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Pattern of HIV-1 Drug Resistance among Adults on ART in Nigeria*

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ABSTRACT

Background: The development of anitiretroviral drug resistance may limit the benefit of antiretroviral therapy. Therefore the need to closely monitor these mutations, especially the use of ART is increasing. This study was therefore designed to determine the ARV drug resistance pattern among ART naïve and expose individuals attending a PEPFAR supported by antiretroviral clinic in Nigeria. **Methodology:** The study participants included patients attending the PEPFAR supported by University College Hospital (UCH), Ibadan ART clinic who have been on HIV treatment for at least one year with consecutive viral load of over 2000 copies/ml as well some ART Naïve individuals with high (>50,000 copies/ml) baseline viral level attending the hospital for pre-ART assessment. Blood sample was collected from each individual for CD4 enumeration, viral load level determination and DNA sequencing for genotypic typing. Antiretroviral drug resistance mutations (DRM) were determined by using the Viroseq software and drug mutations generated by using a combination of Viroseq and Stanford algorithm. DRM were classified as major or minor mutations based on the June 2013 Stanford DR database. **Results:** The most common major NRTI, NNRTI and PI mutation were D67N (33.3%), Y181C (16.7%) and M46L/I (55.6%) respectively. Lamivudine (3TC) and emtricitabine (FTC); nevirapine (NVP) and nelfinavir (NFV) were the most common NRTI, NNRTI, and PI drugs to which the virus in the infected individuals developed resistance. Isolates from 4 patients were resistant to triple drug class, including at least one NRTI, NNRTI and a PI. Only one (4.8%) of the isolates from drug Naïve individuals had major DRM that conferred resistance to any drug. **Conclusion:** Demonstration of high rates of antiretroviral DRM among patients on 1st and 2nd line ART and the presence of DRM in drug Naïve individuals in this study show the importance of surveillance for resistance to ARV in line with the magnitude of scaling up of treatment program in the country.

Keywords: Antiretroviral Therapy; Drug Resistance Mutation; ART Naïve; 1st and 2nd Line ART

1. Introduction

HIV/AIDS continues to be a global health problem since its discovery in 1981 [1] with over 33 million people living with the virus at the end of 2011 [2]. The first case of AIDS was reported in Nigeria in 1986 and the rate of HIV infection in the country increased steadily from 0.6% in 1987 to a peak of 5.8% in 2003. The last HIV national sentinel survey in the country shows that the rate of infection has declined to 3.4% [3], though the prevalence varies by locations from a relatively low rate of 2.1% in the north central and 2.9% in south western zones respectively to a high rate of 7.5% in the north central zone of the country.

According to UNAIDS, the number of new infections globally reduced to 2.5 million in 2011 from 3.2 million in 2001 [2]. Part of the reasons for this success may not be unrelated to the wide spread use of antiretroviral therapy (ART). There are evidences that ART contributes greatly to the reduction of transmission, morbidity and mortality caused by HIV infection [4-6]. This dramatic improvement that is most prominent in the North America, Western Europe and recently Brazil, has led to the advocacy for increased access to antiretroviral drugs in

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resource limited settings [1,7-10]. Many African countries have responded positively and increased access to ART, though with support from international agencies [11-15].

In Nigeria, wide use of ART started in 2002 when the Federal Government launched the pilot HIV treatment program [9,10]. Additional funding for antiretroviral treatment became available in the country through the US government funded by President's Emergency Plan for AIDS Relief (PEPFAR) program and the Global Funds and thus they increased access to ART greatly. To date, over 500,000 patients are on ART in Nigeria, although this number is a far cry to the almost 1.5 million HIV positive individuals who require treatment in the country [16].

The introduction of antiretroviral therapy has substantially changed the natural history of HIV and AIDS. Unlike the 80s and early 90s, people living with HIV/ AIDS [PLWAs] now live better and longer, thus they are able to contribute meaningfully to the economy of their country. However, development of drug resistance may limit the benefit of antiretroviral therapy. Various reports have documented the increase of ARV drug resistance in different countries and regions of the world [17-21]. Although the result of a recent WHO DRM survey which reported that "rate of transmitted DR continues to remain limited in low-and-middle-income countries" [22] is assuring because of the initial skepticism [23,24] by the international community, there is still the need to closely monitor these mutations in each country, especially as the use of ART increases. This study was therefore designed to determine the ARV drug resistance pattern among ART naïve and expose individuals attending the PEPFAR supported by antiretroviral clinic at the University College Hospital, Ibadan, the premier tertiary hospital in Nigeria.

2. Methodology

2.1. Study Site

This study was carried out among patients attending the antiretroviral treatment clinic of the University College Hospital (UCH), Ibadan, Nigeria. The UCH is the foremost teaching hospital located in the southwestern region of Nigeria. The hospital runs 55 weekly specialty clinics with patients' referrals from many states in the southwestern region and from other parts of the country. Antiretroviral treatment started in the hospital in 2002 when the Federal Government of Nigeria introduced ARV program in the country. The treatment program was scaled up in 2004 with support from the US government President Emergency Fund for AIDS Relief (PEPFAR) program through funding provided to the Harvard School of Public Health, Boston, USA. There are over 10,000 HIV infected individuals currently receiving care and treatment in the hospital.

2.2. Study Population

The study participants included patients attending the PEPFAR supported UCH ART clinic who have been on HIV treatment for at least one year with consecutive viral load of over 2000 copies/ml as well some ART Naïve patients attending the hospital for pre-ART assessment. Individuals who commenced therapy before 2005 were excluded from this study because there were reported drug stock-outs during the government of Nigeria pilot treatment program that lasted until late 2004 and some of the patients on that program were reported to have developed drug resistance mutations [20].

2.3. HIV Viral Load Determination (RNA Quantification)

Viral load measurement was carried using the Roche Amplicor version 1.5 with lower and upper detection limits of 400 copies/ml and 750,000 copies/ml respectively.

2.4. HIV Drug Resistance Genotyping

HIV RNA was extracted from 500 ul of plasma using the QIAamp Viral RNA Extraction Mini Spin Kit (Qiagen, Germany). HIV RNA was reverse transcribed to cDNA, amplified and subsequently sequenced using the Viroseq HIV-1 genotyping assay, version 2.0 as previously described by Chaplin *et al*. [25]. Sequences were generated using a 3130 XL genetic analyzer (Applied Biosystems) and the generated sequences were edited and compared with an HXB2 subtype B reference using the Viroseq software and list of mutations generated. The mutations were classified as minor or major base on the June 22, 2013 updated HIV drug resistance data base (http:/hivdb.standard.edu). Resistance to each drug was determined using a combination of the Viroseq and Stanford drug resistance algorithms and resistance to each drug assigned as susceptible, intermediate or resistant.

3. Results

A total of 46 samples were analyzed in this study. The characteristics of the patients whose samples were analyzed are shown in **Table 1**. The mean age of the participants was 43 years (range, 29 - 70) and 58.7% of them were female. However more male patients seem to be failing 2nd line treatment while more female failed 1st line drugs. The average time on ART was 3.2 (range, 0.5 - 5.5) years and 2.8 (range, 1 - 4) years for those failing the 1st line and the 2nd line drugs respectively. There was a gender bias in the time between ART commencement and virologic failure for patients on 1st line regimen. The

average time was 4 years and 1.5 years for female and male patients respectively. Only one of the 21 patients on ART had a major resistance mutation while 71.4% of them had no resistance mutation (**Table 2**). Fifty percent of the patients failing 1st line and 100% of those failing 2nd line had major resistance mutations. About 30% of those failing 1st line drugs did not have any resistance mutation while 19.8% of them had only minor mutations.

Table 3 shows the characteristics of individuals with major resistance mutations. The mean CD4 and median viral load of those with major resistance mutations were lower than those of the study population (**Table 1**). The only ART Naïve individual with a major mutation was a female with CD4 of 23 cells/ul and viral load of 78,792 copies/ml.

The most common major NRTI mutation was D67N followed by T215Y and M41L while the most frequent major NNRTI mutations were Y181C and K103N. Among the PI mutations, the most frequent was M46L/I followed by V82F/S/I and then I47V (**Table 4**). Other mutations detected include: M184V/I (13) M41L (6), E44D (1) T69N (2), L10I/V (16), V11I (3), A98G (7), P225H (1) AND P236L (L). **Table 5** shows the drugs by class to which virus developed resistance. Lamivudine (3TC) and emtricitabine (FTC); nevirapin (NVP) and nelfinavir (NFV) were the most common NRTI, NNRTI, and PI drugs respectively to which the virus in the infected individuals developed resistance. Virus from 4 of the patients were resistant to more than six antiretroviral drugs (**Table 5**) including 3TC, FTC, AZT, d4T, ABC, APV, FOS, IDV, LPV, NFV, TPV. Isolates from the 4 patient samples were resistant to triple drug class, including at least one NRTI, NNRTI and a PI. Interestingly the virus from one of the patients who failed 2nd line treatment was resistant to all the eleven drugs listed above (**Table 6**). Virus from the only ART Naïve individual with major drug resistance mutation was resistant to the PI nelvinavir (NFV).

Table 1. Showing characteristics of study the participants in the study.

ART status	N	Mean age (yrs.)	Average time on ART (yrs.)	Gender		CD4 (cells/ul)		Viral load (copies/ml)	
				Male	Female	Mean	Range	Median	Range
Naïve	21	43.6	NA	9	12	219	14 - 723	102,755	8655 - 2,623,338
1st line failure	16	40.7	2.9	4	12	367	35 - 1165	168,008	3785 - 1,201,535
2nd line failure	9	45.8	2.1	6	3	199	32 - 769	95,261	3899 - 43,926
Total (overall)	46	43.0	NA	19	27	267	14 - 1165	100,417	3785 - 2,623,338

NA: Not Applicable.

Table 2. Resistance mutation types among patients enrolled in the study.

ART status	No tested	No.(%) mutations		Minor mutations		Major mutations	
		No.	%	No.	%	No.	%
Naïve	21	15	71.4	5	23.8	1	4.8
1st line failure	16	5	31.2	3	19.8	8	50.0
2nd line failure	9	0	0.0	0	0.0	9	100
Total (overall)	46	20	43.5	8	17.4	18	39.1

Table 3. Showing some demographic and laboratory parameters of various categories of patients with major drug resistance mutations in the study.

ART status	No with MRM	Gender		CD4 (cells/ul)		Viral load (copies/ml)	
		Male	Female	Mean	Range	Median	Range
Naïve	1	0	1	23	NA	78,792	NA
1st line failure	8	3	5	199	35 - 538	79,132	3785 - 608,333
2nd line failure	9	6	3	199	32 - 769	95,261	3899 - 43,926
Total (overall)	18	9	9	197	23 - 769	79,132	3785 - 60,8333

MRM = Major Resistance Mutation; NA = Not Applicable.

Table 4. Showing frequency of major resistance mutations among the study participants.

Class of drugs	NRTI		NNRTI		PI	
	Mutation	Frequency	Mutation	Frequency	Mutation	Frequency
Mutations	M41L	5 (27.8%)	V179E	1 (5.6%)	L24I	1 (5.6%)
	D67N	6 (33.3%)	G190A	1 (5.6%)	M46L/I	10 (55.6%)
	K70R	1 (5.6%)	K101E	1 (5.6%)	I47V	3 (16.7%)
	L210W	3 (16.7%)	K103N	2 (11.1)	G48V	1 (5.6%)
	T215Y	4 (22.2%)	Y181C	3(16.7%)	I54V	1 (5.6%)
	-	-	Y188L	1 (5.6%)	L76V	2 (11.1%)
	-	-	F227L	1 (5.6%)	V82F/S/I	4 (22.2%)
	-	-	-	-	I84V	1 (5.6%)

Table 5. Showing patterns of drug resistance among the study participants.

Class of drug	Drug	No of sample(s) with resistance to each drug
NRTIs	3TC	10 (55.6%)
	FTC	10 (55.6%)
	AZT	3 (16.7%)
	STAVUDINE	3 (16.7%)
	ABC	3 (16.7%)
	TDF	4 (22.2%)
NNRTIs	DLV	6 (33.3%)
	EFV	6 (33.3%)
	NVP	7 (38.9%)
	ETR	1 (5.6%)
PIs	APV	3 (16.7%)
	FOS	3 (16.7%)
	IDV	4 (22.2%)
	SQV	1 (5.6%)
	LPV	2 (11.1%)
	NFV	7 (38.9%)
	TPV	1 (5.6%)

Table 6. Showing number of patients whose virus had the various drug resistance combination indicated.

Sample of number(s)	Drug resistance
1	NFV
2	DLV, NVP
1	SQV, NFV
2	3TC, FTC, EFV
2	3TC, FTC, ETR
1	DLV, EFV, NVP, TDF
1	3TC, FTC, EFV, NVP
1	3TC, FTC, DLV, NPV, NFV, IDV
1	3TC, FTC, DLV, EFV, NVP, TDF
1	3TC, FTC, DLV, EFV, NVP, NFV, TDF
2	3TC, FTC, DLV, EFV, NVP, NFV, TDF
1	3TC, FTC, AZT, d4T, ABC, APV, FOS, IDV, NFV
1	3TC, FTC, AZT, d4T, ABC, APV, FOS, IDV, LPV, ATV
1	3TC, FTC, AZT, d4T, ABC, APV, FOS, IDV, LPV, NFV, TPV

4. Discussion

This study describes the prevalence and pattern of mutations associated with ARV drug among patients on 1st and 2nd line therapy as well as ART naïve patients in Nigeria. Our results show that more female failed 1st line drugs and a higher average time on ART before failure among female than male patients who failed 1st line. This finding is in accord with an earlier finding by Chaplin *et al*. [26] who observed a potential difference in time to failure based on gender. These earlier workers recommended better drug adherence in women in the first 12 months and gender response to therapy as possible reason for rapid resistance mutations in men. It is therefore reasonable to suggest that problem of poor adherence may also explain why more men seem to be failing 2nd line therapy as observed in this study. The low mean CD4+ cells and high viral load found ART among the naïve individuals is similar to previous reports from Nigeria [25,26] and some other low-and-middle-income

countries [21,27-29]. This may be due to the poor health seeking behavior in Africa where most patients seek medical attention only when their health condition has deteriorated significantly.

Only one [4.8%] of the ART naïve individuals had any major drug resistance mutations with possible resistance to nelvinavir. Although this rate is lower than reports from Europe and some other African counties [28,30-34], it is still a cause for concern because the major source of DR in ART naïve is through transmission of resistance strains. This finding underscores the need for drug resistance surveillance among newly infected individual in order to detect DR transmitted viruses for early intervention. The 4.8% rate of major drug resistance mutations among drug naïve individuals obtained in this study is similar to the rates reported in other low and middle-income countries. A survey conducted by WHO in 20 countries showed an overall transmitted drug resistance virus rate of 3.7% [30]. Globally, the rates of transmission of DR viruses is increasing [20,22,32,35] and therefore the need for pre-ART resistance testing cannot be over emphasized. However, the cost of this testing is enormous and may be difficult to implement in resource limited settings [7].

Although antiretroviral therapy is effective in suppressing HIV-1 replication and prolonging live of infected individuals, some patients are experiencing detectable viral replication even under highly active antiretroviral therapy [36-38]. Several factors such as resistance to current drugs, poor adherence, co-infection with tuberculosis have been associated with this phenomenon [22,39-41]. In this study, 31.2% [5/21] and 19.8% [3/21] of the 1st ART failures had no DR mutation and minor mutations/polymorphisms respectively. The therapeutic failure of these individuals may be due to other factors than DR mutation. The medical records of the patients showed that 50% of these individuals had drug adherence problem, 25% had co-infection with TB and no obvious reason could be attributed to the failure in the remaining 25%. A similar finding was reported by Abar *et al*. among patients failing 1st line therapy in Djibouti [32].

The finding that 50% and 100% of those on 1st line and 2nd line drugs respectively had major drug resistance mutations compared to 4.8% of ART naïve indicates that these mutations developed as a result of ART use rather than transmission of resistant strains. Drug pressure as well as poor drug adherence and drug absorption rate that lead to circulation of sub-optimal blood level of drug are known factors that contribute to the development of drug resistance mutations [22,40,42,43].

The M184V/I mutation was the most common minor mutation found in 72.2% of the samples which is similar to findings of other studies [27,30,44] and known globally as the most common NRTI-resistance mutation [22, 45]. Although the mutations are known to cause high-level *in-vitro* resistance to 3TC/FTC, they are not contraindication to 3TC/FTC due to reduction of viral replication fitness and increase susceptibility to TDF, AZT, and d4T [45]. The most common NRTI, NNRTI and PI associated major resistance mutation detected were D67N [33.3%], Y181C [16.7%] and M46L/I respectively. All the NRTI mutations identified [M41C, D67N, L201W, T215Y, K70R] were TAMS that are known to increase resistance to AZT, tenofovir, d4T, abacavir, and DDI [33,45]. No NRTI conferring multidrug resistance [MDR] was detected. The NNRTI mutation at position 181, Y181C is known to result in high-level ETR and RPV resistance [46-48] while the PI mutation, M46LI is known to have high-level reduced susceptibility or increase resistance to FPV/r and IDV/r [45,49].

The drugs to which each of the virus isolates was resistant to were determined using a combination of the Viroseq and Stanford algorithm. Over 50% of the patients had viruses that were resistant to 3TC or FTC. Only 16.7% of them had viruses that are resistant to AZT while no resistance to d4T was detected. These two drugs are NNRTI backbones for some of the 1st line drugs used in the Nigeria and the results therefore indicate that Nigerian patients are responding well to these drugs and hence can continue to serve as good backbone for 1st line antiretroviral therapy in the country. The high rate of multidrug class resistance found in this study, especially among individuals on 2nd line therapy is of great concern and suggests the need for careful selection of second line drugs based on drug resistance testing. Genotypic testing has been shown to be beneficial in guiding appropriate ART selection [30], hence the significance of this study.

5. Conclusions

We have shown that the high rate of some resistance minor and major mutations occurs in HIV-1 among patients failing first and second line antiretroviral drugs in Nigeria. The study also showed occurrence of resistance mutations in HIV-1 in ARV Naïve patients in our study population. The work therefore emphasizes the importance of surveillance for resistance to ARV in line with the magnitude of scaling up of treatment program in the country.

WHAT IS ALREADY KNOWN ON THIS SUBJECT

Previous studies have shown that development of drug resistance is a major problem associated with wide spread use of antiretroviral drugs for treatment of HIV infected patients. However there is dearth of information on the drug resistance pattern in settings with predominance of non-subtype B of HIV-1 like Nigeria.

WHAT THIS STUDY ADDS TO LITERATURE

The results of this study show the rate and pattern of

antiretroviral drug resistance among HIV-1 infected patients failing first and second line regimens in Nigeria where non-B subtypes of the virus circulate. The high rate of multidrug resistance reported in this study, especially among patients on second line regimen is significant and will be helpful in the choice of drugs for treatment.

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7. Authors' Contribution

GNO and DOO conceived the idea of the study, GNO, PO, IFA and DOO were involved in collection and analysis of data, PO and IFA supervised recruitment and enrolment the patients, GNO and DOO supervised the laboratory investigations. GNO wrote the draft manuscript while all authors reviewed the manuscript and approved the final version. DOO is the guarantor of the paper.

8. Ethical Considerations

Approval for the study was obtained from the University of Ibadan/University College hospital ethical review board. Informed consent was obtained from all participants in the study.

9. Competing Interest

We declare that we have no conflicting interest in the conduct of the study.

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