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Prevalence of residual replication in HIV-1 patients on Tenofovir, Lamuvidine and Dolutegravir (TLD) treatment followed at Al-Nadjma Multipurpose Center from 2021 to 2023

ASBAGUI FAYSALA Oscar [1,*], NAIBI KEITOYO Amedé [2], AHMAT MAHAMAT Ahmat [3], DJELASSEM Franklin [2], OUMAR ALKHATAD Djelani [3], MAHO Foulzi [3], ADAM IBRAHIM Bahar [3], MAHAMAT-NOUR Aguid [3] and BARHIM BOY Otchom [1]

[1] *University of N'Djamena, Doctoral Training in Biology and Human Health B.O.Pox : 1117, N'Djamena, Chad.*
[2] *Livestock Research Institute for Development, B.O.Pox : 433, N'Djamena, Chad..*
[3] *Virology and Molecular Biology Laboratory of the Al-Nadjma/APMS Center: B.O.Pox N'Djamena, Chad.*

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Abstract

Objective: The objective of this study is to determine the prevalence of residual replication in HIV-1-infected patients receiving TLD from 2021 to 2023 followed at the Polyvalent Al-Nadjma center.

Material and method: This was a retrospective and prospective study with an analytical aim which took place at the Polyvalent Al-Nadjma center from April 2022 to December 2023 in the city of N'Djamena. The study began with the interview of 135 patients, 108 of whom signed informed consent to participate in this study. Patients infected with HIV 1 on ART based on the combination of Tenofovir, Lamivudine and Dolutegravir (TLD) for at least 2 years with a load of 999 copies/mL were included. A total of 108 plasmas were collected and analyzed by the RT-PCR technique. The data from the interviews as well as the RT-PCR results were analyzed with R Studio software.

Results: the results of this study revealed a female predominance with 70% (76/108) with a M/F sex ratio of 0.42. The average age of the patients was 40.58 ± 9.58 years. The prevalence of persistent residual replication was 22.22% (n = 24/108), 54.60% (n = 59/108) of patients had virological success, and 19.44% (n = 21/108) of patients had a viral load greater than or equal to 1000 copies/mL and 3.70% (n = 4/108) had an invalid plasma viral load.

Conclusion: The aim of this work was to determine the prevalence of residual replication in patients infected with HIV-1 under Tenofovir, Lamuvidine and Dolutegravir (TLD) followed at the Al-Nadjima/APMS multipurpose center in N'Djamena, Chad. The prevalence of residual replication in this study was 22.22%. This study demonstrated that individuals with a persistent viral load below 1000 copies/mL were at increased risk of therapeutic and virological failure due to the lack of consensus and failure to control the phenomenon of residual replication in patients infected with HIV-1 under TLD

Keywords: Prevalence; Residual replication; HIV-1 patients under TLD; Al-Nadjma Multipurpose Center

1 Introduction

The human immunodeficiency virus (HIV) belongs to the *Retroviridea* family, responsible for acquired immunodeficiency syndrome (AIDS) (Dieuzaert, 2009). HIV infects and destroys memory CD4 T lymphocytes which

* Corresponding author: ASBAGUI FAYSALA Oscar

normally coordinate adaptive T and B responses against pathogens, thus giving rise to a lethal immune deficiency and creating a state of chronic inflammation deleterious for the entire organism.(Rouzoux and al. 2013)

According to UNAIDS, HIV remains a major public health problem due to its prevalence (39 million) worldwide and which remains particularly high in sub-Saharan Africa (25.3 million). In 2022, UNAIDS estimates 29.8 million people will have access to antiretroviral treatment, an increase of 7.8 million [6.9 million-7.9 million] compared to 2010, and new HIV infections have been reduced by 57% since 2010 (UNAIDS, 2022).

The advent of triple antiviral therapy has led to a considerable reduction in morbidity and mortality associated with HIV. The objective of antiretroviral treatment (ARV) is to block viral replication in order to obtain an undetectable plasma viral load (CVP) and then maintain and restore the patient's immune system (ANRS, 2015; Boulassel and al. 2007).

However, the effectiveness of ARVs seems to be compromised by the phenomenon of residual replication and the appearance of resistance to these treatments. Residual replication constitutes an independent risk factor for therapeutic failure and mortality (Dieuzaert, 2009; Fletcher and al. 2014). Residual replication takes place in the “reservoirs” of patients and depends on pharmacological factors (suboptimal ARV plasma concentrations), and the intrinsic power of the ARV treatment to reach these areas. (Delaugerre and al. 2015).

In Chad, 79% of people living with HIV know their serological status and 75% are on antiretroviral treatment (UNAIDS, 2022). Progress in the therapeutic care of patients living with HIV (PLHIV) in Chad has continued in recent years, notably with the development of new, more powerful and better tolerated molecules such as dolutegravir (DTG) in combination with two inhibitors. reverse transcriptase nucleosides (Tenofovir and lamivudine), by ministerial approval and thanks to the directives of the sectoral AIDS control programs in Chad in March 2019.

In Chad, there is no work that has evaluated the residual replication of HIV-1 in patients on TLD. The objective of this study was to determine the prevalence of residual replication in HIV-1 infected patients receiving TLD from 2021 to 2023 followed at the Al-Nadjma/APMS/CHAD Multipurpose Center, in order to better adapt therapeutic strategies .

2 Material and methods

2.1 Study area and period

This was a retrospective and prospective study with analytical purposes which took place at the Al-Nadjma Multipurpose center (APMS) in the commune of the 6th District of the city of N’Djamena in Chad. The geographic coordinates carried out by Globale Position System (GPS): are 12°10'709’’5 north latitude and 15°07'904’’8 east longitude. The patients were people living with HIV infected with HIV-1 under TLD followed regularly for 96 weeks with a plasma viral load less than 1000 copies/mL measured every six months followed as part of the monitoring of HIV-infection. 1.

2.2 Sampling

The sampling was done voluntarily. It began with an interview with patients living with HIV monitored regularly at the Al-Nadjma Multipurpose Center. A total of 135 patients on TLD with a viral load between less than 40 and 999 copies/mL were interviewed. Among them, 108 signed the informed consent form and agreed to participate in this study and 27 refused to participate in the study.

2.3 Sample and data collection technique

Data collection and samples were carried out between April 2022 to December 2023. The information was collected using a structured questionnaire. The data collected were the sociodemographic characteristics of the patients (age, sex and marital status) and biological and clinical data (frequency of residual replication, therapeutic failure and virological success). The blood sample of 2 to 4 mL in 2 tubes containing an EDTA (ethylene diamine tetraacetic acid) anticoagulant per patient was carried out by puncture of the radial vein using a Venoject,® Vacutainers® 7:30 needle. minutes at 9:30 a.m. by a member of the team. The samples were sent to the Molecular Biology laboratory of the APMS center. The samples were centrifuged at 15,000 rpm for 10 minutes. Plasma is collected to be stored at -70 °C for later use.

2.4 Real Time Polymerase Chain Reaction (RT-PCR) protocol

The viral genome was demonstrated using Real Time Polymerase Chain Reaction (RT-PCR) according to the protocol recommended by the company BIOCENTRIC. Extraction of viral RNA from plasma is automated. Being automated we used two kits. The GenoXtract® kit in association with the GXT NA nucleic acid extraction kit version 1.0 (Hain Lifescience, Ref. 12.08.02), marketed by BIOCENTRIC. The extraction technique used is that recommended BIOCENTRIC (Biocentric, 2019a). For amplification the main components of the Generic HIV Viral Charge Amplification Kit (Biocentric) for 220 tests (Ref. TR001-250IC).

For a test, the reaction mixture was composed of the following elements: (3 µL of H2O without nuclease, 5 µL of the enzyme mix, 0.5 µL of the sense primer A, 0.5 µL of the anti-sense primer B, 0.5 µL of probe C) with the "reporter" the FAMTM fluorophore at 5' and the non-fluorescent MGB "quencher" at 3' and 0.5 µL of IC primers/Cy5-probes) (Biocentric, 2019b).

After preparing the reaction mix solution, 10 µL of RNA eluate was added to each well already containing reaction mix. Place all samples in the thermal cycler (FluoroCycler® seconds of primer hybridization at +62 °C and 30 seconds of elongation at +72 °C then the final elongation of 7 minutes between 50 °C to 60 °C. The FluoroSoftware® XT-IVD allowed us to generate rapid results with a summary report (Biocentric, 2019b).

2.5 Statistical analyzes of the data

The data from the interviews as well as the results of the plasmas tested were entered into an Excel spreadsheet in Microsoft Office 2010 software then converted to CSV and then exported to R Studio software version 4.0.4.2021 for analyses. Concerning the analytical statistics, the Chi-square test and the Pearson test were used to compare the proportions (frequency of residual replication, therapeutic failure and virological success) and the variations in prevalence linked to the detection of replication. viral residual as well as for their significance. The significance threshold was set at 0.05 and the P-value calculated using the Pearson test.

3 Results

3.1 Characteristics of the patients studied

During the study, 135 patients were enrolled, 108 of whom agreed to participate with a plasma viral load below 1000 copies/mL.

Women were predominant with 70% (76) compared to 30% (32) of men with a M/F sex ratio of 0.42. The average age of the patients was 40.58 ± 9.58 years. The extreme ages were 18 and 72 years. The age group of 40 to 50 years old was the most represented with 41%, followed by that of 29 to 39 years old with 34%.

In this study, married people were the most represented at 48.18%, followed by widowers with a rate of 30.55%.

3.2 Patient plasma viral load results

After 96 weeks (24 months) of treatment under TLD the following results were obtained in the patients who participated in this study. A prevalence of 54.60% (n = 59/108) of patients in virological success, 22.22% (n = 24/108) were in persistent residual replication and 19.44% (n = 21/108) of patients had a viral load greater than or equal to 1000 copies/mL and 3.70% (n = 4/108) had an invalid plasma viral load. These results are highly significant with a P-value = 0.005

Table 1 Plasma viral load of patients

CV/copies/mL	N	Prevalence	CI at 95%	P-value	Interpretation
Undetectable CVp	59	54.6	[-58.26 ; 50.94]	0.00512	Very Significant
CVp <1000	24	22.22	[-23.14 ; 21.3]		
CVp ≥1000	21	19.44	[-20.19 ; 18.69]		
Invalid	4	3.70	[-4.03 ; 3.94]		

Legend: CVP: plasma viral load, CV: viral load, N: the number, CI: confidence interval,

Table 2 shows variations in the prevalence of persistent residual replication among patients. In this study, the prevalence of persistent residual replication (RR) was 16.66% in female patients and 6.48% in male patients. These results are significant at the 5% level with a P-value = 0.05981 using the Pearson test.

Table 2 Distribution of RR patients by sex

Persistent RR		N	Prevalence	CI at 95%	P-value	Interpretation
Female	Yes	18	16.66	[-17.25 ; 16.07]	0.05981	significant
	No	58	53.7	[-57.27 ; 50.12]		
Male	Yes	7	6.48	[-6.61 ; 6.35]		
	No	25	23.15	[-24.13 ; 22.16]		

Legend : RR : residual replication

The study revealed a prevalence of 38.88% in female patients with virological success compared to 15.74% in males. These results are very significant at the 5% level with a P-value = 0.0014 using the Pearson test (table 3).

Table 3 Distribution of patients with virological success according to sex

Virological success		N	Prevalence	CI at 95%	P-value	Interpretation
Female	Yes	42	38.88	[-41.06 ; 36.69]	0.0014	Not significant
	No	34	31.48.7	[-33.06 ; 29.8]		
Male	Yes	17	15.74	[16.28 ; 15.19]		
	No	15	13.88	[-13.97 ; 13.78]		

During this study, 14 female patients were in virological failure, representing a prevalence of 12.96%. The prevalence of virological failure was 6.48% among male patients. These results are not significant with a rate of 5% in the Pearson test (P-value = 0.0654) (Table 4)

Table 4 Distribution of patients with virological failure according to sex

Virological failure		N	Prévalence	CI at 95%	P-value	Interpretation
Féminine	Yes	14	12.96	[-13.35 : 12.96]	0.0654	Not significant
	No	65	60.18	[-64.43 ; 55.92]		
Male	Yes	7	6.48	[-6.60 ; 6.35]		
	No	25	23.14	[-24.12 ; 22.15]		

4 Discussion

4.1 Characteristics of the patients studied

During the study, 135 patients were enrolled, 108 of whom agreed to participate with a plasma viral load below 1000 copies/mL.

Women were predominant with 70% (76) compared to 30% (32) of men with a M/F sex ratio of 0.42. The average age of the patients was 40.58 ± 9.58 years. These results are close to those published by Naibi in 2017 in patients undergoing antiviral treatment at the Souro Sano University Hospital Center in Bobo-Dioulasso in the Republic of Burkina Faso. But different from those found by Zied and al.2020 in Oman who found 59% of their patients were men with an average age of 44 years. This difference could be explained by the size of the samples and the duration of their studies which was longer than ours as well as the size of their samples which was larger than ours.

The average age of the patients was 40.58 ± 9.58 years. The extreme ages were 18 and 72 years. The age group of 40 to 50 years old was the most represented with 41%, followed by that of 29 to 39 years old with 34%. Our results are comparable to those of Allahna and al., 2019 in patients on antiretroviral treatment with a low level of persistent viremia who found 39.5% in the age group of 40 to 49 years. These results can be explained by the fact that this age group is the most sexually active and is more affected by HIV and many of them are entering the AIDS phase.

In this study, married people were the most represented, with a prevalence of 48.18%, followed by widowers with a rate of 30.55%. These results are lower than those Zoungrana and al. 2018 in patients infected with HIV-1 on second-line antiretroviral treatment with persistent low-level residual viremia at the Souro Sano University Hospital Center in Bobo-dioulasso in the Republic of Burkina Faso. These results can be explained by the fact that 90% of patients who agreed to participate in the study claimed to have another sexual partner apart from their husbands or wives, that is to say an extramarital romantic relationship. These statements may support the high prevalence of married patients in this study.

4.2 Patient plasma viral load results

After 96 weeks (02 years) of treatment under TLD the following results were obtained in the patients who participated in this study. A prevalence of 54.60% (n = 59/108) of patients in virological success, 22.22% (n = 24/108) were in persistent residual replication and 19.44% (n = 21/108) of patients were in virological failure with a viral load greater than or equal to 1000 copies/mL and 3.70% (n = 4/108) had an invalid plasma viral load. Our results are lower than those of 85% (n = 102/120) published by Nyaku and al 2019 in patients with virological success, but lower than those of 40% (n = 60/153) obtained by Zied and al, 2020 in patients with virological success. On the other hand, the prevalence of residual replication of 22.22% found in our study is higher than that of 2.9% published by Underwood and al., 2019 in the Republic of Mexico in a cohort study of patients and close to that 25% obtained by García –Gasco and al, 2008 in their series of residual viremia. Our study showed that 19.44% of patients were in virological failure. Our results are lower than those of 16% reported by Vendenhende and al, 2015 in virological failure reported in a cohort of patients regularly followed in France. Normally, patients on the TLD regimen should have an undetectable plasma viral load but this is not in our study context. Several reasons could explain the phenomenon of residual replication leading to virological failure. In a descriptive cohort conducted by Maldarelli and al, 2011 showed that 80% of individuals infected with HIV-1 under antiretroviral treatment had residual viremia between 1 copy/mL to 49 copies/mL stable after 60 weeks of treatment. Maldarelli and al, 2011, explained that detectable residual viremia was due to the stable periodic release of HIV from latently infected cells, probably from sanctuary sites that are poorly targeted by antiretroviral treatment causing this residual replication. . Furthermore, residual viremia causes chronic immune activation, associated with increased morbidity and mortality in HIV-infected patients (Boulassel and al. 2008; Kuller and al. 2008. These statements may explain this increase in the prevalence of persistent residual replication and the virological failures observed in the patients who participated in this study.

In this study, the prevalence of persistent residual replication was 16.66% in female patients and 6.48% in male patients. These results are far from unanimous. For Ryscavage and al, 2014. Low and very low viremia in HIV-1-infected patients should not be considered because they are essentially "blips" but all but 18-24% become persistent low-level viral loads. They conclude that if the frequency of persistent low-level viral load still remains high, without viral suppression, it is necessary to think about the alternative therapeutic regime and review the treatment as well as its duration. McMahon and al, 2010 showed that intensification of antiretroviral therapy with a potent HIV-1 integrase inhibitor cannot decrease persistent viremia in subjects receiving suppressive therapy, indicating that cell cycling rapidly infected with HIV-1 are not present. They assert that eradicating persistent residual viremia will require new therapeutic approaches. On the other hand, Shen and al. 2008 noticed that some of their HIV-1 infected patients on therapy were well compliant and even responsive to antiretroviral treatment but they noticed that their patients did not have undetectable CVP but rather progressed towards residual replication. They demonstrated that this residual replication takes place in the “reservoirs”. These reservoirs are the sanctuaries (central nervous system), anatomical reservoirs (intestine, intestinal lymphoid tissues), genital (compartment) and lymphoid system.

5 Conclusions

The objective of this study was to determine the prevalence of residual replication in patients infected with HIV-1 on Tenofovir, Lamuvidine and Dolutegravir (TLD) followed at the Al-Nadjima/APMS Multipurpose Center in N'Djamena, Chad. The overall prevalence of residual replication in this study was 22.22%. The virological success observed in the patients from this study was 54.6%. This study demonstrated that individuals with a persistent viral load below 1000 copies/mL are at increased risk of virological failure. Currently, there is no consensus on how to manage patients with low-level residual viremia or residual replication. To successfully eliminate this residual replication, it is essential to

first identify all stable reservoirs and develop therapeutic strategies for each reservoir in order to resolve the phenomenon of residual replication in patients under TLD which normally should have an undetectable plasma viral load.

Compliance with ethical standards

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Declaration of conflicts of interest

All authors declare no conflict of interest related to this present study.

Authors Contribution

AFO, designed the research protocol. Protocol Revised by NKA and BBO. AFO AIB, AMA, and OD carried out the collection and processing of the samples. AMA and MF supervised the analyses. NKA and DF analyzed and interpreted the results. All authors contributed to the revision of this manuscript.

Statement of ethical approval

Authorization for ethical clearance of this study was obtained from the National Bioethics Committee of Chad (CNBT)

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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