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Human immunodeficiency virus type-1 (HIV-1) genetic diversity and prevalence of antiretroviral drug resistance mutations in treatment-naïve adults in Jos, North Central Nigeria

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The presence of human immunodeficiency virus (HIV) type-1 diversity has an impact on vaccine efficacy and drug resistance. It is important to know the circulating genetic variants and associated drug-resistance mutations in the context of scale up of antiretroviral therapy (ART) in Nigeria. The objective of this study was to determine the genetic diversity of HIV-1 and the prevalence of antiretroviral (ARV) drug resistance mutations among antiretroviral treatment-naïve HIV-1 infected patients in Jos, North Central Nigeria. Plasma samples were collected from 105 ARV drug-naïve patients enrolled for HIV care at the Jos University Teaching Hospital (JUTH) HIV Treatment Center between October 2010 and April 2011. One hundred (100) samples were successfully amplified. Viral subtyping was done using REGA subtyping tool and by phylogenetic analysis using PAUP software. The drug resistance mutations were determined using the Stanford University HIVdb sequence interpretation algorithm. HIV-1 subtypes identified were; CRF02_AG (48.0%), G (41.0%), CRF06_cpx (6.0%) and A1 (5.0%). 8% of the patients' isolates had at least one major resistance mutation in the *RT* gene: Nucleoside reverse transcriptase inhibitors: M41L (1%), K65KR (1%), M184IM (1%), M184V (2%) and T215ADNT (1%), non-nucleoside reverse transcriptase inhibitors: K103N (2%), K101E (1%), G190A (1%), P225HP (1%), Y181I (1%), Y188L (1%), and Y181C (1%). Among antiretroviral (ARV) naïve patients in Jos, North Central Nigeria, the common HIV-1 subtypes was CRF_02 and G. And the prevalence of drug resistance mutations was found to be high (8%). Further study and national surveillance will be critically important to understand the clinical impact of transmitted resistance mutations on ART naïve individuals in resource limited settings.

Key words: HIV-1 subtypes, antiretroviral (ARV), treatment-naïve, drug-resistance, mutation, accessory and polymorphisms, Nigeria.

INTRODUCTION

Most human immunodeficiency virus type-1 (HIV-1) strains infecting individuals in the Americas, Europe and Australia belong to subtype B, whereas the majority of the remaining viral subtypes are found among persons in sub-Saharan Africa (Robertson et al., 2000). The genetic diversity within HIV-1 presents a challenge for global management of HIV infection even as antiretroviral (ARV) drugs become increasingly available. The use of highly active antiretroviral therapy (HAART) in developed countries has led to a marked reduction in the mortality rate among HIV infected patients. Until recently, access to life-saving ARVs was limited in low and middle-income countries. However, the introduction of affordable ARV combinations through local and international efforts has led to a significant increase in access to ARVs. Treatment failure is mostly related to emergence of drug-resistant variants and its transmission to uninfected individuals raises serious clinical concerns for initiation of therapy. The prevalence of resistant mutations in newly infected individuals range between 10 to 25% in Europe and the Americans (Wensing et al., 2005), while in developing countries like Nigeria, where access to ARVs is increasing, resistance in the treatment-naïve HIV-1 infected is rarely reported (Vergne et al., 2006). Drug resistance arises from mutations in the *pol* (protease-PR and reverse transcriptase-RT) genes that encode the molecular targets for the drugs. The viral polymorphisms are due to the high rate of HIV-1 replication, low fidelity of RT (Wei et al., 1995), recombination (Nukoolkarn et al., 2004) and variants accrual during the infectivity period (Hirsch et al., 1998). The emergence of amino acid substitutions associated with resistance can be classified as major (primary) and accessory (secondary) mutations. Some major mutations may lead to several fold decrease in sensitivity to one or more antiretroviral drugs by single (mutation) appearance. Accessory mutations may not result in a significant decrease in sensitivity but are associated with an increase in viral fitness. Thus, the appearance of major mutation in a genome already containing accessory mutations could influence the speed with which highly resistant viruses are selected during therapy. Studies have revealed that HIV-1 subtypes A1, CRF02-AG and G predominate the epidemic in Nigeria (Chaplin et al., 2011; Ajoge et al., 2012). Given the increasing genetic heterogeneity of HIV-1 in West Africa and the increasing use of antiretroviral drugs, it is important to characterize *pol* gene sequences from HIV-1 subtypes occurring among drug-naive populations. Recent studies have documented that Nigeria has a prevalence of 1.6% in untreated patients (Hamers et al., 2011).

Nigeria has the second highest burden of HIV infection in the world, second only to South Africa. The report of the 2010 HIV survey showed that the North Central Zone had the highest HIV prevalence (7.5%) of all the six geo-political zones in the country (GARPR, 2012). The current number of Nigerians on ARVs has been estimated to be 620,000 (personal communication). This figure has not been disaggregated by geo-political zone; hence the number of persons on ARVs in north central zone in currently not known. However, with the rapid scale up of ARV use and limited infrastructure for care and monitoring, Nigeria may face an increase in the emergence of HIV-1 resistant strains among the drug-naïve population. In this study, we characterized HIV-1 subtypes and determined prevalence of drug resistance mutations in treatment-naïve patients in Jos, North Central Nigeria.

MATERIALS AND METHODS

Patients

This study was carried out at the AIDS Prevention Initiative in Nigeria (APIN) supported HIV clinic at the Jos University Teaching Hospital (JUTH), Jos. The entry point for all patients was either through HIV counseling and testing (HCT) or referred HIV positive patients from other services within the hospital and the surrounding community. One hundred and five (105) HIV-1 infected treatment-naive patients were recruited sequentially after obtaining informed consent between October 2010 and April 2011. The Jos University Teaching Hospital Ethics Committee approved the study protocol. A questionnaire was used to collect basic demographic data from each study participant. Criteria for inclusion in this study was patients who had no previous ARV exposure and were aged 18 years and above. Blood samples were collected in athylenediaminetetraacetic acid (EDTA) lined containers and plasma was extracted and cryopreserved. The samples were subsequently shipped in ice parked containers to the Kenya Medical Research Institute HIV-Resistance Laboratory, Kisian Kisumu, where genotypic testing using In-house Genotyping System was done. Out of 105 samples tested, 100 were successfully amplified for genotypic drug resistance testing

HIV-1 genotyping

Isolation and amplification of HIV-1 partial pol gene by RT-PCR and nested PCR

Two amplification protocols for HIV *pol* gene were used as previously described (McNulty et al., 2007). Adhering to a unidirectional flow, the HIV viral RNA was extracted from plasma using the QIAamp Viral RNA mini kit (Qiagen, Hilden, Germany) followed by reverse-transcription of the RNA into cDNA using the outer primers Prt-F1-forward (2253-2275 nucleotides, nt) and RT-R1 reverse (nt 3370-3348) for RT-PCR. The cDNA were amplified by nested-PCR with primer Prt-F2 (forward, 2265-2288 nt) and RT-R2 (reverse, 3326-3304 nt). The amplified DNA fragments from nested-PCR were verified by visually comparing the intensity of each sample's band to that of the DNA mass ladder's bands of

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known DNA quantity for expected size by electrophoresis in 1.0% agarose gel stained with 0.5 µg/ml ethidium bromide and photographed under ultraviolet illumination. The fragments were purified using the QIAquick PCR purification kit (Qiagen, Hilden Germany) in spin columns, and direct population-based sequencing performed on both strands using BigDye® Terminator v3.1 Cycle Sequencing kit on an automated ABI 3130X1 Genetic Analyzer (Applied Biosystems).

Sequence analyses and determination of drug resistance mutation

The generated nucleotide sequences were viewed using the Sequence Analysis Software v3.7, and aligned and edited using Sequencher version 5.0, which assembles the six overlapping sequence segments for the six primers to form a contiguous sequence. Sequences with frame shifts or stop codons were excluded from analysis. The quality of the generated sequences was checked using Sequence Quality Assessment Tool (SQUAT). The sequences in fasta format were then subjected to the Stanford HIVdb algorithm (http://hivdb.stanford.edu/) for subtyping and determination of mutations conferring various antiretroviral drugs resistances. Mutations in the sequences were defined as differences from the consensus B reference sequence and were further characterized as RTI and PI associated resistance mutations.

Subtyping and phylogenetic analyses

HIV-1 subtyping was performed using REGA HIV-1 subtyping tool V2.0 from Stanford HIV drug resistance database (http://hivdb.stanford.edu/), a worldwide subtype references were obtained from Los Alamos database (http://hiv-web.lanl.gov), and sequences were aligned against the known reference strains. Phylogenetic inferences were performed by the neighbor-joining (NJ) method as implemented in PAUP* version 4.0 (Swofford et al., 2002).The reliability of the tree topology was tested with 1000 bootstrap analyses and bootstraps of ≥70% were considered significant. The bootscanning method was used to detect and study recombination, as implemented in the SIMPLOT software, version 2.5 (Saminen et al*.,* 1995). Figure 2 shows a rooted tree of the 84 selected pol sequences for clarity with group O sequence O.CM.91 as an out group. The recombination was further confirmed with Recombination Identification Program (RIP) version 3.0 available online (http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html). Subtypes assignments by this method are shown to the right of the tree with isolates clustered around the various references.

RESULTS

Baseline characteristics of the study population

A total of 105 patients were sequentially recruited and categorized. Samples for five patients could not be amplified and they were excluded from analysis. The mean age (years) was 37±9.5. The proportion of women and men were 55 and 45%, respectively. 98% of the patients reported a heterosexual mode of transmission while 2% was via blood transfusion. None of the patients were men who had sex with men (MSM) or injection drug users (IDU). 66% of the participants resided in Plateau state, while 34% resided in other states. 69% were married, 19% were single (never married), while 8% were separated/divorced and 5% were widowed. 11, 22, 36 and 32% of the subjects had no formal education, elementary, secondary and tertiary education respectively. WHO disease staging of the subjects was: stage I, 24%; II, 21%; III, 36%, and IV, 19%. 50% of the spouses of the participants were HIV positive, while 50% were HIV negative. The participants whose spouses were on ARV were 14%, and those whose spouses were not on ARV were 86%. The median baseline CD4 cell count was 186 cells/mm^3 (IQR: 12-737).

Phylogenetic analyses of the PR and RT Sequences using PAUP v. 4.0

Figure 1 summarizes the distribution of different genetic subtypes in the *pol* region using 21 references from Los Alamos HIV Sequence database. Figure 2 shows results of the phylogenetic analyses of the sequences and illustrates the different clusters within the isolates. The subtypes AG and G clustered around references from Nigeria and other West and Central African neighboring regions. Phylogenetic analysis of the partial pol sequences showed that 48/100 (48%) of the isolates were CRF02_AG, 41/100 (41%) subtype G, 6/100 (6%) CRF06_ cpx and 5/100 (5%) subtype A1 (Figure 1).

Reverse transcriptase sequence variability of isolates

After the analysis of PR and RT sequences, 8% of the patients (8/100) had at least one resistance mutation (95% CI: 3.9 to 16.2%). The distributions of the subtypes and profiles of drug-resistance mutations according to drug classes (Table 1), and the mutations conferring to resistance to NRTIs included M41L (1%), K65KR (1%), M184IM (1%), M184V (2%) and T215ADNT (1%), while mutations that conferred resistance to NNRTIs comprised K101E1%), G190A1%), P225HP (1%), Y181I (1%), Y188L- (1%, K103N (2%) and Y181C (2%) (Table 2). Of the eight patients, seven females harbored viruses with drug resistance mutations to both RT inhibitors. Of four individuals that harbored multiple mutations, three of them were females (Table 1). The frequency of the accessory mutations in the RT region are as shown in Figure 3 and were as follows; R211K (65%), V179IE (8%), G196EG- (8%), V118I (5%), V106I (5%), A98SG (2%), T69N-(2%), L228HL (2%), and E44DE (1%), V90I (1%), E138A (1%) and L210M (1%).

Protease sequence variability in isolates

There was very low prevalence of major mutations detected in the non-B strains to protease inhibitors from

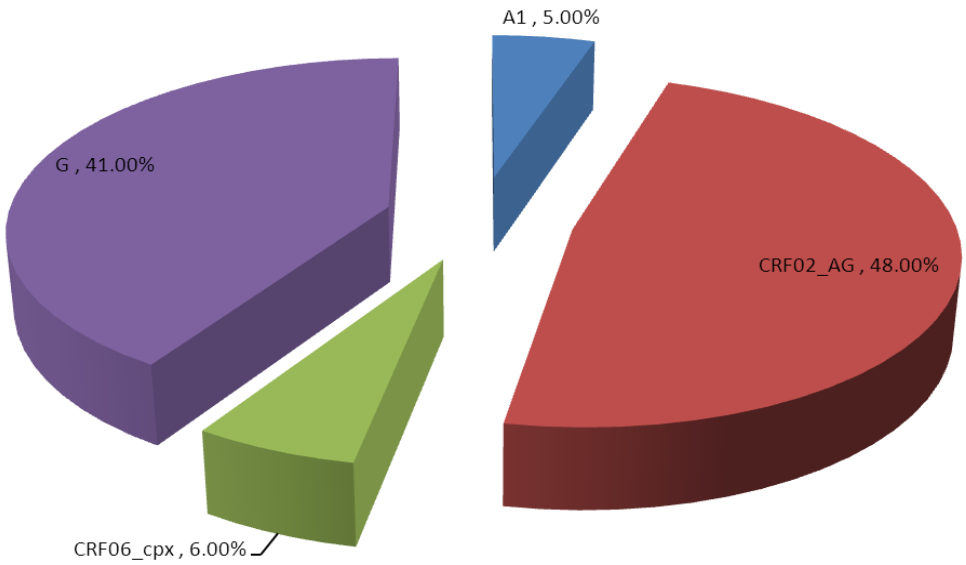

Figure 1. Analysis of HIV-1 genetic diversity of the *pol* gene. Phylogenetic analysis of the PR and RT sequences revealed distribution of HIV-1 subtype's diversity circulating among ARV treatment-naïve patients. The prevalence of observed subtypes are: 48% of the sequences were subtype CRF02_AG, 41% were subtype G, 6% subtype were CRF06_cpx, and 5% subtype A1. Subtypes AG and G were predominant in circulation than subtypes CRF06_cpx and A1.

the 100 samples. In contrast, many minor mutations were found at the following positions, in order of decreasing frequency (Figure 3); K20VMI (97%), M36I (94 %), E35DEGQ (42%), V82IV (40%), L63P (25%), L10I/V (20%), V77I (5%), I62V (3%), L10M (1%), V11LV (1%), L33F (1%), Q58E (1%) and L90V (1%). The single mutations observed were; L33F, L90V, Q58E, V11LV, and L10M. The substitution of valine at codon 90 and methionine at codon 10 against the known amino acid with subtype B was rare to non-B subtype. The study observed that each sequence had at least one known accessory PI drug resistance mutation (Figure 4).

DISCUSSION

Primary HIV drug resistance represents a challenge for the treatment of HIV infection because it can reduce the efficacy of first-line ART and has impact clinical outcomes. It is established that primary drug-resistance will emerge in the region where ART has been widely available for years (Shekelle et al., 2007), and that HIV-1 subtypes have been associated with drug resistance mutations. We analyzed the PR and RT gene-coding regions of 100 HIV-1 isolates from treatment-naive patients. This is the largest number of isolates among treatment naïve patients studied so far in North Central Nigeria. Sixty-three (63%) of our patients had CD4 count of ≤200 cell/mm^3, which may suggest long-standing infection and were therefore eligible to commence antiretroviral treatment using the existing national treatment guidelines at the time of the study.

The WHO disease staging was also evidence of advanced infection as majority of the patients were classified in stages III and IV. The frequency of those whose spouses were on ARV was 14% and this may be explained by the fact that patients enrolled in the ARV Centre upon disclosure of their HIV serostatus invite their spouses for provider-initiative HIV counseling and testing in the program if their HIV status turns out to be positive.

Interestingly, the most predominant strains circulating in the zone were subtype CRF02_AG with proportion of 48% followed by subtype G-41%, CRF06_cpx 6% and subtype A-5%, which were comparable with previous studies in Nigeria, Chad, Niger and Cameroon (Peeters et al., 2000; Vidal et al., 2003; Chaplin et al., 2011). The subtype AG clustered around the isolates from Ibadan, Nigeria and Cameroon. The high prevalence of CRF02_AG and G may explain differences in infectivity, elevated viral replication suggesting a higher rate of transmission. The fact that HIV-1 subtype G was observed with multiple drug resistance mutations could suggest higher rates of mutation and replicative capacity within the studied population. The diversity of HIV-1 viruses in Nigeria is becoming more evident, although the prevalence of subtype AG and G appears to be on the rise.

The presence of resistance mutations is a central

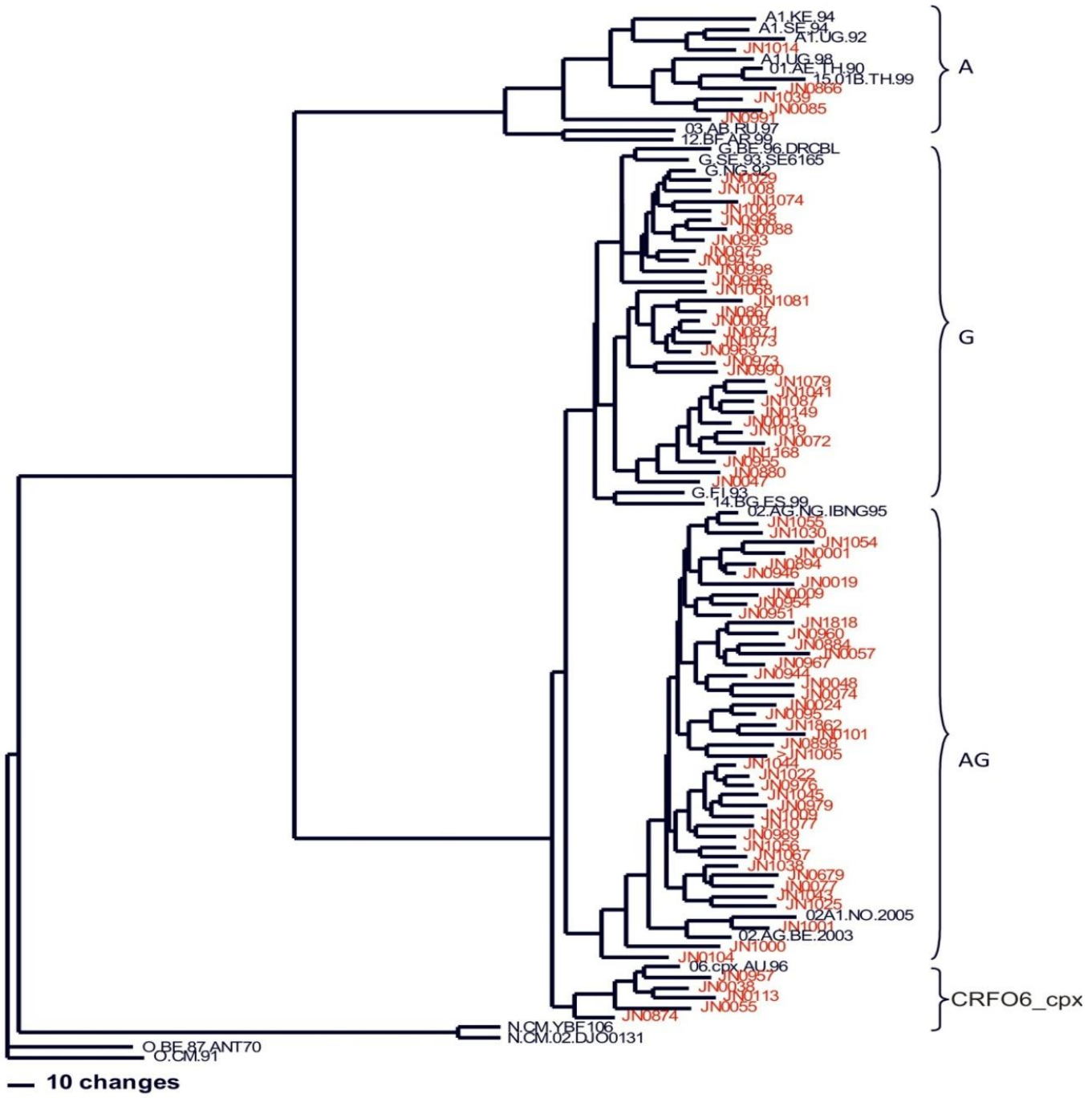

Figure 2. Phylogenetic analysis of Isolate from antiretroviral treatment-naïve patients. Neighbor– Joining (NJ) Rooted Tree of selected 84 HIV-1 isolates colored red by prefix “JN” with 21 aligned sequences (coloured black) from Los Alamos HIV database using PAUP version 4. Clustering of HIV-1 isolates among ARV treatment-naïve patients: the HIV-1 pol gene sequences were compared including Nigerian sequences obtained from Los Alamos HIV sequence data base. The subtype CRF02_AG and G clustered significantly around sequences from Nigeria (02.AG.NG.IBNG95 Ibadan isolates), Belgium (02.AG.BE.2003) and Jos Isolate (G.NG.92) respectively. Subtypes A1 and CRF06_cpx clustered around references from Thailand (15.01B.TH.99), Uganda (A1.UG.98) and 06.cpx.AU.96 respectively. Isolates JN1000 and JN0104 did have an earlier branching compared to other AG isolates suggesting an early earlier existing transmitted isolates. An additional pruning analysis, which consists of removing respective reference sequences from an alignment and rerunning the phylogenetic analysis, revealed that our CRF02_AG, subtype G sequences all maintained their distinct clustering patterns even when their respective reference sequences were absent. This further confirms the stability of the tree topology and subtype assignments. In addition, the subtype A and CRF06_cpx sequences clustered separately, and among themselves from the crown groups of AG and G viruses. Taken together, these data imply that CRF02_AG, G subtype A1, and CRF06_cpx have been in circulation for some time.

Table 1. Distribution of HIV-1 subtypes and mutations conferring resistance to NRTIs and NNRTIs among treatment-naïve patients.

PTID	Age	Sex	CD4 Cell count	Duration of HIV-1 Infection (AI)	HIV-1 Subtype	Spouse on ARV	RT major mutation	
							NRTI	NNRTI
JN1041	36	F	18	LI	G	Yes	M184IM	K101E, Y181I
JN0055	31	F	31	Recent	CRF06_cpx	No	-	Y188L
JN0679	34	M	93	LI	CRF02_AG	No	K65KR	Y181C
JN0866	27	F	21	LI	A1	Yes	T215ADNT	-
JN0990	39	F	272	LI	G	Yes	M184V	K103N, P225HP
JN0088	45	F	389	LI	G	Yes	M184V	Y181C, G190A
JN0110	29	F	237	LI	CRF02_AG	No	M41L	-
JN0114	43	F	21	LI	CRF02_AG	No	-	K103N

PTID, Patient identity; AI, avidity index; LI, long infection; ARV, antiretroviral; RT, reverse transcriptase; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

Table 2. Frequency of NRTI and NNRTI drug resistance mutations among treatment-naïve patients.

NRTI mutation	Number	%	NNRTI	Number	%
M41L	1	1.00	K101E	1	1.00
K65KR	1	1.00	K103N	2	1.00
M184IM	1	1.00	Y181I	1	1.00
M184V	2	2.00	Y181C	2	2.00
T215ADNT	1	1.00	Y188L	1	1.00
			G190A	1	1.00
			P225HP	1	1.00

NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non nucleoside reverse transcriptase inhibitor. Frequency of major NRTI and NNRTI mutations are reported in the total study population of 100 patients

problem to treatment efficacy. Of 100 sequenced samples, 8% of the subjects harbored at least one primary mutation that confers resistance to either NRTI or NNRTI (Table 1). The high prevalence of NNRTIs resistance among cases with transmitted resistance is a potential problem as the national guidelines recommends efavirenz based HAART as a preferred first-line therapy. NNRTI is known to have low genetic barriers, with which a single mutation may confer high level resistance to more than one drug in the class (K103N, Y18C/I, Y188L). Multiple major RT mutations were observed in four patients. Among the major drug-resistance mutations, the most common multiple mutations observed were NNRTI resistance mutations as observed in three three female patients (Table 1); this may suggest transmitted resistance since their spouses were known to be on ARV drug. The presences of T215ADNT mutation which is a revertant, suggests a previous infection with HIV-1 strains containing T215Y/F or represents a transition on the reversion from T215F/Y.

The observed prevalence of 8% ARV drug-resistance mutations in drug naïve patients is in contrast to 1.6% prevalence previously reported (Hamers et al*.,* 2011). This study reveals that most individuals had long established infection (89% data not shown), although the exact time of infection is unknown. Therefore, the observed prevalence of resistance may have been influenced by the long duration of HIV-1 infection and another possibility, is that high TDR is expected in patients whose spouses have poor adherence to ART or spouses who have multiple sexual contacts other than their spouse. Theoretically, newly infected patients are more likely to harbor more resistant viruses than chronic or long established infection because transmitted resistance may have reversed after a while without drug pressure (Perelson et al., 1996). This might be explained by the fact that wild type virus may become predominant in the absence of selective drug pressure when individuals are infected with both wild type and mutant viruses. However, several studies have demonstrated the persistence of mutations of major resistance for periods as long as five to seven years (Barbour et al*.,* 2004). The observed multiple primary resistance mutations were common in subtype G suggesting low genetic restrictions. Also identified was the replacement of leucine (L) with methionine (M) instead of tryptophan (W) at drug

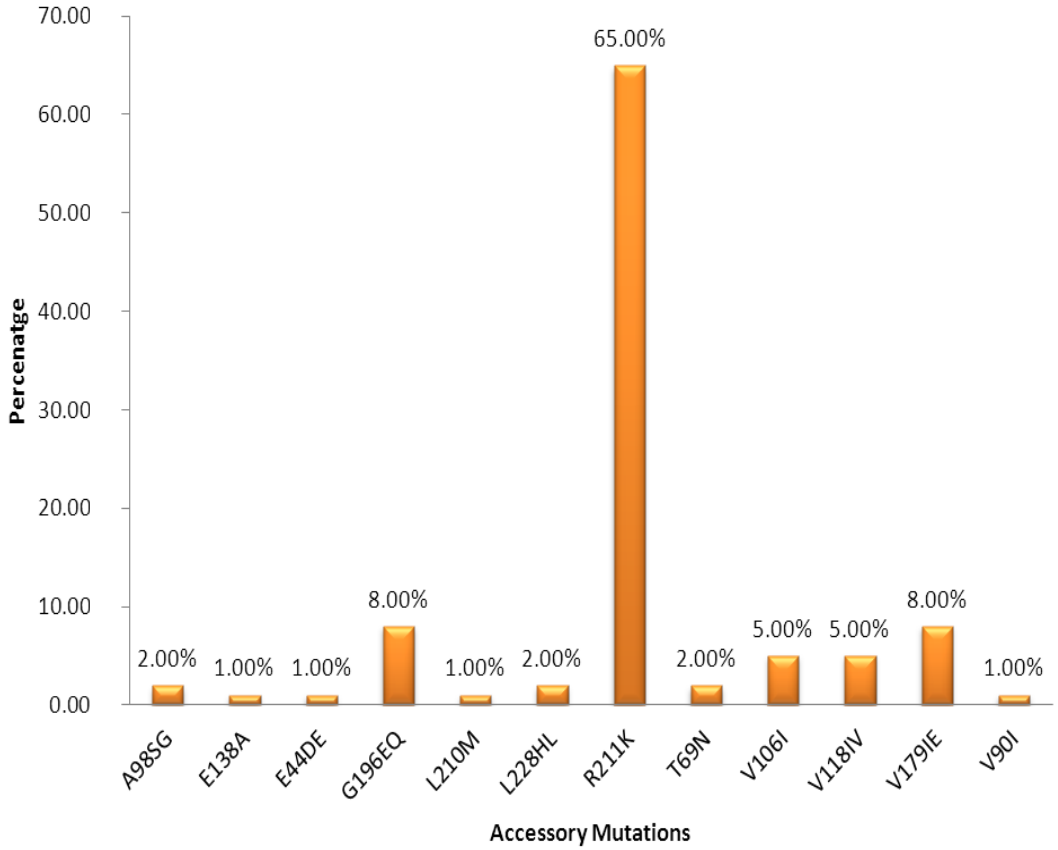

Figure 3. Prevalence of RT (NRTI and NNRTI) accessory mutations among the ARV treatment naïve patients. This figure shows the result of the sequence analysis blasted into the Stanford HIV consensus drug resistance database reveals some accessory (minor) mutations that confer some form of resistance to antiretroviral drugs. In NRTI the highest prevalence of amino acid substitution was observed in position R211K (65%), followed by Q196EQ (8%), L228HL (2%), A98S (2%), and E138, E44DE and L210M as 1% each. The frequency of amino acid position in NNRTI are in order of decrease; V179IE (8%), V106 and V1185 (5%), T69N (2%), and V90I1 (1%).

resistance position 210W; which is a rare finding. This mutation could be a reversion from thymidine analogue mutation-II (TAM) L210W mutation which confers high level resistance to some NNRTIs (stavudine and zidovudine).

The frequency of (TAM) M41L was low with only 1% of patients having virus with any TAM, and is associated with most nucleoside analogues except lamivudine. Overall, the protease gene region was less conserved than the RT gene because of its genome flexibility. Again, protease can afford to retain its altered dynamics via the compensatory mutations (Arts and Haduza, 2012). However, accessory mutations in the protease gene are known to reduce “genetic barrier” to drug resistance by increasing the selection of the resistant strains in the presence of other mutations (Wainberg and Brenner, 2012). Several other accessory mutations that are not classically associated with high level resistance were observed in *RT* gene; E44DE (1%), V90I (1%), A98SG (1%), E138K (1%), L210M (1%), L228HL (2%), T69N (2%), V118I (5%), V106I (5%), G196EG (8%), 179IE (8%) and R211K (65%). These mutations by themselves do not have a substantial effect on phenotype but have contributory role in the development of resistance mutations by modifying the effects of drug selected mutations, possibly by compensatory changes in the enzyme backbone. It has been reported that some of these RT positions (NRTI-44, 69, 211, 228; NNRTI-90, 98, 106,118,179) actually confer intermediate resistance to some available nucleoside and non-nucleoside analogues with diverse compensatory roles (Johnson et al., 2011). The prevalence of these mutations in the HIV-1 isolates infecting an individual suggests that the outcome of drug treatment may adversely affect the study population.

There were high frequencies of accessory mutations observed in protease gene, and the predominant mutations were at positions K20VMI, M36I, E35DEGQ

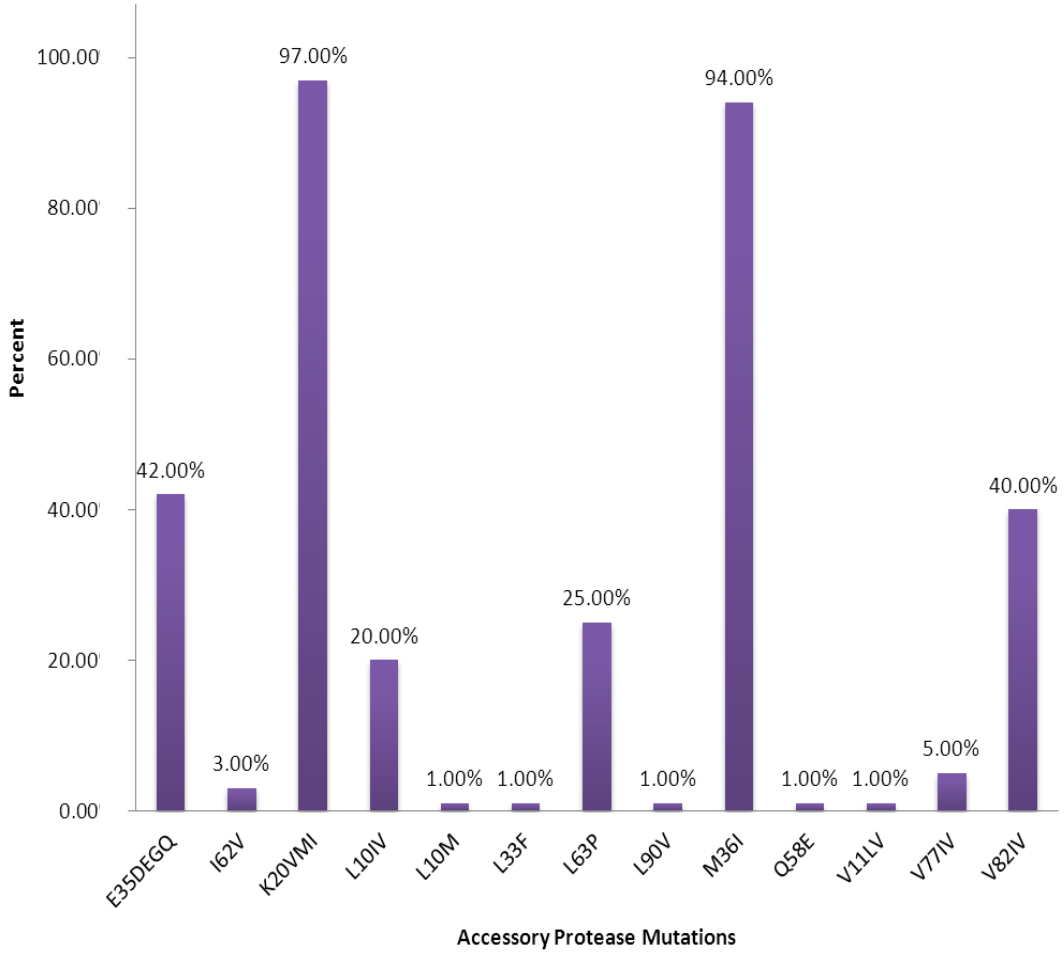

Figure 4. Prevalence of Protease Accessory mutations among the ARV treatment naïve patients. The figure shows the prevalence of accessory mutations associated with protease inhibitors in decreasing order; K20VMI (97%), M36I (94%), E35DEGQ (42%), V82VI (40%), L63P (25%), L10IV (20%), V77IV (5%), I62V (3%), and L10M, V11LV, L33F, Q58E, and L90V as 1% each. The most common substitutions were K20VMI, M36I, E35DEGQ and V82I. The single mutations observed were; L33F, L90V, Q58E, V11LV, and L10M. The substitution of valine at codon 90 and methionine at codon 10 against the known amino acid with subtype B is rare to non-B subtype.

and V82IV. The presence of these minor mutations do not lead to high level resistance when occurring alone but they either improve viral fitness or increases the drug resistance level in the presence of major PI mutations (Nijhuis et al., 1999; van Maarseveen et al., 2006; Scherrer et al., 2012).

From comparative analyses, the study also demonstrates that in HIV-1 subtypes CRF02_AG and subtype G, the specific codon positions are often utilized differently, either in contrast with one another or with subtype B in encoding amino acids, hence leading to distinct functional motifs that may influence divergent pathways to drug resistance. These findings emphasize the significance of subtype specific determinants of susceptibility to antiretroviral therapy and immune response (Wainberg and Brenner, 2012). However, it is important to keep in mind that late detection of HIV disease and accessing of ARVs are the characteristics of most patients attending the tertiary treatment center in Nigeria. Over 60% of individuals enrolled in this study were diagnosed with <200 cells/mm^3, and were therefore late presenters. The observed result reflects transmitted drug resistance (TDR) levels characteristics of individuals with established infection or advanced HIV disease. In Nigeria, the population of treated patients in the scale-up exercise is not well monitored, and poor adherence to ARV medication could lead to emergence of drug resistance and drug failure. This may suggest that the transmission of resistance mutations from treated individuals to treatment-naïve patients is likely to occur more frequently.

In conclusion, this study indicates that ARV resistant

variants and drug-resistance mutations exist among HIV-1 infected treatment naïve patients in Jos, North Central Nigeria. The diversity of subtypes has implications for treatment and vaccine design strategies. Population surveillance for ARV drug resistance is important and should be included in all implementation programs. The findings from this study also underscores the need for targeted genotyping the HIV-1 viral genome before beginning of ARV treatment and recommendations of appropriate choice of first-line regimens in resource limited countries.

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