

Virological and Molecular Profile of People Living with the Human Immunodeficiency Virus Starting Dolutegravir Based Antiretroviral Treatment in Kinshasa, Democratic Republic of Congo

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Abstract

Context: Despite the new recommendations “Test and Treat”, the virological and molecular parameters remain important information for Antiretroviral Treatment and adequate for monitoring of patients infected with HIV/AIDS. **Objective:** the Objective of this study is to present the virological and molecular profile of People Living with HIV starting Antiretroviral Therapy in Kinshasa in the era of the Dolutegravir. **Methods:** This was a transversal study to determine virological and molecular profile of People Living with HIV (PLHIV) starting an ARV Treatment. The patient’s inclusion period was from October 04, 2021 to February 15, 2022. A sample of 5 ml of blood was taken in a tube with EDTA anticoagulant for Molecular Biology analyzes (Viral Load and Sequencing) in all HIV patients, after reading and signing informed consent. The population was made up of adult patients over the age of 18, infected with HIV and starting ART. **Results:** 119 patients (56.3% of women) were included in this study, thus a sex-ratio of 1.29. The average age of patients included was 39.87 ± 12.36 years. The most represented age group is that of 36 to 45 with 37 patients (31.9%) followed by that from 26 to 35

years with 24 patients (20.7%). Out of 119 patients, 21 patients had an undetectable Viral Load (VL). The median value of VL was 4.16 log₁₀ RNA copies/ml. 114 samples were successfully amplified. Subtype A was dominant with 23 cases (20.2%); followed by the subtype C and CRF02_AG each with 14.0%, and D (10.5 %). K65R (1.8%), T69P/N (4.4%), K70R (7.9%) and M184V (7.0%) mutations were listed as existing mutations for Nucleotide Transcriptase Inhibitors. **Conclusion:** 38 patients (31.93%) started the TARV with a positive virological prognosis. The subtype A remains dominant in Kinshasa with 23 cases (20.2%); followed by the subtype C and CRF02_AG each with 14.0%. For Inhibitors of Transcriptase Reverse Nucleotide; K65R, T69P/N, K70E/R and M184V mutations were found in patients' naive of ARV Treatment.

Keywords

Virological Profile, Molecular Profile, PLHIV, Start of ART, Kinshasa

1. Introduction

After more than four decades of fighting the infection of the Human Immunodeficiency Virus (HIV) and the Acquired Immuno Deficiency Syndrome (AIDS), these are still major public health problems around the world. According to the United Nations Organization of Nations to Combat HIV/AIDS (UNUSA), in 2020, 37 million were estimated [30.2 millions - 45.1 millions] the number of People Living with HIV/AIDS (PLHIV) and 1.5 millions [1.0 million - 2.0 million] people were newly infected with HIV/AIDS in the same year [1]. Similarly, the World Health Organization (WHO) estimates that Sub-Saharan Africa remains the most affected region which carries the heaviest burden of the epidemic with 26 million PLHIV and represents 70% of all deaths related to AIDS in the world [2].

Despite the new recommendations "Test and Treat" adopted by HIV/AIDS control programs in the Democratic Republic of the Congo (National Multisectorial Program to Combat HIV/AIDS-PNMLS and the National HIV/AIDS and National Program ISTS-PNLS) [3] [4], virological (Viral Load) and molecular (circulating strains of the virus) parameters remain important information for Antiretroviral Treatment (ART) and adequate monitoring of patients infected with HIV/AIDS [5] [6]. They, both, are significant parameters for the decision to start ART and of which molecules are suitable according to the molecular diversity of HIV [5]. They are also very important in the epidemiological surveillance, the diagnosis of children under 18 months, and the adherence to treatment at the change of line of treatment [5].

Hence the objective of this study was to present the virological and molecular profile of People Living with HIV starting an Antiretroviral Therapy in Kinshasa in the era of the Dolutegravir.

2. Methods

2.1. Study Design, Patient and Samples Framework

This study is a cross-sectional and descriptive to determine the virological and molecular profile of People Living with HIV (PLHIV) starting an ARV Treatment (ART) in Outpatients Treatment Centers (OTC) for HIV in Kinshasa, Democratic Republic of Congo (DRC). The patient's inclusion period was from October 04, 2021 to February 15, 2022, during which all adult patients initiating an ART in an included OTC were included. Sixteen OTCs had been included for study based on their expertise in the management of PLHIV, their technical tray of care and their accessibility [7].

In the OTCs, 5 ml sample of blood were taken in a tube with EDTA anticoagulant from the vein in the bend of the elbow for molecular biology analyzes (viral load and sequencing) in any positive HIV patient by serology according to The national protocol, after reading and signing informed consent. Patients were included consecutively randomly according to their presence in the OTCs during consultations.

All molecular data has been recorded on the previously tested survey sheets.

2.2. Study Population

The population was made up of adults over the age of 18 during inclusion, infected with HIV and initiating an ART in the OTC during the inclusion period.

2.3. Inclusion Criteria

All the patients coming to initiate ART in the selected centers during the inclusion period were included in the cohort upon signing the informed consent.

2.4. Parameters of Interest

The parameters of interest retained for this study were: sex, age, viral load (VL) and sequencing data.

2.5. Molecular Analyzes

After sampling, the samples were taken, while respecting temperature, at the Molecular Biology laboratory of the Faculty of Medicine, University of Kinshasa for analysis.

DNA was extracted from 200 µl of Buffy Coat using the Qiamp DNA Mini Kit QIAGEN® for DNA extraction [8]. A Nested PCR in the *gag* and *pol* regions was carried out for confirmation of the serology of all the patients included [9] [10]. A reverse transcription PCR (RT-PCR) and a nesting PCR were made to amplify the regions of interest for the protease and for the Reverse Transcriptase (RT) for sequencing [11] [12].

RNA was extracted from 140 µl of plasma in the Molecular Biology laboratory using the Qiamp RNA Mini Kit QIAGEN® for RNA extraction [13]. A Quantitative Real-Time PCR (qPCR) was carried out to quantify proviral HIV in the

samples according to the protocols previously described [11] [14] [15].

The extract RNA was also used for sequencing. A Reverse Transcription PCR (RT-PCR) and a Nested PCR were made to amplify the regions of interest for the protease and for the Reverse Transcriptase (RT) for sequencing. The PCRs were carried out under the conditions previously described [11] [12]. These fragments were sequenced by the Sanger sequencing method. The pairing of the fragments obtained (sens and anti-sens) was carried out with the Vector NTI Advance® 11.5 software (Invitrogen, Life Technologies) and compared with the Database of Stanford University (www.hivdb.stanford.edu) [11] [12].

Table 1 presents the different primers and probes used for the different amplifications.

2.6. Ethical Consideration

This study was approved as a whole by the Research Ethics Committee of the School of Public Health, Faculty of Medicine, University of Kinshasa (ESP/CE/115/2021). The authorization to access the OTCs was obtained from the competent authorities of the various institutions included. Before inclusion, the fully written consent was obtained from each patient. The blood samples were taken by the technical teams of the centers. The results of the biochemical analyzes were returned to the centers at the end of the exams.

2.7. Statistical Analyzes

The analyses were carried out using the SPSS version 26 software (Statistical Package for Social Sciences, IBM). Only the available data was analyzed, the missing data was considered completely random. The continuous variables were presented on average \pm standard deviation and compared using the Student T test. The 95% respective proportions and intervals were calculated for categorical data.

3. Results

One hundred nineteen (119) patients were included in this study in accordance with the inclusion criteria; 67 (56.3%) are of female sex while 52 (43.7%) are male thus giving a sex-ratio of 1.29 in favor of women. **Figure 1** presents the above data.

The average age of patients included is 39.87 ± 12.36 years with extremities from 18 to 69 years. The most represented age group is that of 36 to 45 years with 37 patients (31.9%) followed by that from 26 to 35 years with 24 patients (20.7%), that of 46 to 55 years with 22 Patients (19.0%) and that from 18 to 25 years old with 19 patients (16.4%). These data are presented by **Figure 2**.

All the 119 positive patients per serology were positive by PCR for the “*pol*” region while 115 samples were positive for the “GAG” region. The 4 negative samples on the “*gag*” region were positive on the “*env*” region. **Table 2** presents the results of the nested PCR diagnostic.

Table 1. Primers and probes for amplifications.

Types of PCR	Primers	Sequences
Nested PCR for diagnostic on DNA		
HLA	GH26 Forward	5'-GTGCTGCAGGTGTAAACT-3'
	GH27 Reverse	5'-CACGGATCCGGT-3'
Gag	GAG1 Forward	5'-GGTACATCAGGCCATATCACC-3'
	GAG4 Reverse	5'-ACCGGTCTACATAGTCTC-3'
Pol	POLITG1 Forward	5'-CCCTACAATCCCCAAAGTCAAGG-3'
	POLITG4 Reverse	5'-TACTGCCCTTCACCTTTCCA-3'
Env	ENV1 Forward	5'-GAGGATATAATCAGTTTATGG-3'
	ENV4 Reverse	5'-AATTCCATGTGTACATTGTACTG-3'
Nested gag	GAG2 Forward	5'-GAGGAAGCTGCAGAATGGG-3'
	GAG3 Reverse	5'-GGTCCTTGTCTTATGTCC-3'
Nested pol	POLITG2 Forward	5'-TAAGACAGCAGACAAATGGCAG-3'
	POLITG3 Reverse	5'-GCTGTCCCTGTAATAAACCCG-3'
Nested env	ENV2 Forward	5'-GATCAAAGCCTAAAGCCATG-3'
	ENV3 Reverse	5'-CAATAATGTATGGGAATTGG-3'
Quantitative PCR (qPCR) for Viral Load on RNA		
qPCR	HIV1MG Forward	5'-GCCTCAATAAAGCTTGCCTTGA-3'
	HIV1MG Reverse	5'-GGCGCCACTGCTAGAGATTTT-3'
	HIV1MG Probe	FAM-5'-AAGTAGTGTGTGCCCGTCTGTTRTKTGA CT-3'-BHQ1
PCR for Sequencing		
RT-PCR Prot	5' prot 1	5'-TAATTTTTTTAGGGAAGATCTGGCCTTCC-3'
	3' prot 1	5'-GCAAATACTGGAGTATTGTATGGATTTTCAGG-3'
Nested PCR Prot	5' prot 2	5'-TCAGAGCAGACCAGAGCCAACAGCCCCA-3'
	3' prot 2	5'-AATGCTTTTATTTTTTCTTCTGTCAATGGC-3'
RT-PCR RT	MJ3	5'-AGTAGGACCTACACCTGTCA-3'
	MJ4	5'-CTGTTAGTGCTTTGGTTCCTCT-3'
Nested PCR RT	A(35)	5'-TTGGTTGCACTTTAAATTTTCCATTAGTCCTATT-3'
	NE1(35)	5'-CCTACTA AACTTCTGTATGTCATTGACAGTCCAGCT-3'
RT-PCR Alt Prot	5' eprB	5'-AGAGCTTCAGGTTTGGGG-3'
	3' eprB	5'-GCCATCCATTCCCTGGCTT-3'
Nested PCR Atl Prot	5' prB	5'-GAAGCAGGAGCCGATAGACA-3'
	3' prB	5'-ACTGGTACAGTTTCAATAGG-3'
RT-PCR Atl RT	RT1	5'-CCAAAAGTTAAACAATGGCCATTGACAGA-3'
	RT4	5'-AGTTCATAACCCATCCAAAG-3'
Nested PCR Alt RT	RT18	5'-GGAAACCAAAAATGATAGGGGAATTGGAGG-3'
	RT21	5'-CTGTATTTCTGCTATTAAGTCTTTTGATGGG-3'

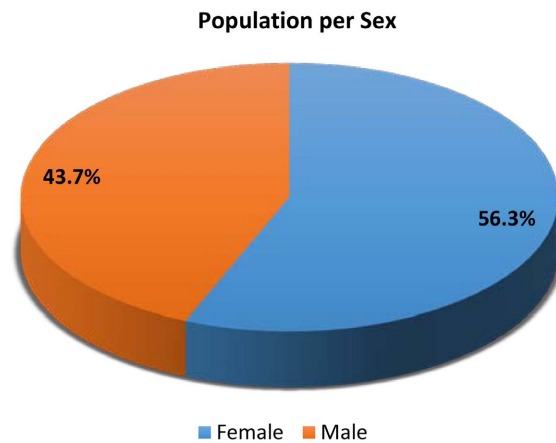


Figure 1. Distribution of the population by gender at the inclusion.

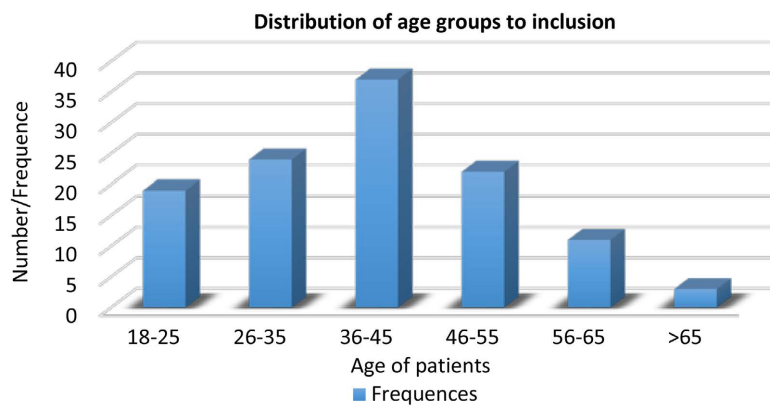


Figure 2. Distribution of age groups to inclusion.

Table 2. Nested PCR results for HIV diagnosis.

Samples	PCR HLA		Region gag		Region pol		Region env	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
119	119	0	115	4	119	0	4	0

Out of 119 patients, 21 patients had an undetectable Viral Load (VL) or lower than 50 copies of RNA/ml. The median value of the VL was 4.16 log₁₀ RNA/ml copies (14360.50 RNA/ml copies) with extreme values from 0.0 log₁₀ to 7.11 log₁₀ RNA/ml copies. These data is presented in **Table 3**.

The regions of Reverse Transcriptase (RT) and the Protease were amplified and sequenced respectively for 114 (95.79%) and 111 (93.28%) patients. One hundred and fourteen (114) samples were therefore successfully amplified. Subtype A is dominant with 23 cases (20.2%); followed by the subtype C and CRF02_AG respectively with (14.0%), D (10.5%), G (5.3%), followed by H, CRF01_AE and U respectively (4.4%) as described on **Figure 3** and **Table 4**. No mutation was found for DTG; K65R (1.8%), T69P/N (4.4%), K70R (7.9%) and M184V (7.0%) mutations were listed as existing mutations for nucleotide inhibitors of Reverse Transcriptase (**Table 4**).

Table 3. Values of the viral loads of patients to inclusion.

	Viral Load (copies of RNA/ml)	
	Values	Log ₁₀
Median	14360.5	4.16
Lower limit	1	0.00
Upper limit	12905352.0	7.11
Viral Load intervals		
	Values	Percentage
Undetermined VL	21	17.65
VL < 3.0 log ₁₀	38	31.93
3.0 log ₁₀ < VL < 5.0 log ₁₀	40	33.61
VL > 5.0 log ₁₀	20	16.81

Table 4. Molecular data of patients on D0.

Molecular data of patients on D0		
<i>Prevalence of circulating subtypes</i>		
Subtypes	Number	Percent
A	23	20.2
B	3	2.6
C	16	14.0
D	12	10.5
E	1	0.9
F	2	1.8
G	6	5.3
H	5	4.4
J	3	2.6
K	4	3.5
CRF01	5	4.4
CRF02	16	14.0
CRF05	2	1.8
CRF06	2	1.8
CRF11	1	0.9
CRF25	3	2.6
CRF45	3	2.6
CRF56	2	1.8
U	5	4.4
Total	114	100.0

Continued

<i>Prevalence of mutations of interest</i>		
INTR	Number	Percent
K65R	2	1.8
T69P	5	4.4
K70E/R	9	7.9
M184V	8	7.0

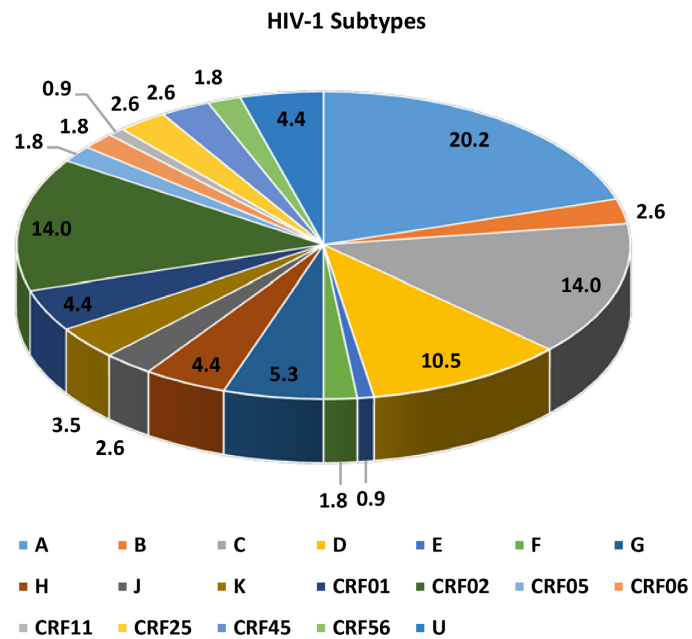


Figure 3. Distribution of HIV-1 subtypes in Kinshasa.

4. Discussion

The objective of this work was to present the virological and molecular profile of People Living with HIV (PLHIV) starting Antiretroviral Treatment (ART) in Kinshasa in the era of the Dolutegual. A total of 119 PLHIV had been included, in accordance with the criteria, for this study starting ART in 16 Outpatient Treatment Centers (OTC) disseminated in the 4 districts of Kinshasa, Democratic Republic of Congo.

Sixty-seven (67) patients, or 56.3%, are of female sex while 52 (43.7%) are male thus giving a sex-ratio of 1.29 in favor of women. These results presenting a sex-ratio in favor of the female sex are similar to the trend related to the various studies published for Kinshasa in recent years which tend to feminize HIV infection in Kinshasa and even in Central Africa [5] [7] [11] [12] [16].

The average age of patients included is 39.87 ± 12.36 years with extremities from 18 to 69 years. The most represented age group is that of 36 to 45 years with 37 patients (31.9%) followed by that from 26 to 35 years with 24 patients (20.7%), that of 46 to 55 years with 22 Patients (19.0%) and that from 18 to 25

years old with 19 patients (16.4%). These values, which present the age group of 36 to 45, are shared by the various authors who have published on PLHIV in Kinshasa in recent years [5] [7] [11] [12] [16].

All the 119 positive patients per serology were positive by PCR for the “pol” region while 115 samples were positive for the “gag” region. The 4 negative samples on the “gag” region were positive on the “env” region. Diagnostic PCR was used later to confirm the positivity of questionable and indeterminate samples [9] [10].

Twenty-one (21) patients out of the 119 patients included, 17.65%, had an undetectable VL or lower than 50 copies of RNA/ml. This brings into question the concept and definition of “patients starting treatment” or even “patient naive of treatment”. It has been shown that, in precarious situations, patients change treatment centers without notice; they leave an OTC to register as a naive patient in another center which has a better tray of management of PVVIH [17].

The median value of the VL was 4.16 \log_{10} RNA/ml copies (14,360.50 RNA/ml copies) with extreme values from 0.0 \log_{10} to 7.11 \log_{10} RNA/ml copies. Despite the various awareness of different programs, patients are still starting their treatment with high VLs. Twenty (20) patients, or 16.81%, started the ART with a higher VL at 5.00 \log_{10} RNA/ml copies; this is already synonymous with a poor prognosis for treatment [5] [17] [18] [19]. The majority (33.61%) of patients started the ART with a VL included between 3.00 \log_{10} and 5.00 \log_{10} RNA/ml copies; This is a mitigated prognosis for good management of PVVIH [5] [17] [18] [19]. Only 38 patients (31.93%) started the ART with a positive prognosis. These data corroborate those of the literature on the profile of PLHIVs starting an ART in Kinshasa and even in some cases in Central Africa [5] [11] [20].

The regions of Reverse Transcriptase (RT) and the Protease were amplified and sequenced respectively for 114 (95.79%) and 111 (93.28%) patients. One hundred and fourteen (114) samples were therefore successfully amplified. The amplification rate of the two regions was almost 96% for samples. The differences in amplification between Protease and RT are mainly in the sizes of regions of interest to amplify and diversity that exists [12] [21] [22]. Similar data has been presented in different literatures [12] [21] [22].

Subtype A is dominant with 23 cases (20.2 %); followed by the subtype C and CRF02_AG respectively with (14.0%), D (10.5%), G (5.3%), followed by H, CRF01_AE and U respectively (4.4%). These results are similar to the various subtypes found in the literature for Kinshasa where subtype A maintains itself dominant compared to other subtypes C, D and G [11] [12]. The presence of recombined forms is explained by the dynamism of HIV infection in Kinshasa, the possibilities of crossed infections due to the population movement of villages to metropolitan areas, but also by the return of expatriates and immigrants to the capital [23].

No mutation was found associated with resistance to the DTG; K65R (1.8%), T69P/N (4.4%), K70E/R (7.9%) and M184V (7.0%) mutations were listed as existing mutations associated with resistance to Nucleotide Inhibitors of Reverse

Transcriptase (NIRT). The K65R mutation (1.8%) is associated with resistance against Lamivudine (3TC) and Tenofovir (TDF); The T69P/N mutation (4.4%) is associated with resistance to all NIRT; The K70E/R (7.9%) is associated with TDF; The M184V (7.0%) at 3TC [11] [12] [23]. Almost 8% of naive patients start the first -line TARV with a predisposition to failure for treatment such as recommended for the DRC since 2019 (Tenofovir + Lamivudine + Dolutegravir) [24] [25].

Limitation of the Study

This present study was limited to some centers of Kinshasa. Therefore, generalization of the results should be done carefully. However, being the first study in the era of Dolutegravir transition, this does not take any value out of the findings.

5. Conclusion

Thirty-eight (38) patients (31.93%) started the ART with a positive virological prognosis. The subtype a remains dominant in Kinshasa with 23 cases (20.2%); followed by C and CRF02_AG subtype respectively with (14.0%). No mutation was found associated with resistance to the Dolutegravir. For Nucleotide Inhibitors of Reverse Transcriptase; K65R (1.8%), T69P/N (4.4%), K70E/R mutations (7.9%) and M184V (7.0%) were found in naive treatment patients.

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Conflicts of Interest

The authors do not declare any conflict of interest for this study.

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List of Abbreviations and Acronyms

ART: Antiretroviral Treatment; **ARV:** Antiretroviral; **DRC:** Democratic Republic of Congo; **DTG:** Dolutegravir; **HIV:** Human Immunodeficiency Virus; **OTC:** Outpatient Treatment Center; **PLHIV:** Person Living with the Human Immunodeficiency Virus.