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Profile of People Living with Human Immunodeficiency Virus Initiating Treatment in the Dolutegravir Era in Kinshasa, Democratic Republic of Congo

Berry I. Bongenya[1,2*], Benoit O. Kabengele[3], Marie-Thérèse A. S. Sombo[4], Guy M. M. Bumoko[4], Hippolyte N. T. Situakibanza[3], Fridolin K. K. Kodondi[5], Gauthier K. Mesia[6], Mariano M. Lusakibanza[6], Jean Marie N. Kayembe[3], Georges L. Mvumbi[7], Baudouin B. Buassa[7], Richard L. Kalala[7], Erick N. Kamangu[2,7*]

[1]Faculty of Medicine, Bel Campus Technological University, Kinshasa, Democratic Republic of Congo
[2]"HIV/AIDS Focus" Research Group, Kinshasa, Democratic Republic of Congo
[3]Department of Internal Medicine, Faculty of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of Congo
[4]Department of Neurology, Faculty of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of Congo
[5]Laboratory of Biochemistry, Faculty of Pharmaceutical Sciences, University of Kinshasa, Kinshasa, Democratic Republic of Congo
[6]Clinical Pharmacology Unit, Department of Pharmacology, Faculty of Medicine and Pharmaceutical Sciences, University of Kinshasa, Kinshasa, Democratic Republic of Congo
[7]Service of Molecular Biochemistry, Department of Basic Sciences, Faculty of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of Congo
Email: *bongenyaberry@gmail.com, *erick.kamangu@unikin.ac.cd

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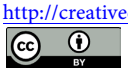
Abstract

Background: For several decades, the introduction of AntiRetrovirals (ARVs) has improved the symptomatology of People Living with HIV (PLHIV), a spectacular reduction in morbidity and mortality, an improvement in life expectancy and quality of life of PLHIV. **Objective:** The objective of this study was to determine the profile of PLHIV initiating AntiRetroViral Treatment (ART) in the era of Dolutegravir in Kinshasa. **Methods:** Cross-section of a prospective cohort to determine the profile of PLHIV initiating ART in Kinshasa. The inclusions were from October 04, 2021 to February 15, 2022. Confirmation of the diagnosis was carried out by Nested PCR. The inclusion criteria were: being at least 18 years old, confirmed HIV positive, naïve to ART, consenting and having signed an informed consent. The parameters of interest followed for the present study were: age, sex, religion, level of study, marital status, occupation, height, weight, body mass index (BMI), the clinical profile, the opportunistic infections as well as the para-clinical assessment



(biochemistry and molecular biology). **Results:** 67 (56.3%) women and 52 (43.7%) men were included, thus 119 patients, all confirmed positive for HIV by Nested PCR on the gag, pol and env regions. The average age of the patients included is 39.87 ± 12.36 years and the most represented age group is that of 36 to 45 years with 37 patients (31.9%). The average height was 1.66 ± 0.08 meters, with an average weight of 56.41 ± 13.30 kg, giving an average Body Mass Index (BMI) of 21.54 ± 5.17 kg/m^2. The majority of patients were married (46.1%), of Protestant religion (70.7%), with secondary education (66.7%), and working in the informal sector (29.4%). 49 patients (41.5%) were in clinical stage 3 and 55 patients (47.0%) had a normal clinical status. Malaria (45.4%) and tuberculosis (29.4%) were the most common Opportunistic Infections. The mean values of the patients' assessed biochemical parameters were within the ranges. The median VL value was 4.16 $\log_{10}$ RNA copies/ml. Subtype A (20.2%) is dominant. Mutations K65R (2 cases), T69P/N (5 cases), K70R (9 cases) and M184V (8 cases) were listed. **Conclusion:** In Kinshasa, PLHIV start ART late. The biochemical parameters evaluated are within normal ranges, with high VLs. Subtype A remains predominant and there are mutations conferring resistance to ART.

Keywords

Profile, PLHIV, Starting of ART, Dolutegravir, Kinshasa

1. Introduction

The Human Immunodeficiency Virus (HIV) infection and Acquired Immunodeficiency Syndrome (AIDS) still remain a major public health problem throughout the world, in general, and, in particular, in Sub-Saharan Africa (SSA) [1]. For several decades, the introduction of AntiRetrovirals (ARVs) has led to an improvement in the symptoms of People Living with HIV (PLHIV), a spectacular reduction in morbidity and mortality, an improvement in life expectancy and the quality of lives of PLHIV [2].

The AntiRetroViral Treatments (ART) used today allow a significant improvement in the survival of PLHIV, a slowing down of immune degradation, as well as a spectacular reduction in the frequency of Opportunistic Infections (OI) [3] [4]. These infections constitute the main part of the symptomatology of HIV infection responsible for heavy morbidity and mortality of PLHIV, especially in countries with limited resources [4].

The assessment of the biological parameters constitutes a real-time witness on the one hand of the state and the evolution of an infection, and on the other hand of the effectiveness and the tolerance of the treatment to the said infection. The biological assessment of initiation and follow-up of HIV infection, which is often accompanied by an immunological, virological and molecular assessment, is an important complement to the clinical evaluation of the infected patient [3].

The clinic of HIV infection has been the subject of several publications in our

community [4]-[9]. Nevertheless, there is little knowledge about PLHIV starting Anti-Retroviral Treatments (ART) in our environment for the last few years, especially since the introduction of Dolutegravir in 2019. Hence the objective of this work was to determine the profile of People Living with HIV initiating AntiRetroViral Therapy in the era of Dolutegravir in Kinshasa, Democratic Republic of Congo.

2. Methods

2.1. Study Design, Patient and Sample Setting

This cross-sectional study is a prospective cohort to determine the profile of People Living with HIV (PLHIV) starting ARV Treatment (ART) in Outpatients Treatment Centers (OTC) for HIV in Kinshasa, Democratic Republic of Congo (DRC). The patient inclusion period was from October 04, 2021 to February 15, 2022, where all patients initiating ART in an HIV OTC were included. The following centers had been included in the study after random selection, because of their expertise in the care of PLHIV and their accessibility: Center IST Matonge, Center IST Victoire, Legion PIR, Saint Clément Health Center, Bondeko Ya Hospital Sika, Monkole Hospital Center, Lufungula Camp Police Central Hospital, Saint Alphonse Health Center, Ngaba Mother and Child Center, Elonga Health Center, Esengo Health Center, Bolingo Health Center, Masina Pilot Health Center, Center Reference Hospital of Camp Kabila, Ngondo Maria Health Center, Central Military Hospital of Camp Kokolo [10].

In the OTCs, a sample of 5 ml of blood was collected in a tube with EDTA anticoagulant from the vein in the bend of the elbow for the various analyzes in any HIV positive patient by serology according to the national protocol, after reading and signature of informed consent. Patients were randomly included consecutively according to their presence in the OTCs during the consultations.

Sociodemographic data, medical history, previous exposures as well as biological and clinical data were recorded on the worksheets previously tested by the supervision team.

Confirmation of routine diagnosis using Rapid Diagnostic Tests (RDTs) was performed by Nested PCR on genomic Deoxyribose Nucleic Acid (DNA) [11] [12].

2.2. Study Population and Inclusion Criteria

The source population of this work was adults over the age of 18 at inclusion, infected with HIV and initiating ART in the OTC during the inclusion period (October 04, 2021 to February 15, 2022). The inclusion criteria were as follows: being at least 18 years old at inclusion, confirmed HIV positive by RDT, naïve to ART, consenting and having signed an informed consent.

2.3. Parameters of Interest

The parameters of interest followed for the present study were: age, sex, religion,

residence, level of study, marital status, occupation, socio-economic level, height, weight, the body mass index (BMI), the clinical profile, the opportunistic infections as well as the para-clinical assessment (biochemistry and molecular biology). All parameters were recorded in the patient's files from the centers and the results of the analysis.

2.4. Biochemical Analyzes

After collection, the samples were transported, respecting the temperatures, to the Molecular Biology Laboratory of the Department of Basic Sciences at the Faculty of Medicine of the University of Kinshasa (UNIKIN) where they were homogenized and separated into 2 tubes: for Biochemistry and for Molecular Biology. The Biochemistry tube was sent to the Biochemistry Laboratory of the Faculty of Pharmaceutical Sciences for analysis, while the Molecular Biology tube was stored at −20˚C in the Molecular Biology Laboratory of the Faculty of Medicine for subsequent analysis.

The different biochemistry analyzes were carried out on a spectrometer (HumaLyzer Primus, Human, Germany) with the different respective analysis kits. Hemoglobin was evaluated using a hematology analyzer (HumaCount 60TS, Human, Germany) with specific reagents. All the biochemistry analyzes were carried out according to the protocols in application at the Biochemistry Laboratory.

2.5. DNA Extraction and Nested PCR for Diagnosis

DNA was extracted from 200 µl of Buffy coat to maximize lymphocyte concentration using the QIAGEN® QIAamp DNA Mini Kit for DNA extraction [13]. The DNA thus extracted was stored in a freezer at −20˚C before being used for amplification.

Nested PCR on genomic DNA was performed on all samples to confirm the serological status of patients included in the study. Conventional HLA PCR was used to confirm the presence of the extracted genetic material. Nested PCRs were performed on the gag and pol region to confirm the presence of the proviral DNA inserted into the genomic DNA. Nested PCR on the env region was performed in case of discordant gag and pol results. The various primers as well as the amplification conditions have been previously described in the literature and are presented in **Table 1** and **Table 2** [11] [12].

2.6. RNA Extraction and Viral Quantification

RNA was extracted from 140 µl of plasma at the Molecular Biology Laboratory using the QIAamp RNA Mini Kit QIAGEN® for RNA extraction [14]. Extracted samples were stored at −20˚C until use.

After extraction, a Quantitative Real-Time PCR (qPCR) was performed to determine the amount of HIV RNA in the samples according to previously described protocols [15] [16]. The primers used are HIV1MGForward, HIV1MGReverse, with the HIV1MGProbe probe and the TaqMan One-Step

Table 1. Primers and sequences for PCR.

Type of PCR	Primers	Sequences
Nested DNA PCR for diagnostics		
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'-TACTGCCCCTTCACCTTTCCA-3'
env	ENV1 Forward	5'-GAGGATATAATCAGTTTATGG-3'
	ENV4 Reverse	5'-AATTCCATGTGTACATTGTACTG-3'
Nichée gag	GAG2 Forward	5'-GAGGAAGCTGCAGAATGGG-3'
	GAG3 Reverse	5'-GGTCCTTGTCTTATGTCC-3'
Nichée pol	POLITG2 Forward	5'-TAAGACAGCAGACAAATGGCAG-3'
	POLITG3 Reverse	5'-GCTGTCCCTGTAATAAACCCG-3'
Nichée 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'-AAGTAGTGTGTGCCCGTCTGTTRTKTGACT-3'-BHQ1
PCR for Sequencing		
RT-PCR Prot	5' prot 1	5'-TAATTTTTTAGGGAAGATCTGGCCTTCC-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'-TTGGTTGCACTTTAAATTTTCCCATTAGTCCTATT-3'
	NE1(35)	5'-CCTACTAACTTCTGTATGTCATTGACAGTCCAGCT-3'
RT-PCR Alt Prot	5' eprB	5'-AGAGCTTCAGGTTTGGGG-3'
	3' eprB	5'-GCCATCCATTCCTGGCTT-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'-GGAAACCAAAAATGATAGGGGGAATTGGAGG-3'
	RT21	5'-CTGTATTTCTGCTATTAAGTCTTTTGATGGG-3'

Table 2. Cycle and temperature for PCR.

	PCR gag/pol	PCR env/Nested pol	PCR HLA/Nested gag	Nested env	RT-qPCR quantitative
Reverse Transcription					50˚C/30min.
Enzyme Activation					95˚C/10min.
Initial Denaturation	95˚C/9min.	95˚C/9min.	95˚C/9min.	95˚C/9min.	
Denaturation per cycle	94˚C/1min.	94˚C/1min.	94˚C/1min.	94˚C/1min.	95˚C/15sec.
Hybridation per cycle	50˚C/1min.	50˚C/1min.	55˚C/1min.	45˚C/1min.	60˚C/1min.
Elongation per cycle	72˚C/1min.	72˚C/1min.	72˚C/1min.	72˚C/1min.	
Final Elongation	72˚C/10min.	72˚C/10min.	72˚C/10min.	72˚C/10min.	
Hold (en ˚C)	4	4	4	4	
Number of cycle	40	35	30	30	50

enzyme. The region targeted by this PCR is part of the Long Terminal Repeat (LTR) which is the more or less protected region of the HIV type 1 genome described. The calibration curve was plotted with commercial standard controls previously quantified from 10^2 to 10^7 in increments of a logarithm of 10. The slope is acceptable for a curve between −3.40 and −3.10 according to the applicable protocol Applied Biosystems (ABS) 7500 Fast Real-Time PCR System thermal cycler. The various primers as well as the amplification conditions have been previously described and are presented in Table 1 and Table 2.

2.7. Sequencing and Molecular Identification

The extracted RNA was also used for sequencing. Reverse Transcription PCR (RT-PCR) and Nested PCR were performed to amplify regions of interest for Protease and Reverse Transcriptase (TR) for sequencing. The PCRs were carried out under the conditions previously described [17] [18]. These fragments were sequenced by the Sanger sequencing method. The pairing of the fragments obtained (sense and antisense) was carried out with Vector NTI Advance® 11.5 software (Invitrogen, Life technologies) and compared with the Stanford University database (https://hivdb.stanford.edu/) [17] [18]. Minor base adjustments were made where necessary to align sequence readings.

2.8. Ethical Consideration

This study was approved by the research ethics committee of the School of Public Health, Faculty of Medicine, University of Kinshasa (ESP/CE/115/2021). Authorization to access the Outpatients Treatment Centers was obtained from each competent authority of the different institutions selected for the study. Prior to inclusion, fully informed consent was obtained from each patient. The samples in the OTCs were taken by the technical teams of the centers. The results of the biochemical analyzes were returned to the centers as part of the usual management of PLHIV.

2.9. Statistical Analyzes

Analyzes were performed using SPSS version 26 software. Only available data were analyzed, missing data were considered completely random. Continuous variables were presented as mean ± standard deviation and compared using Student's t-test. Proportions and their respective 95% confidence intervals (CI) were calculated for categorical data.

3. Results

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

3.1. Anthropometric Data

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

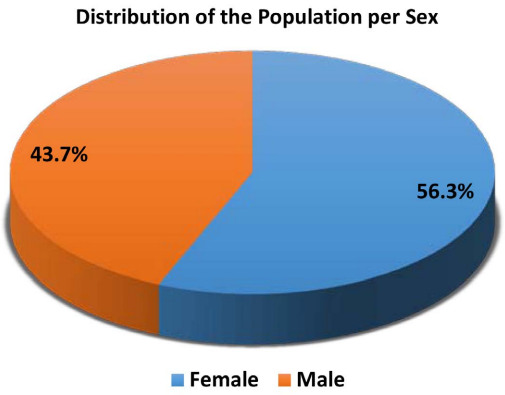


Figure 1. Distribution of the population by gender.

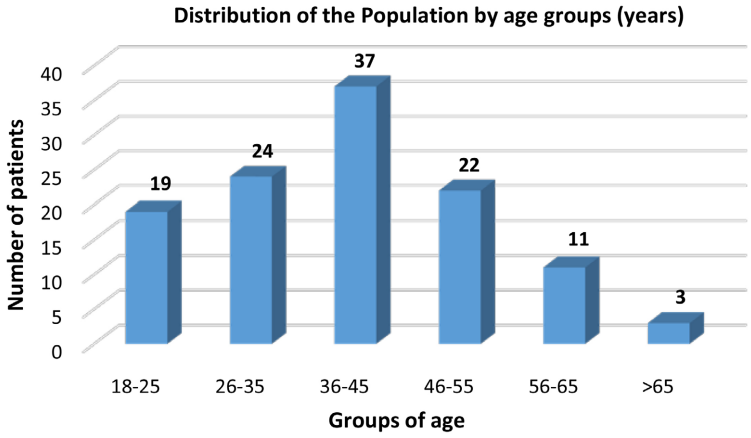


Figure 2. Distribution of the population by age groups.

The average height was 1.66 ± 0.08 meters with extremities of 1.50 to 1.75 meters. The average weight of the patients on D0 was 56.41 ± 13.30 kg with extremities of 30 to 106 kg. The average value of the Body Mass Index (BMI) through the study was 21.54 ± 5.17 kg/m^2 with extremities of 12 to 30 kg/m^2. The BMI interval, in kg/m^2, the most represented is that of 18.5 to 24.9 with 19 patients (45.2%) followed by that of 15 to 18.5 with 12 patients (28.6%), that of 25 to 29.9 with 9 patients (21.4%) and that of 30 to 34.9 with 2 patients (4.8%) (Figure 3).

The mean temperature of the patients at inclusion was 36.69˚C ± 0.68˚C with extremities of 36.0˚C to 38.70˚C.

All the means of the anthropometric data are presented in Table 3.

3.2. Sociodemographic Data

The majority of patients (46.1%) were married, followed by single (33.0%), widowed (13.9%) and divorced (7.0%).

Protestants were more numerous (70.7%) in the study population, followed by Catholics (20.7%) and Muslims (1.7%).

More than three-fifths of patients (66.7%) have secondary level, followed by 16.7% of patients who have primary level, 9.6% with university level and 7.0% who are illiterate.

Most patients (29.4%) in the study informally, followed by 23.5% who are traders, 14.3% housewives, 13.4% unemployed, 8.4% police and 5.0% military.

All the socio-demographic data presented above are listed in Table 4.

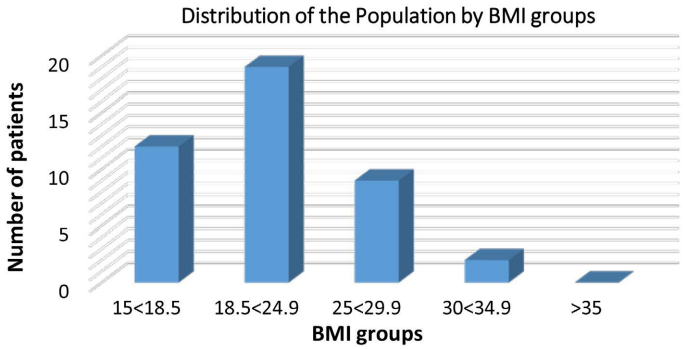


Figure 3. Distribution of the population by BMI groups.

Table 3. Mean values of patient data at baseline (D0).

Parameters	Patients			
	Mean	Standard Deviation	Minimal Values	Maximal Values
Age (years)	39.87	12.36	18.00	69.00
Height (meter)	1.66	0.08	1.50	1.75
Weight (kg)	56.41	13.30	30.00	106.00
BMI (kg/m^2)	21.54	5.17	12.00	30.00
Temperature (˚C)	36.69	0.68	36.00	38.70

Table 4. Socio-demographic parameters.

Socio-demographic parameters	Patients	
	Values	Percentage
Marital Status **(*N* = 115)**		
Single	38	33.0
Married	53	46.1
Divorced	8	7.0
Widowed	16	13.9
Religion **(*N* = 116)**		
Catholic	24	20.7
Protestant	82	70.7
Muslim	2	1.7
Others	8	6.9
Level of Education **(*N* = 114)**		
Analphabet	8	7.0
Primary Level	19	16.7
Secondary Level	76	66.7
University Level	11	9.6
Post-University Level	0	0
Occupation **(*N* = 119)**		
Jobless	16	13.4
Informal employment	35	29.4
Trader	28	23.5
House hold	17	14.3
Driver	2	1.7
Military	6	5.0
Police man/woman	10	8.4
Engineer	1	0.8
Student	4	3.4

3.3. Clinical

Forty-nine patients (49), or 41.5%, were in clinical stage 3; followed by 40 patients (33.9%) who were in clinical stage 1, 18 patients (15.3%) in clinical stage 2 and 11 patients (9.3%) in clinical stage 4. Fifty-five (55) patients, or 47.0%, had a normal clinical condition; followed by 39 patients (33.3%) who had a good clinical state, 22 patients (18.8%) a bad clinical state and 1 patient (0.9%) a pre-moribund clinical state. These clinical data are presented in Table 5.

Table 5. Clinical aspects of patients on D0.

Clinics of patients	Frequency	Percentage
Clinical Stage according to WHO (N = 118)		
Stage 1	40	33.9
Stage 2	18	15.3
Stage 3	49	41.5
Stage 4	11	9.3
Clinical state of the patient (N = 117)		
Normal	55	47.0
Good	39	33.3
Bad	22	18.8
Pre-Moribund	1	0.9
Moribund	0	0

The most common known medical antecedents are: tooth decay and sleep disorder (30.2%), alcoholism and tuberculosis (28.4%), appetite disorder (26.1%), neuro-peripheral disorders (17.9%), smoking, hypertension and hypotension (17.2%). These data are comprehensively presented in Table 6.

The most common known prior morbidities are: malaria (35.3%), high blood pressure and heart disease (14.7%), drug abuse and tuberculosis (12.2%), and smoking (9.5%). These data are presented more fully in Table 7.

3.4. Opportunistic Infections

The Opportunistic Infections (OIs) most commonly found in PLHIV initiating ART are: Malaria with 54 cases (45.4%), Tuberculosis (29.4%), Cutaneous pruritus (23.5%), urinary infections (21.8%), oral candidiasis and skin eruptions (20.2%). Table 8 presents the exhaustive list of Opportunistic Infections found in the study.

3.5. Biochemistry

The mean values of the biochemistry parameters of the patients at the start of ART were as follows: 31.61 ± 20.71 IU/L for ALT, 25.81 ± 19.96 IU/L for AST, 79.35 ± 49.49 IU /L for Amylase, 108.13 ± 62.17 mg/dl for Total Cholesterol, 2.77 ± 1.27 mg/dl for Creatinine, 72.53 ± 22.23 mg/dl for Glycemia, 10, 30 ± 2.33 g/dl for Hemoglobin, 7.91 ± 1.75 g/dl for Total Protein, 131.23 ± 68.80 mg/dl for Triglycerides, and 33.61 ± 26.27 mg/dl for Urea. These data are shown in Table 9.

3.6. DNA Extraction and Nested PCR for Diagnosis

DNA was extracted for the 119 blood samples collected by the QIAGEN® Protocol for DNA and amplified by Nested PCR. After amplification, 119 samples

Table 6. Known medical history of patients at inclusion.

Known medical history (N = 116)	Frequency	Percentage
Sleep disorder	35	30.2
sexual disorder	6	5.2
Neuro-peripheral disorder	21	17.9
Smoking	20	17.2
Meningitis	0	0
Epilepsy	3	2.6
Tooth decay	35	30.2
Paralysis	3	2.6
Suicidal ideation	7	6.0
Appetite disorder	30	26.1
Behavior trouble	6	5.2
Alcoholism	33	28.4
Tuberculosis	33	28.4
Stroke	4	3.5
Otitis	7	6.0
Rheumatism	11	9.5
Hypertension	20	17.2
Nervousness	12	10.4
Hypotension	20	17.2

Table 7. Prior morbidities known to patients on inclusion.

Prior morbidities known (N = 116)	Frequency	Percentage
Epilepsy	4	3.5
Encephalopathy	3	2.6
Malaria	41	35.3
Malnutrition	10	8.8
Diabetes	2	1.7
High Blood Pressure	17	14.7
Alcoholism	9	7.8
Heart disease	17	14.7
Sickle cell disease	0	0
Meningitis	2	1.7
Anemia	2	1.7
Sleep Deprivation	10	8.6
Drug Abuse	14	12.2
Head trauma	1	0.9

Continued

Tuberculosis	14	12.2
Sepsis	3	2.6
Depression	2	1.7
Stroke	4	3.4
Smoking	11	9.5

Table 8. Opportunistic Infections encountered in patients on inclusion.

Opportunistic Infections	Patients (N = 119)	
	Values	Percentage
Oral candidiasis	24	20.2
Vaginal mycosis	13	10.9
Vaginal pruritus	16	13.4
Cutaneous pruritus	28	23.5
Shingles	4	3.4
Rash	24	20.2
Dermatitis	18	15.1
Diarrhea	17	14.3
Intestinal parasitosis	12	10.1
Rhinitis	11	9.2
Tuberculosis	35	29.4
Malaria	54	45.4
Urinary tract infection	26	21.8
Non-specific STI	5	4.2
Others	11	9.2

Table 9. Biochemistry values of patients at inclusion.

Parameters	Patients		Normal Values
	Mean	Standard Deviation	
ALT (IU/L)	31.61	20.71	0 - 41 UI/L
AST (IU/L)	25.81	19.96	0 - 31 UI/L
Amylase (IU/L)	79.35	49.49	≤90 UI/L
Total cholesterol (mg/dl)	108.13	62.17	110 - 200 mg/dl
Creatinine (mg/dl)	2.77	1.27	0.5 - 1.5 mg/dl
Blood glucose (mg/dl)	72.53	22.23	60 - 110 mg/dl
Hemoglobin (g/dl)	10.30	2.33	≥12 g/dl
Total protein (g/dl)	7.91	1.75	6.6 - 8.2 g/dl
Triglycerides (mg/dl)	131.23	68.80	35 - 185 mg/dl
Urea (mg/dl)	33.61	26.27	15 - 45 mg/dl

were positive for HLA PCR, confirming the presence of genetic material in all samples. One hundred and nineteen samples were positive 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. Hence, all 119 samples were confirmed positive for HIV by nested PCR (Table 10).

3.7. RNA Extraction and Quantification by Real-Time PCR (qPCR)

RNA was extracted for the 119 samples from decanted whole blood plasma collected by the QIAGEN® protocol for RNA and amplified by qPCR. After amplification, 21 samples showed an undetectable Viral Load, less than 50 RNA copies/ml. The median CV value was 4.16 $\log_{10}$ RNA copies/ml (14360.5 RNA copies/ml) with the lower and upper ends respectively equaling 0.0 $\log_{10}$ and 7.11 $\log_{10}$ copies of RNA/ml. The above results are shown in Table 11.

3.8. Sequencing and Molecular Identification

After sequencing of the different samples, the Reverse Transcriptase (RT) and Protease regions 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 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 2. No mutation was found for DTG; mutations K65R (2 cases), T69P/N (5 cases), K70R (9 cases) and M184V (8 cases) were listed as existing mutations for Nucleotide Reverse Transcriptase Inhibitors (Table 11).

4. Discussion

The objective of this study was to present the profile of People Living with HIV (PLHIV) initiating AntiRetroViral Treatment (ART) in Kinshasa in the era of Dolutegravir. One hundred and nineteen (119) PLHIV were included, in accordance with the criteria, for this study initiating treatment in 16 Outpatients Treatment Centers (OTC) disseminated in the four districts of Kinshasa, Democratic Republic of Congo.

Sixty-seven (67) patients, or 56.3%, are female while 52 (43.7%) are male, giving a sex ratio of 1.29 in favor of women. These data are similar to those reported by various studies for Kinshasa [10] [19] [20] [21]. The feminization of HIV is a reality in Kinshasa which is evolving towards a generalization due to the constraint which obliges women to be screened, especially during prenatal consultation and premarital testing [17] [18] [22].

Table 10. Results for the Nested PCR for HIV diagnostic.

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

Table 11. D0 patient molecular data.

D0 Patient Molecular Data		
Prevalence of circulating subtypes		
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***
Prevalence of mutations of interest		
INTR	**Number**	**Percent**
K65R	2	1.8
T69P	5	4.4
K70E/R	9	7.9
M184V	8	7.0

The average age of the patients included is 39.87 ± 12.36 years with extremities of 18 to 69 years. The most represented age group is that of 36 to 45 years with 37 patients (31.9%) followed by that of 26 to 35 years with 24 patients (20.7%), that of 46 to 55 years with 22 patients (19.0%) and that of 18 to 25 years with 19 patients (16.4%). These data correspond to those of the literature reported by various authors for Kinshasa [10] [17] [18] [19] [20] [21]. The age

range of 15 to 49 years is that of very sexually active people reported by previous data [10] [17] [18] [19] [20] [21].

The majority of patients (46.1%) were married, followed by single (33.0%), widowed (13.9%) and divorced (7.0%). This profile is similar to that presented by various authors where married patients were in the majority in the populations followed [10] [17] [18] [19].

Protestants (70.7%) were more numerous in the study population, followed by Catholics (20.7%) and Muslims (1.7%). Religion in Kinshasa in particular and in the Democratic Republic of Congo (DRC) in general is gaining more and more importance on the way of life and habits commonly found in the environment, it even influences the decisions to be taken concerning the acceptance of HIV status, adherence to treatment and compliance with AntiRetroViral Treatment by the faithful of our different religious denominations. The predominance of the Protestant church has been presented in previous studies for Kinshasa [10] [21] [23] [24].

More than three-fifths of patients (66.7%) have secondary level, followed by 16.7% of patients who have primary level, 9.6% with university level and 7.0% who are illiterate. These results correspond to those reported for Kinshasa [8] [10] [19] [25]. The level of education plays a key role in the understanding, adherence and adherence to AntiRetroViral Treatments, even during the follow-up and care of patients after adherence. This generally low level reflects the level of knowledge and also the behavior of the population [19].

Most of the patients (29.4%) in the study work in the informal sector, followed by 23.5% who are traders, 14.3% housewives, 13.4% unemployed, 8.4% police and 5.0% military. These results reflect the impact of the social and economic situation of the population of Kinshasa due to precariousness, and the lack of stable and remunerative employment [10] [26].

Forty-nine patients (49), or 41.5%, were in clinical stage 3; followed by 40 patients (33.9%) who were in clinical stage 1, 18 patients (15.3%) in clinical stage 2 and 11 patients (9.3%) in clinical stage 4. Fifty-five (55) patients , or 47.0%, had a normal clinical condition; followed by 39 patients (33.3%) who had a good clinical state, 22 patients (18.8%) a bad clinical state and 1 patient (0.9%) a pre-moribund clinical state. Most patients start their ART at high stages according to WHO and with advanced clinical signs which has a direct impact on the patient's treatment prognosis. This calls into question the quality of the therapeutic management because of the deteriorated clinical signs of the patients. These observations are general for Kinshasa and are presented by different authors [18] [19] [20] [26].

The most common known medical history was: dental caries and sleep disorder (30.2%), alcoholism and tuberculosis (28.4%), appetite disorder (26.1%), neuro-peripheral disorders (17.9%), smoking, hypertension and hypotension (17.2%). The sleep disorder can be justified by anxiety attacks experienced by PLHIV due to the non-acceptance of their serological status, which complicates adherence to treatment.

The most common known prior morbidities were: malaria (35.3%), high blood pressure and heart disease (14.7%), drug abuse and tuberculosis (12.2%), and smoking (9.5%). In the endemic malaria environment where the city of Kinshasa is located, it is natural that malaria is the most common morbidity among patients.

The Opportunistic Infections most found in PLHIV initiating ART were: Malaria with 54 cases (45.4%), Tuberculosis (29.4%), Cutaneous pruritus (23.5%), urinary tract infections (21 .8%), oral candidiasis and skin eruptions (20.2%). Different authors have presented the same Opportunistic Infections in populations of PLHIV for Kinshasa [8] [27]-[32]. The state of immunosuppression of patients favors endemic infections such as malaria, those linked to the environment such as tuberculosis and those linked to personal hygiene, oral candidiasis and dermatoses [29] [32].

The mean values of the biochemistry parameters of the patients at the start of ART are as follows: 31.61 ± 20.71 IU/L for ALT, 25.81 ± 19.96 IU/L for AST, 79.35 ± 49.49 IU /L for Amylase, 108.13 ± 62.17 mg/dl for Total Cholesterol, 2.77 ± 1.27 mg/dl for Creatinine, 72.53 ± 22.23 mg/dl for Glycemia, 10, 30 ± 2.33 g/dl for Hemoglobin, 7.91 ± 1.75 g/dl for Total Protein, 131.23 ± 68.80 mg/dl for Triglycerides, and 33.61 ± 26.27 mg/dl for Urea. Most of the biochemistry values of PLHIV within the limits of acceptable ranges for a normal patient. Excluding blood glucose and total cholesterol levels which are towards the lower ranges; the other biochemical parameters are more towards the upper ranges. Only the Creatinine values are completely above the normal range. Similar results have been presented previously for PLHIV in Kinshasa [33] [34].

After amplification, 119 samples were positive for HLA PCR, confirming the presence of genetic material in all samples. One hundred and nineteen samples were positive for the "pol" region while 115 samples were positive for the "gag" region. This difference is explained by the size of the sequences to be amplified because the "pol" region is longer and more easily amplified [35] [36]. The 4 negative samples on the "gag" region were positive on the "env" region. Hence, the 119 samples were confirmed positive for HIV by Nested PCR. Discrepancies between pol and gag were reassessed on the env region to confirm patient positivity. Nested DNA PCR has been shown to be more accurate and specific than rapid serology tests [11] [12] [35] [36].

After RNA amplification for Viral Load (VL), 21 samples were undetectable, less than 50 RNA copies/ml. The median VL value was 4.16 $\log_{10}$ RNA copies/ml (14360.5 RNA copies/ml) with the lower and upper ends respectively equaling 0.0 $\log_{10}$ and 7.11 $\log_{10}$ copies of RNA/ml. In the present cohort, the median VC is already high at inclusion, which is a poor prognosis for ART. Patients in our setting generally start ART with VLs greater than 3.00 $\log_{10}$ RNA copies/ml; which is already a poor prognosis for good care of PLHIV. This corroborates the data in the literature on PLHIV starting ART in Kinshasa and in Central Africa [17] [20] [37] [38].

After sequencing of the different samples, regions of Reverse Transcriptase (RT) and 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. This difference in the rate of amplification in the 2 regions is observed in several previous works, and can be justified by the length of the RT region which is twice that of the Protease [17] [18] [39] [40] and even by the diversity of strains circulating in Kinshasa [17] [18] [41] [42]. Subtype A was dominant with 23 cases (20.2%); followed by 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%). Subtype A remains dominant over other subtypes in Kinshasa over the years [17] [18]. Recombinant forms such as CRF_02 (14.0%) and CRF_01 (4.4%) remain present among the wild strains. The diversity of strains in Kinshasa remains highly evolving and dynamic. These results are similar to those found in the literature for Kinshasa [17] [18] [37] [42]. No mutations were found for DTG; mutations K65R (1.8%), T69P/N (4.4%), K70R (7.9%) and M184V (7.0%) have been listed as existing mutations for Nucleotide Reverse Transcriptase Inhibitors. These mutations are generally associated with resistance to Lamivudine-3TC (K65, T69, M184) and Tenofovir-TDF (K65, T69, K70) [17] [18] [40] [42]. As a result, nearly 8% of naïve patients who start ART in Kinshasa according to national recommendations (Tenofovir + Lamivudine + Dolutegravir) have a prognosis of ART failure because of the pressure of K70 on Tenofovir.

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

In Kinshasa, People Living with HIV (PLHIV) who start AntiRetroViral Treatment (ART) are mostly married, of Protestant faith, with a high school education and working in the informal sector. They start ART late in the advanced clinical stage (stage 3) with already established malaria and/or tuberculosis. Biochemical parameters are within normal ranges for the most part, with Viral Loads generally high. Subtype A remains predominant among PLHIV and there are mutations conferring resistance to first-line ART.

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Conflicts of Interest

The authors declare no conflict of interest for this study.

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List of Abbreviations and Acronyms

AIDS: Acquired Immunodeficiency Syndrome, **ART:** AntiRetroViral Treatment, **ARV:** AntiRetroViral, **BMI:** Body Mass Index, **CI:** Confidence Interval, **DNA:** Deoxyribose Nucleic Acid, **DRC:** Democratic Republic of Congo, **DTG:** Dolutegravir, **HIV:** Human Immunodeficiency Virus, **OTC:** Outpatient Treatment Center, **PCR:** Polymerase Chain Reaction, **PLHIV:** Person Living with Human Immunodeficiency Virus, **RDT:** Rapid Diagnostic Test, **RNA:** Ribose Nucleic Acid, **RT-PCR:** Reverse Transcription PCR, **SSA:** Sub-Saharan Africa.