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HIV-1 Drug Resistance Mutations in Patients Failing 1st Line Therapy in a Comprehensive Care Center in Nairobi, Kenya

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Abstract

Background: HIV-1 drug resistance is an emerging challenge for HIV-1 infected clients who are on antiretroviral therapy (ART). In Kenya, as in many other developing countries, ART is now accessible to clients who need it. However, they must be done a CD4 test first and if the count is <300, then ART is commenced. With the initiation of ART comes the challenge of adherence to medication, a factor that is impacted greatly by the understanding of the client of the importance of adherence and the financial ability to keep their appointments, especially if the clients come from a distant location. Objective: To identify HIV-1 drug resistance mutations inclientsfailing1st line antiretroviral therapy in Nairobi, Kenya. Methodology: A cross sectional study was carried out where whole blood samples were collected from clients attending a HIV care and treatment clinic in Nairobi. Clients who had been on ART for more than 6 months and had a viral load greater than 1000 were enrolled in the study. A total of 52 client samples were successfully sequenced in the reverse transcriptase region and analyzed. Results: After analysis of the generated sequences, it was seen that 43 (82.6%) of the clients had HIV-1 drug resistance mutations conferring resistance to one or more nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse-transcriptase inhibitors (NNRTIs). Majority of the clients (46%) were infected with HIV-1 subtype A viruses. Conclusion: The findings of the study showed that a significant proportion of the clients on ART had developed resistance mutations to one or more drugs that are used as 1st line therapy in Kenya. There is need for continuous education of the population on importance of adherence to medication. There is also need for clinicians to be trained on using viral load and HIV drug resistance testing, where available, as methods of monitoring treatment failure so that clients can be

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switched to alternative medication immediately the need arises, so as to improve their treatment outcomes.

Keywords

HIV-1, Treatment Failure, Antiretroviral Therapy, Drug Resistance, Subtype

1. Introduction

1.1. Impact of HIV/AIDS

Global HIV/AIDS statistics show that as of 2012, there were 2.3 million new HIV-1 infections [1]. Sub-Saharan Africa accounts for more than 68% of the global prevalence of HIV, with women and the youth carrying the biggest burden of the disease [2]. Since the discovery of HIV-1 in Kenya in 1984, it continues to be a big healthcare burden for the country. The roll out of antiretroviral therapy (ART) has led to a significant reduction in HIV/AIDS related mortality [3].

1.2. Use of ART in Management of HIV/AIDS

It is estimated that the cost of first line ART in some low and middle-income countries has been greatly reduced to around US $140 per person per year [1]. In 2013 the World Health Organization (WHO) revised the guidelines of ART initiation meaning that the number of eligible people for treatment initiation increased to approximately 28.6 million [4]. As of January 2011, there were 410,000 patients on ART in Kenya. The increase in ART coverage in the country is expected to lead to an increase in drug-resistant strains [5]. Stigma and cultural practices that affect ART uptake in Kenya may affect ART compliance, resulting in an increased emergence of drug-resistant mutants, which are a potential source of drug resistance. To mitigate against the development of HIV/AIDs drug resistance, the government of Kenya developed a guideline for clients who are due for initiationof ART. These include providing the clients with information on importance of ART and adherence to medication [6].

1.3. Regimen Used for ART Management in Kenya

Initiation of ART in Kenya has been primarily based on CD4 count measurements [6]. It has also been shown that initiation of life-long triple therapy in pregnant mothers, regardless of their CD4 count is beneficial to the mother and drastically reduces the chances of transmission of the virus from the mother to the child [6].

Currently the first-line treatment of HIV-1 and AIDS is based on the combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI), which is also known as highly active antiretroviral therapy or HAART [7]. Most resource-limited countries use the standard first line regimens that were recommended by WHO [8]. In Kenya, initiation of ART in drug naïve clients is based on Tenefovir + Lamivudine + Efavirenz/Nevirapine [6]. In pregnant women where clients are unable to tolerate tenofovir then Zidovudine + Lamivudine + Efavirenz/Nevirapine have been used [6].

1.4. Development of Resistance to ART

The most important enzyme in HIV multiplication in the body is reverse transcriptase (RT). The two main types of drugs used to inhibit RT are nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). NRTIs resemble nucleosides and are activated by the host to convert them to their triphosphate form. When bound in the active site, they terminate polymerization because they lack a 3' hydroxyl group [9]. NNRTIs are structurally different from the nucleoside RT inhibitors. They bind near the catalytic site of reverse transcriptase and alter the enzymes ability to change conformation. This increased enzyme rigidity prevents its normal polymerization function. Resistance to NNRTIs occurs when there are mutations in the binding pocket, and affinity for the drug is reduced.

According to the Kenya HIV-Drug resistant report of 2011 [10], in order to establish treatment failure the following should be seen: New or recurrent WHO stage 4 condition or certain WHO clinical stage 3 conditions e.g. tuberculosis, plasma viral load above 1000 copies/ml and fall of CD4 count to baseline or persistent CD4 levels below 100 cells/mm^3. Virological failure occurs earlier, which is later followed by immunological and clinical failure. Diagnostically, the reverse is true in resource-limited settings, because of availability diagnostic resources. Viral load is the most sensitive prognostic tool to identify treatment failure and is used in resource-rich settings [11]. Although resistance testing is not widely employed in developing countries due to the costs involved, the need will increase due to emergence of drug resistance as antiretroviral therapy is scaled up [12].

2. Materials and Methods

2.1. Enrolment of Clients

This study was carried out to detect HIV-1 drug resistance mutations in ART-experienced clients who were seen to have virological and clinical failure. Blood samples were collected from clients aged 18 years and above and on 1st line treatment for more than 6 months, with a CD4 count of below 350 cells/ml and/or patients with persistently high viral load in spite of being adherent to ART. Consenting was done before sample collection and testing. The study commenced after obtaining ethical clearance from the KEMRI/National ethical review committee. After getting informed consent from the clients, 3 ml of whole blood was collected. Plasma was separated and stored at –80˚C until when RNA was extracted from them.

2.2. Laboratory Methods

Viral RNA was extracted from the cryopreserved plasma samples using the Qiagen® RNA extraction kitas per the manufacturer's specifications. A single step RT and nested PCR was done using in-house primers targeting the protease and reverse transcriptase regions of the HIV-1 pol gene as previously described [11]. The reverse transcriptase gene was amplified and sequenced. The generated sequences were analyzed for subtype diversity using the REGA subtyping tool [13] and BLAST tool [14]. Drug resistance was determined using the International Aids Society (IAS) algorithm and the Stanford University HIV database.

Fifty-two samples were successfully sequenced and analyzed. The clients were aged 18 to 72 years with (Median age of 36years). Of the 52 subjects 36 were female and 16 male. The Median CD4 count of the subjects was 172 cell/ul (CD4 range 10 - 350 cells/ul), with a mean of 178 cells/ul.The viral load of the clients ranged from 1234 to 2465798 copies (median: 58070 copies).

2.3. Results

After sequencing of the RT region and analysis of the same, it was determined that 43 (82%) of the clients had mutations conferring resistance to one or more NRTIs and NNRTIs. Analysis of the sequences showed that 36 (83.7%) of the clients with drug resistance mutations had NRTI drug mutations while 40 (93%) of the clients with drug resistance mutations had NNRTI related drug resistance mutations. Thirty three (76.7%) of these clients had both NRTI and NNRTI related drug resistance mutations (**Table 1**).

3. Discussion

The study showed that 82.6% of the clients enrolled in this study had HIV-1 drug resistance mutations conferring resistance to one or more NRTI and NNRTI drugs. This was high but was expected because these clients were failing clinically and virologically. These clients had an age range of 18 to 72 years with (Median age of 36 years). Many clients who access ART clinics feature in the age bracket of 15 - 49 years, which is the age bracket hardest hit by the HIV epidemic [15].

In sub-Saharan Africa, the common HIV-1 drug resistance profiles include the M184V mutation which is linked to resistance to nucleoside reverse transcriptase inhibitors, and the K103N mutation which is linked to resistance to non-nucleoside reverse transcriptase inhibitors; NNRTIs). The less common HIV-1 drug resistance mutations that have been seen in sub-Saharan Africa include Thymidine analogue mutations (TAMs) and the K65R mutation, which are less common [16]. In this study, this was also evident (**Table 1**), with a variety of other mutations being reported as well.

Table 1. HIV-1 drug resistance mutations in clients failing treatment in the study population.

Sequence ID	Gender	First Line Therapy	Viral Load	CD4 Count	NRTI Mutations	NRTI Mutations Drugs	NNRTI Mutations	NNRTI Mutations Drugs	Sequence Subtype
31	F	3TC, NVP, TDF	823453	78	M41L, D67N, M184V, L210W, T215Y	3TC, ABC, AZT, D4T, DDI, FTC, TDF	K101E, G190A	EFV, NVP, RPV	A1
61	F	3TC, D4T, NVP	789708	88	D67N, K70R, V75M, M184V, T215F, K219Q	3TC, ABC, AZT, D4T, DDI, FTC	A98G, Y188L, G190A	EFV, NVP, RPV	A1
96	F	3TC, EFV, NVP	789456	87	M184V	3TC, FTC	K103N	EFV, NVP	D
106	F	3TC, NVP, AZT	1657	348	NONE	NONE	K103N	EFV, NVP	D
119	F	3TC, NVP, TDF	68790	132	K65R	DDI, TDF	V90I, K103N, V108I	EFV, NVP	D
126	F	3TC, D4T, NVP	13232	205	NONE	NONE	Y188L, G190A	EFV	A1
142	M	3TC, AZT, EFV	6467	297	D67N, T69N, K70R, M184V, T215F, K219E	3TC, ABC, AZT, D4T, DDI, FTC	K101P, K103N	EFV, ETR, NVP, RPV	D
145	F	3TC, AZT, EFV	2456789	12	K65T, M184V,	3TC, FTC	V108I, Y181C	NVP	A1
157	F	3TC, AZT, EFV	856789	76	NONE	NONE	K103N	EFV, NVP	D
167	M	3TC, AZT, EFV	10344012	10	K67R, K70R, M184V, K219E	3TC, ABC, D4T, DDI, FTC, TDF	K101E, K103N, Y181G, G190A, H221Y	EFV, ETR, NVP, RPV	A1
171	F	3TC, D4T, EFV	955046	28	M41L, M184V, T215F	3TC, AZT, D4T, FTC	V106M, Y188C, G190A	EFV, NVP	A2
252	M	3TC, D4T, EFV	23456	290	NONE	NONE	K103N	EFV, NVP	D
288	F	3TC, NVP, TDF	42345	280	M41L, K65N, M184V, L210W, T215Y	3TC, ABC, AZT, D4T, DDI, FTC, TDF	Y188C, H221Y	NVP	A1
307	M	3TC, NVP, TDF	3000	324	NONE	NONE	K103N	EFV, NVP	D
322	M	3TC, AZT, EFV	45689	150	D67N, T69N, K70R, M184V, T215F, K219Q	3TC, ABC, AZT, D4T, DDI, FTC	K103N, V108I,H221`Y	EFV, NVP	A1
384	F	3TC, AZT, EFV	1543	348	NONE	NONE	K103N	EFV, NVP	D
412	M	3TC, D4T, NVP	675778	64	M41L, V75T	D4T	V90I, V106M, V179D	EFV, NVP	C
430	F	3TC, EFV, NVP	35467	125	M184V	3TC	NONE	NONE	B
433	F	3TC, AZT, EFV	1324	348	NONE	NONE	K103N	EFV, NVP	D
439	M	3TC, EFV, TDF	255677	77	K65R	DDI, TDF	V90I, K103N, V108I	EFV, NVP	A1

Continued

466	F	3TC, D4T, NVP	893123	71	M41L, M184V, T215Y	3TC, AZT, D4T, FTC	K103N	EFV, NVP	C
516	M	3TC, D4T, NVP	230144	110	M41L, M184V, T215F	3TC, AZT, D4T, FTC	V106M, Y188C, G190A	EFV, NVP	D
544	F	3TC, AZT, EFV	5128	290	M41L, L74I, M184V, T215Y	3TC, ABC, AZT, D4T, DDI, FTC	K103N, V108I, H221Y, K238T	EFV, NVP	A1
604	M	3TC, AZT, EFV	10344	237	A62V, K65R, K70R, M184V, K219E	3TC, ABC, D4T, DDI, FTC, TDF	K101E, K103N, Y181C, G190A, H221Y	EFV, ETR, NVP, RPV	A1
605	F	3TC, D4T, EFV	345678	104	M41L, D67N, L74I, M184V, L210W, T215F	3TC, ABC, AZT, D4T, DDI, FTC, TDF	K101E, G190A	EFV, NVP	A1
627	F	3TC, D4T, NVP	8457	220	M41L, D67N, T69N, K70R, M184V, T215Y	3TC, ABC, AZT, D4T, DDI, FTC	A98G, Y181F	ETR, NVP, RPV	A1
652	F	3TC, D4T, NVP	61200	176	M41L, D67N, V75I, M184V, T215Y,	ABC, AZT, D4T, DDI, FTC	A98G, K103N, K238T	EFV, NVP	A1
661	F	3TC, AZT, EFV	1750	350	K65R, K219R	DDI, TDF	V190I, V1O8I, Y181C, H221Y	NVP	D
687	F	3TC, D4T, NVP	1845	341	A62V, K65R, K70R, M184V	3TC, ABC, D4T, DDI, FTC, TDF	K101E, Y181C, G190A, H221Y	EFV, ETR, NVP, RPV,	A1
792	F	3TC, D4T, NVP	324567	76	K60R, K70T, M184V	3TC, ABC, DDI, FTC, TDF	K103N, Y181C	EFV, NVP	A1
867	F	3TC, D4T, NVP	2133	335	M41L, D67N, T69D, K70R M184V, T215F, K219Q	3TC, ABC, AZT, D4T, DDI, FTC	Y181I	ETR, NVP, RPV	A1
872	M	3TC, AZT, EFV	897986	63	M184V, T215F	3TC, FTC	K101E, K103N, E138A, H221Y, K238T	EFV, NVP	A1
948	F	3TC, EFV, NVP	812345	13	M184V	3TC, FTC	NONE	NONE	G
1393	M	3TC, NVP, AZT	80123	156	K65R, M184V	3TC, ABC, DDI, FTC, TDF	K103N, E138G, V179T, Y181C	EFV, NVP	D
1508	M	3TC, NVP, TDF	96458	134	M184V	3TC, FTC	V90I, A98AG, K101EK, G190A	EFV, NVP, RPV	A1
1591O R 1592	M	3TC, D4T, NVP	21324	186	K65R, D67N, M184V, K219E	3TC, ABC, D4T, DDI, FTC, TDF	V108I, Y181C, H221HY	ETR, NVP	A1
1788	F	3TC, AZT, EFV	7456	262	M184V	3TC, FTC	K103KN, V179T	EFV, NVP	A1
2017	M	3TC, NVP, AZT	96458	172	M184V	3TC, FTC	K103N	EFV, NVP	D

Continued

2174	F	3TC, NVP, AZT	13245	228	D67G, K70R, M184V, K219Q	3TC, FTC	K101E, G190S	EFAVIRENZ, NEVIRAPINE	D
9427	F	3TC, EFV, TDF	4240	336	M184V, K219E	3TC, FTC	NONE	NONE	A1
10615	M	3TC, EFV, AZT	58070	169	M184V	3TC, FTC	K103N, V106A, Y181C, G190A, H221Y, F227L	EFV, ETR, NVP, RPV	A1
11480	F	3TC, D4T, NVP	30405	187	D67N, K70R, M184V, T215N	3TC, AZT, D4T, FTC	K103N	EFV, NVP	A1
12256	F	3TC, NVP, TDF	120108	107	M184V	3TC, FTC	K103N	EFV, NVP	D

Of the 52 subjects that were enrolled in the study, 36 were female and 16 were male. The Median CD4 count of the subjects was 172 cell/ul (CD4 range 10 - 350 cells/ul), while the viral load ranged from 1234 to 2,465,798 copies (median: 58,070 copies). This showed that the clients were failing treatment and hence the high HIV-1 drug resistance rates were not unexpected. Additionally, most of the clients enrolled in the study were infected with HIV-1 subtype A viruses (46%). This was not unexpected as HIV-1 subtype A viruses are the common viruses circulating in Kenya [17].

This study serves to show the importance of monitoring treatment failure using CD4 and viral load in resource limited settings as they serve as prognostic markers for detecting resistance. In addition, it shows that importance of use of HIV drug resistance testing in determining the specific drug resistance mutations and the equivalent drugs that the patients are failing. This is important in switching to more efficient therapies.

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