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Virological and Molecular Profile of People Living with HIV after 24 Weeks of Treatment with Dolutegravir in Kinshasa

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Abstract

Context: The appointment of the M6 is crucial because it is an indicator of the prognosis of the evolution of the care and the decision-making on the continuation of the AntiRetroViral Treatment. **Objective:** The objective of this study is therefore to present the virological and molecular profile of People Living with HIV under treatment with Dolutegravir 6 months after being put on ART in Kinshasa. **Methods:** The present study is a cross-sectional view at the sixth month of a prospective cohort to determine the virological and molecular profile of People Living with HIV (PLHIV) after 6 months of ART based on Dolutegravir (DTG) in Kinshasa. A sample of 5 mL of blood was taken from all HIV patients included. The collection of biological data was carried out under the same conditions as at inclusion. After extraction, Quantitative Real-Time PCR was carried out to determine the quantity of HIV RNA in the samples according to the protocols previously described. Reverse Transcription PCR (RT-PCR) and Nested PCR were carried out to amplify the regions of interest for Protease and Reverse Transcriptase for sequencing. **Results:** The median VL value was 2.92 $\log_{10}$ RNA copies/mL. With 17.75% of patients experiencing major failure of first-line treatment. Subtype A is dominant with 13 cases (20.98%); followed by CRF_02AG (16.13%), subtypes C (14.52%), D (9.68%) and K (6.45%). The K65R (3 cases), T69P/N (6 cases), K70R (9 cases) and M184V (8 cases) mutations were listed as existing muta-

tions for Nucleotide Reverse Transcriptase Inhibitors. **Conclusion:** After 6 months of ART, 59.67% of People Living with HIV on Tenofovir-Lamivudine-Dolutegravir is in therapeutic success while 40.33% are in a state of treatment failure. Subtype A remains dominant in the population of PLHIV. Resistance mutations were detected for Lamivudine and Tenofovir, but none for Dolutegravir.

Keywords

Virological Profile, Molecular, PLHIV, 6 Months of ART, Dolutegravir, Kinshasa

1. Introduction

After more than 4 decades, infection with the Human Immunodeficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS) remains a public health problem throughout the world according to reports from the Joint Program of United Nations on HIV/AIDS (UNAIDS). Since 2014, UNAIDS and its partners have launched the 90-90-90 targets at the International Conference on HIV/AIDS in Melbourne. The goal was therefore to be able to diagnose 90% of all HIV-positive people in a given region, to provide AntiRetroViral Treatment (ART) to 90% of diagnosed People Living with HIV/AIDS (PLHIV) and to obtain viral suppression (therapeutic success) for 90% of patients on AntiRetroViral Treatment (ART) [1] [2] [3] [4]. Therefore, to achieve this last objective, the realization of the Viral Load (VL) proves to be a necessity in the care of PLHIV.

According to UNAIDS in 2020, the Democratic Republic of Congo (DRC) had approximately 520,000 PLHIV [5] [6] [7]. Despite major advances in the fight against HIV, the epidemic continues to seriously harm public health in all regions. The difficult socio-economic environment of the country did not allow the achievement of the various objectives set by UNAIDS for the care of PLHIV, in particular with regard to the suppression of VL in patients on ART in the different cohorts of the country.

In the DRC, the National Multisectoral Program for the Fight against HIV/AIDS (PNMLS) and the National Program for the Fight against HIV/AIDS and Sexually Transmitted Infections (PNLS) are the bodies that organize the care of PLHIV at the national level [5] [6] [7]. According to the published rules, the patient follow-up schedule, within the framework of the published standards, in the different centers, for the first year, recommends, after initiation of ART, appointments in the first month (M1) of care, at the third month (M3), at the sixth month (M6), at the ninth month (M9) and at the twelfth month (M12) [6] [7]. For patient follow-up, the M6 appointment, being an appointment at the end of the first year of ART, is crucial because it is an indicator of the prognosis of the evolution of care and decision-making on the continuation of ART.

The objective of this study is therefore to present the virological and molecu-

lar profile of People Living with HIV under treatment with Dolutegravir 6 months after starting ART in Kinshasa.

2. Methods

2.1. Study Design, Patient Setting and Samples

The present study is a cross-sectional view at the sixth month of a prospective cohort to determine the virological and molecular profile of People Living with HIV (PLHIV) after 6 months of ARV Treatment (ART) based on Dolutegravir (DTG) in Kinshasa, DRC. The period for the sixth month (M6) follow-up appointment for the included patients was from April to August 2023.

During the M6 appointment, a sample of 5 mL of blood was taken for estimation of the Viral Load and sequencing in all HIV patients included. Sample collection was carried out under the same conditions as at inclusion.

2.2. Study Population

The population of the present study was the patients included in the cohort who responded for their M6 appointment in the period from April to August 2023, all having been previously seen during the inclusion period.

2.3. Parameters of Interest

As at inclusion [8], the parameters of interest at M6 were: age, sex, as well as molecular assessment.

2.4. RNA Extraction and Viral Quantification

At the Molecular Biology Laboratory, RNA was extracted from 140 µl of plasma using the QIAamp RNA Mini Kit QIAGEN® for RNA extraction [9]. 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 [10] [11]. **Table 1** presents the conditions for the different amplifications.

2.5. Sequencing and Molecular Identification

Reverse Transcription PCR (RT-PCR) and Nested PCR were performed on the extracted RNA to amplify regions of interest for Protease and Reverse Transcriptase (TR) for sequencing. The PCRs for sequencing were carried out under the conditions previously described in the literature [12] [13].

2.6. Ethical Consideration

This study was approved in its entirety by the research ethics committee of the School of Public Health, Faculty of Medicine, University of Kinshasa (ESP/CE/115/2021). Permission to access the centers was obtained from each competent authority. The samples in the centers were taken by the technical teams of the centers.

Table 1. Primers and probes for amplifications.

PCR Types	Primers and Probe	Sequences
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'

2.7. 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 (CIs) were calculated for categorical data.

2.8. Operational Definitions [8]

Virological Failure: Virological failure is defined as a persistent Viral Load (VL) measurement greater than 1000 RNA copies/mL (3.00 $\log_{10}$ RNA copies/mL) after 6 months of ART. There are 3 types of Virological failure according to the quantity of viral RNA detected in the sample:

- Minimal failure (3.00 $\log_{10}$ < VL < 4.00 $\log_{10}$ RNA copies/mL),
- Moderate failure (4.00 $\log_{10}$ < VL < 5.00 $\log_{10}$ RNA copies/mL),
- Major or severe failure (VL > 5.00 $\log_{10}$ RNA copies/mL).

3. Results

During the M6 appointment, 62 patients were registered, including 38 women (61.3%) and 24 men (38.7%), thus giving a sex ratio of 1.58 in favor of women. Figure 1 presents the above data.

The median VL value was 2.92 $\log_{10}$ RNA copies/mL (840 RNA copies/mL). The lower and upper extreme values were respectively equal to 0.0 $\log_{10}$ and 5.99 $\log_{10}$ RNA copies/mL. A total of 59.67% of patients were in treatment success with a VL of less than 3.0 $\log_{10}$ RNA copies/mL, with 17.75% of patients experiencing major failure of first-line treatment. The results of the VLs mentioned above are presented in Table 2.

All 62 samples were successfully amplified. Subtype A is dominant with 13 cases (20.98%); followed by CRF_02AG (16.13%), subtypes C (14.52%), D (9.68%) and K (6.45%) as described in Table 3. No mutations conferring resistance n was found for DTG after 6 months of ART. The K65R (3 cases), T69P/N (6 cases), K70R (9 cases) and M184V (8 cases) mutations were found as existing mutations for Nucleotide Reverse Transcriptase Inhibitors (Table 3).

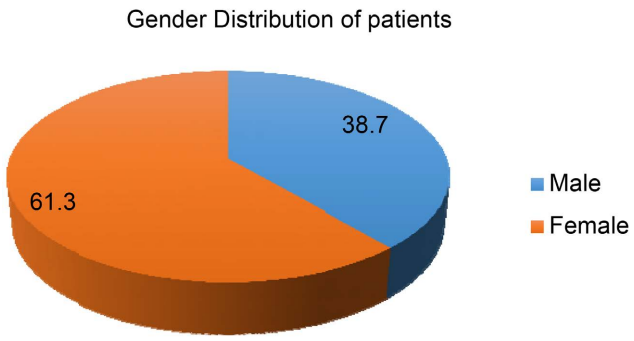

Figure 1. Gender and age group of patients in M6.

Table 2. Viral Load Values of patients at M6.

	Viral Load (copies of RNA/mL)	
	Values	**Log_{10}**
Median	840	2.92
Inferior Limit	1	0.00
Superior Limit	971,874	5.99
Intervalsof Viral Load		
	Values	**Percent**
VL Indeterminate	25	40.32
VL < 3.0 $\log_{10}$	12	19.35
3.0 $\log_{10}$< VL < 5.0 $\log_{10}$	14	22.58
VL > 5.0 $\log_{10}$	11	17.75

Table 3. Molecular data of patients in M6.

Molecular data of patients in M6		
Prevalence of circulating subtypes		
Sous-types	**Number**	**Percent**
A	13	20.98
B	1	1.61
C	9	14.52
D	6	9.70
E	1	1.61
F	1	1.61
G	3	4.85
H	2	3.22
J	1	1.61
K	4	6.45
CRF01	3	4.85
CRF02	10	16.13
CRF05	1	1.61
CRF06	1	1.61
CRF11	1	1.61
CRF25	1	1.61
CRF45	1	1.61
CRF56	1	1.61
U	2	3.26
Total	**62**	**100.0**
Prevalence of mutations of interest		
INTR	**Number**	**Percent**
K65R	3	4.84
T69P	6	9.68
K70E/R	9	14.52
M184V	8	12.90

4. Discussion

The objective of this study was to present the virological and molecular profile of People Living with HIV (PLHIV) after 6 months of ART based on Dolutegravir (DTG). The total number of patients who kept the M6 follow-up appointment was 62 patients with a predominance of the female sex in the order of 38 women (61.3%) against 24 men (38.7%). The predominance of the female gender in the present study corroborates the existing data in the literature. The majority of studies carried out in Kinshasa, the Democratic Republic of Congo (DRC), or

even in Central Africa mention this predominance of the female sex in the populations of PLHIV monitored, which is due to several demographic, health and social factors [7] [14] [15].

After RNA amplification to determine plasma Viral Load (VL), the median VL value was 2.92 $\log_{10}$ RNA copies/mL (840 RNA copies/mL) with the lower and upper ends respectively equal has 0.0 $\log_{10}$ and 5.99 $\log_{10}$ RNA copies/mL. The VL was undetectable for 25 patients giving a rate of 40.32% of patients with undetected VL. With 19.5% of patients having a VL of less than 3.00 $\log_{10}$ RNA copies/mL, the therapeutic success rate is 59.67%. The virological failure rate of first-line treatment according to the recommendations of the World Health Organization (WHO) is 40.33%, with 17.75% of patients in major failure (greater than 5.0 $\log_{10}$ copies of RNA/mL). These data are justified by the fact that more than half of patients start ART with a poor remission prognosis [8] [15] [16]. At M6 of the present cohort, the failure rate of 40.33% is higher than those presented for the city of Kinshasa in previous years [12] [17]. This demonstrates the need for virological and molecular monitoring of patients on ART, and that this monitoring should continue to figure in the care recommendations even for countries with limited resources.

After sequencing the different samples, 62 samples were successfully amplified. Subtype A is dominant with 13 cases (20.98%); followed by CRF02_AG (16.13%), subtypes C (14.52%), D (9.68%) and K (6.45%). Wild strains still dominate over recombinant strains. No mutations were found for DTG after 6 months of ART. However, the mutations K65R (3 cases), T69P/N (6 cases), K70R (9 cases) and M184V (8 cases) were listed as existing mutations for Nucleotide Reverse Transcriptase Inhibitors. Even after the loss of patients, subtype A still remains dominant in the cohort of PLHIV on ART in Kinshasa, followed by CRF02 and subtype C. This is the profile presented for Kinshasa through the various available literatures [12] [13] [18]. After 6 months of ART, no mutation was found to induce resistance to DTG. This confirms, according to various literatures, the strong genetic barrier attributed to DTG, reason why the molecule was introduced for countries with limited resources [19] [20]. However, the mutations found in PLHIV are mainly associated with resistance to Nucleotide Reverse Transcriptase Inhibitors (INTR); specifically to Lamivudine-3TC (K65, T69, M184) and Tenofovir-TDF (K70) [12] [13] [21]. As a result, 14.52% of patients on ART, or 36% of patients with virological failure, are in a situation of treatment failure because of the mutation which confers resistance to TDF. There is a strong correlation ($p < 0.00$) between the treatment failure and the combination of mutation M184-K70. These results corroborate data presented subsequently on the correlations of certain mutations and treatment failures [12] [13]. This therefore invites a correction of the first-line formula based on local scientific evidence.

5. Conclusion

After 6 months of first-line AntiRetroViral Treatment based on Dolutegravir,

59.67% of People Living with HIV on Tenofovir-Lamivudine-Dolutegravir are in therapeutic success while 40.33% are in a state of treatment failure. Subtype A remains dominant in the population of PLHIV in Kinshasa. Resistance mutations were detected for Lamivudine-3TC (K65, T69, and M184) and Tenofovir-TDF (K70), but none for Dolutegravir-DTG.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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