfuvirtide-naive isolates remain clinically sensitive.

Acquired resistance centres round a 10 amino acid motif between residues 36 and 45 in gp41 that forms part of the binding site of enfuvirtide. The 10 amino acid motif is critical for viral fusion, and enfuvirtide-resistant mutants show poor replicative capacity compared with wild type. Reversion to a wild-type, drug-sensitive state has been reported following enfuvirtide withdrawal (Michael *et al.,* 2004). From a therapeutic perspective, viral entry is one of the most attractive points for intervention in the viral life cycle, since drug activity is independent of intracellular access. The HIV entry process has three discrete steps: attachment, co-receptor binding and fusion, each representing a unique drug target The initial step in the entry process involves attachment of the viral envelope glycoprotein (gp120) to the CD4 cell surface receptor on helper T-cells, and on other susceptible cell types (Kowalski *et al.,* 1987; Lasky *et al.,* 1987; Earl *et al.,* 1990; Gallaher *et al.,* 1995).

The host-derived viral lipid membrane is studded with virus-encoded trimeric envelope structures consisting of each of two glycoproteins, gp120 and gp41. The structure is formed via intramolecular disulphide bridges and by non-covalent intermolecular bonds. In each envelope structure, a trimer of gp120 molecules makes up the cap and the stalk is formed from a gp41 trimer that is anchored in the viral lipid bilayer. Upon binding of gp120 to CD4 and chemokine receptors, a conformational change in the structure of gp41 occurs so that the membranes of virus and cell are brought into close proximity, resulting in membrane fusion and subsequent infection (Michael *et al.,* 2004). Many inhibitors, known as attachment inhibitors, have been designed to block the binding of gp120 to the CD4 receptor and a number are in pre-clinical or clinical development including BMS-806 and BMS-043, PRO 2000, (Vermeire *et al.,* 2003) TNX-35525 and PRO542 (Trkola *et al.,* 1995; Nagashima *et al.,* 2001; Vermeire *et al.,* 2003).

The emergence of resistance to antiretroviral agents for the treatment of HIV-1 infection has fuelled the search for new drug classes with a novel mechanism of action (Hammer *et al.,* 2006). Chemokine (C-C motif) receptor 5 (CCR5) antagonists interfere with viral–cellular interactions in the entry process. Preceding HIV-1 entry, viral

envelope glycoprotein (gp120) binds to the CD4 receptor, resulting in a conformational change that allows the subsequent interaction with a CCR5 or chemokine (C-X-C motif 4) receptor 4 (CXCR4) expressed on the surface of the target cell (Deng *et al.,* 1996; Dragic *et al.,* 1996; Feng *et al.,* 1996) Further molecular rearrangements initiate gp41-mediated membrane fusion. Three CCR5 antagonists entered clinical evaluation, of which one was discontinued because of toxicity (AAplaviroc, GSK), one is currently still in clinical investigation (Bennett *et al.,* 2008) (VVicriviroc, Schering-Plough) and one is US Food and Drug Administration (FDA)/ European Medicine Agency-approved and marketed (mMaraviroc, Pfizer).

However, these drugs have not yet been introduced in Kenya and most developing countries. Nevertheless, since it is known patients at later stage of infection on progression to AIDS viruses develop X4 tropic virus, it is not surprising that viruses resistant to these drugs have already been observed by switching their coreceptor usage from R5 to X4 (Westby *et al.,* 2007; Landovitz *et al.,* 2008).

The use of these compounds could significantly improve the therapy outcome of individuals infected with HIV-1 if no drug resistance associated mutations (DRAMs) are present. However, the susceptibility and the development of resistance to these inhibitors by some HIV subtypes viruses are not known (Tsibris *et al.,* 2008a). For maraviroc, the mutations A316T and I323V have been reported to confer partial resistance for subtype B viruses. Complete resistance to this entry inhibitor occurs when both mutations (A316T/I323V) are present (Westyby *et al.,* 2007). However some of the mutations associated to drug resistance often occur naturally hence posing a challenge on the future status on this new class of drugs.

WAY FORWARD

It is clear from the currently available reports that among the untreated HIV-1 patients, the prevalence of known drug-resistance mutations is very low, when compared to the alert cut-off (5%), which has been defined by the ad hoc working group of the world health organisation (WHO) (Lazzari *et al.,* 2004). In view of expanded treatment access to ARV amongst the Kenya HIV-infected population, there is a need to assess the utility and feasibility of integrating HIV drug-resistance surveillance into the national ARV treatment programmes. Accelerated roll-out of ART could also lead to the emergence and transmission of ARV-resistant viruses. Although HIV drug resistance cannot be prevented completely, there might be a possibility of combating its spread and impact. Implementation of WHO evaluation on Early Warning indicators for HIV drug resistance development is an ideal strategy in assessing and monitoring of HIV drug resistance in poor resource countries. Moreover, in Kenya there are very few reference laboratories that currently have the facility to perform genotyping / phenotyping techniques. Hence larger studies and continuous surveillance are needed among drug naive HIV infected individuals, particularly with recent infections. Also generating a HIV drug-resistance database using Kenyan strains will be very important for the future clinical management of HIV disease.

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