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Low level of HIV-2 replication in patients on long-term antiretroviral therapy in Togo

Abstract

In the absence of virological follow-up in people infected with HIV-2, is the ongoing clinical and immunological follow-up in our resource-constrained countries efficient? In this study we document the immuno-virological follow-up of patients infected with HIV-2 in Togo. Thus, a cross-sectional study was carried out at the BIOLIM laboratory of the University of Lome. The sample consisted of HIV-2 infection patients on ART, followed in care centres in Lome, Togo. Confirmation of the HIV type, CD4$^+$ T-cells counting, HIV-2 viral load, HIV-1 viral load and HIV-2 resistance genotyping were carried out.

Based on the results of serological retesting, 30 HIV-2 infected patients, including 8 HIV-1 and HIV-2 co-infected, were included. The median age was 52 years IQR [40.7-61.0years]. The median duration on antiretroviral therapy was 7 years IQR [2,00-8,75 years]. At baseline, all patients were on PI-based treatment, 80.0% of them were on lopinavir / ritonavir. The median CD4$^+$ T-cells level was 586 cells per µl IQR [442-732 cells]. The proportion of subjects with detectable HIV-2 viral load (VL> 50 copies / ml) was 13.3% (4/30) with an average VL of 5533 copies / ml. Resistance genotyping of HIV-2 in the RT and Prot regions of the *pol* gene in virologically challenged subjects revealed the presence of resistance mutations respectively for the INRT class (M184V, Q151M, K65R, V111I) and PI (I54M, I50V, V47A). Under current conditions, HIV-2-infected patients will face a long-term limit to the choice of treatment due to the onset and accumulation of ARV resistance mutations.

Keywords: HIV-2, viral load, drug-resistance mutations, Togo

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Abbreviations: ART, antiretroviral therapy; ARV, antiretroviral; PIs, protease inhibitors; NRTI, nucleoside reverse-transcriptase inhibitor; PLWHIV, people living with HIV; BIOLIM, biology and immunology laboratory; LTRs, long terminal repeat; IQR, inter-quartile range

Introduction

In West Africa, where HIV-2 infects up to 1-2 million people,[1] antiretroviral therapy (ART) is becoming increasingly available and ART "scaling-up" programs proliferate. Significant numbers of HIV-2-infected individuals will have access to and will be treated with antiretroviral (ARV) drugs developed against HIV-1 infection.[2] However, HIV-2 is intrinsically resistant to the non-nucleoside reverse transcriptase inhibitors and to enfuvirtide, and reports suggest that HIV-2 may be partially resistant to some protease inhibitors (PIs) and has a low genetic barrier to nucleoside reverse-transcriptase inhibitor (NRTI) resistance.[3–6]

In Togo, country located in West Africa, HIV-1 and HIV-2 circulate with a large majority of people infected by HIV-1. In the country, all people living with HIV (PLWHIV) are monitored in care centres accredited by the national AIDS program (PNLS/Togo). Monitoring of PLWHIV is done using a national guide adapted from World Health Organization (WHO) recommendations .[7]. However, due to the lack of HIV-2 viral load supply, PLWHIV-2 on ART in our settings, do not have access to virological Follow-up. The aim of this study is to evaluate, based on viral load data, the quality of management of patients infected with HIV-2 in our current conditions in Togo.

Materials and methods

Data and samples

We conducted a cross-sectional study from January to June 2017 and we included HIV-2 mono-infected patients and those infected with both HIV-2 and HIV-1 followed-up in Lome, the capital city of Togo. All patients were on ART at least for one year. For each patient, 10 ml of venous blood was collected using EDTA tube at the time of enrolment to confirm the HIV status and monitor the immunological and virological status. Using a survey form, we also recorded socio-demographic data, HIV related disease staging according to WHO classification criteria and information on ART. After performing the biological analysis, we completed the form with the number of CD4$^+$ T-cells and the value of HIV-2 RNA viral load. In case of double-infection, we added the result of HIV-1 RNA viral load.

Laboratory methods

The HIV status of patients enrolled in this study was confirmed using INNO LIA HIV I/II Score (Innogenetics NV Belgium). Serological tests were performed at the national reference center for HIV test and sexual transmitted infections (CNR-VIH/IST) at the university teaching hospital Sylanus Olympio (CHUSO).

CD4 T-Cells measurement was done at the molecular biology and immunology Laboratory (BIOLIM), located at Health Science Faculty of University of Lome with flow cytometry standard on BD FACSCalibur using BD MultiTest reagents and MultiSet software (BD BioSciences).

The HIV-2 RNA viral load was performed with an in house Generic HIV-2 BioCentric (Bandol France) test. It is a two main technical steps test based on an automated method of extraction of retroviral RNA followed by Real-Time PCR amplification of extracted RNA. The test targets region located at Long Terminal Repeat (LTRs) and detection threshold is 10copies/ml for a sample portion of 1000µl.[8] In case of dual infection, the HIV-1 viral load measurement was undertook in the same laboratory using Abbott m2000rt after

automatically extraction with Abbott m2000sp (Abbott Pack, Illinois, USA). Protease and reverse transcriptase sequencing was performed in samples with a plasma HIV-2 viral load above 50 copies/ml at the virology laboratory Bichat-Claude Bernard in France using in house methods as previously described.[9]

Statistical analysis

Data were analysed using descriptive statistics and ++ exact Fisher test, + independence Chi-2 test.

Ethical aspects

The national ethics committee of Togo approved this survey. Patients were informed and had given their written consent before being included.

Results

We included 30 PLWHIV-2 and followed-up in 11 care centres in Lome. Among them, 22(73.3%) were HIV-2 infected patients and 8 dual HIV-1/2 infected. Patients' characteristics are shown in Table 1. The sex ratio M/F was 0.60%. The median age at enrolment was 54 years (Inter-quartile range (IQR): 41-61 years) and 49.5 years (IQR: 40-56 years) respectively for HIV-2 infected patients and dual HIV-1/2 patients. Most of patients (86.7%) were in stage 1 of the WHO classification. Median duration of ART was 7 years (IQR: 2-9 years) in HIV-2 mono-infected patients versus 8 years (IQR: 5-9 years) in dual HIV-1/2 infected patients. Adherence to treatment was found in 73.3% of patients.

Details on treatment and biological characteristics of patients are shown in Table 2. Thus 25(83.3%) patients received a first line treatment based on protease inhibitor (PI) regimen. At enrolment, all patients were on PI regimen comprising in 93.3% of them, Tenofovir (TDF) and Lamivudine (3TC) as nucleoside reverse transcriptase inhibitors (NRTI). The PI used was Lopinavir boosted by ritonavir in 80% of cases. Five patients experienced change in their first line treatment protocol.

The median $CD4^+$ T-cells count for HIV-2 infected patients and dual HIV-1/2 patients was 595 cells per µl IQR [474.2-771.5 cells] and 535 cells per µl IQR [387.2 -608.8 cells] p(0.899) respectively, and 33.3 % of subjects had a $CD4^+$ T-cells count of less than 500 cells per µl.

Table 1 Socio-demographic, clinical and duration on ART of HIV-2 and Dual in HIV-1 and HIV-2 infected patients

	HIV 2		HIV 1&2		p-Value
	n	%	n	%	
Gender					
Female	13	59	6	75	0.6722++
Male	9	41	2	25	
Age (Years)					
Median [IQR]	54	[40-61]	49	[40-56]	0.5331++
ART Duration (Years)					
≤5	8	36.4	2	25	0.6974+
>5	14	63.6	6	75	
WHO Stage					0.9999++
1	19	86.4	7	87.5	
2	3	13.6	1	12.5	
ART Observance					0.3568++
Yes	17	77.3	5	62.5	
No	5	22.7	3	37.5	

++, exact fisher test; +, independence Chi-2 Test; IQR, inter-quartile range

Of 30 patients included, 26(86.7%) had undetectable HIV-2 viral load. In the 4(13.3%) patients detected (CV>50 copies/ml), the HIV-2 viral load ranged from 1013 to 9874 copies/ml with a mean of 5533 copies/ml. Dual infected patients (n=8) were all, undetectable in HIV-2 viral load measurement , amongst them, 3 had a detectable HIV-1 viral load but less than 200 copies/ml.

In the 4 patients with detectable viremia, protease and reverse transcriptase sequencing were successful for 2(50%) samples. HIV-2 resistance genotyping in the reverse transcriptase and protease region of the *pol* gene revealed the presence of resistance mutations respectively for the NRTI class (M184V, Q151M, K65R, V111I, Y115F) and PI (I54M, I50V, V47A) (Table 3).

Discussion

We aimed to carry out the measurement of the HIV-2 load in order to use the results to assess the quality of immunological and clinical follow-up available in our currents conditions in Togo. We found more than 80% HIV-2 infected patients in virological success. Compared with HIV-1 infection, HIV-2 infection is characterized by a much longer asymptomatic stage, lower plasma viral loads, slower decrease in $CD4^+$ T-cells count, decreased mortality rate associated with AIDS, and lower rates of mother-to-child transmission, and sexual transmission.[10] Our findings are in line with these characteristics. However, long-term ART could increase the $CD4^+$ T-cells level and avoid progress to AIDS and related deaths.

The age of the patients confirmed the low transmission of HIV-2 from mother to child even we found a case of 12-year-olds vertically infected. We noted that the age ranges of the patients with HIV-2 and dual HIV-1/2 infections are different from that of HIV-1 infected patients reported in Togo.[11–13] and many other African countries.[14,15] While the highest rate of HIV infected adults was from 35 years old in Togo,[16] people older than 40 years were persons infected with HIV-2 and dual HIV-1/2 in our study population. As reported in Nigeria,[17] there was no significant difference between the HIV-2 and HIV- 1/2 dually infected patients.

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Table 2 Biological and treatment characteristics of HIV-2 and dual HIV-1 and HIV-2 infected patients

Patient code	HIV status	Years on ART	CD4 Cells /µl	ART at enrolment	Other ART received	HIV-2 Viral load copy/ml	HIV-1 viral load copy/ml
BLP01	HIV-2	1	707	TDF-3TC-LPV/r	-	0	-
BLP02	HIV-2	1	1713	TDF-3TC-LPV/r	-	0	-
BLP03	HIV-2	1	965	TDF-3TC-LPV/r	-	0	-
BLP06	HIV-2	6	391	TDF-3TC-LPV/r	-	3837	-
BLP09	HIV-2	8	593	TDF-3TC-LPV/r	-	1013	-
BLP10	HIV-2	6	576	TDF-3TC-LPV/r	-	0	-
BLP11	HIV-2	8	845	TDF-3TC-LPV/r	ABC-DDI-LPV/r	0	-
BLP12	HIV-2	2	626	TDF-3TC-LPV/r	-	0	-
BLP13	HIV-2	2	1042	TDF-3TC-LPV/r	-	0	-
BLP16	HIV-2	1	781	TDF-3TC-ATV/r	-	0	-
BLP18	HIV-2	7	470	TDF-3TC-ATV/r	AZT-3TC-LPV/r	0	-
BLP19	HIV-2	12	551	TDF-3TC-LPV/r	-	0	-
BLP20	HIV-2	2	597	TDF-3TC-ATV/r	-	0	-
BLP21	HIV-2	8	274	TDF-3TC-LPV/r	-	9874	-
BLP24	HIV-2	7	469	TDF-3TC-LPV/r	-	0	-
BLP25	HIV-2	7	536	TDF-3TC-LPV/r	-	0	-
BLP27	HIV-2	8	743	TDF-3TC-LPV/r	ABC-DDI-LPV/r	0	-
BLP28	HIV-2	12	732	TDF-3TC-LPV/r	ABC-DDI-LPV/r	0	-
BLP31	HIV-2	9	884	TDF-3TC-ATV/r	D4T-3TC-NVP	0	-
BLP35	HIV-2	2	369	TDF-3TC-LPV/r	-	0	-
BLP37	HIV-2	9	388	TDF-3TC-ATV/r	TDF-3TC-LPV/r	0	-
BLP36	HIV-2	7	487	ABC-3TC-LPV/r	AZT-3TC-ABC// D4T-3TC-LPV/r	7408	-
BLP05	HIV-1&2	9	653	TDF-3TC-LPV/r		0	0
BLP07	HIV-1&2	1	360	TDF-3TC-LPV/r		0	47
BLP23	HIV-1&2	9	594	ABC-3TC-LPV/r	ABC-DDI-LPV/r	0	143
BLP26	HIV-1&2	9	726	TDF-3TC-LPV/r		0	0
BLP29	HIV-1&2	2	373	TDF-3TC-ATV/r		0	0
BLP30	HIV-1&2	11	551	TDF-3TC-LPV/r	D4T-3TC-NVP	0	0
BLP32	HIV-1&2	11	392	TDF-3TC-LPV/r	D4T-3TC-NVP	0	0
BLP33	HIV-1&2	6	519	TDF-3TC-LPV/r	D4T-3TC-NVP	0	60

TDF, tenofovir; ABC, abacavir; D4T, stavudine; DDI, didanosine; AZT, zidovudine; LPV/r, lopinavir/ritonavir; NVP, nevirapine; 3TC, lamivudine; ART, antiretroviral Therapy

Table 3 Treatment characteristics and drug resistance mutations in patients experiencing virological failure

Patient code	Duration on ART (years)	Viral Load Copy/ml	RT mutations	Drugs affected	PR mutations	Drugs affected	ART at enrolment
P21	8	9874	K65R M184V V1111	ABC 3TC	I50V V47A	DRV LPV/r	TDF-3TC-LPV/r
P36	7	7408	M184V Y115F Q151M V1111	3TC ABC TDF AZT	I54M	DRV LPV/r	ABC-3TC-LPV/r

RT, reverse transcriptase; PR, protease; ART, antiretroviral therapy; DRV, darunavir; LPV/r: lopinavir/ritonavir; ABC, abacavir; TDF, tenofovir; 3TC, lamivudine; AZT, zidovudine

The slightest virulence of HIV-2[18] and the use of ART explain easily the fact that patients are found in stage 1 of the WHO classification. Thus, a previous study reported in 2013, in both ARV-naive and starting ART and followed-up in clinical centres in West Africa, that overall, 16.7% of HIV-2 patients on ART had an advanced clinical stage (WHO IV or CDC-C).[19] Unfortunately we had no information on the clinical stage of the patients at ART initiation. In the same study, the median CD4 count at the ART initiation was 166 cells/mm^3, IQR (83-247) among HIV-2 infected patients and 146 cells/mm^3, IQR (55-249) among dually seropositive.[19] Certainly because of long-term triple therapy, median CD4$^+$ T-cells levels was above 500 cells/µl, contrary to those reported .[9]

We found that in long-term ART, 86.7% of HIV-2 infected patient included in this study were virally suppressed at enrolment. Compare to HIV-1 infected patient in the country, with the same duration on ART, less than 50% of them are found undetectable.[20] It has been shown in West Africa cohort study that 46.5% of HIV-2 infected patients were well controlling infection.[21] This seems due to a better immune response including better protection and less immunopathology.[22]

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Despite the low-level of HIV-2 viral load in patients enrolled in our study we found (n=4) (13.3%) subjects with detectable viremia. Similar results have been reported in others studies about patient on long term ART.[9,23] This viral replication may be due to emergence of virus with drug-resistance mutations. Other mechanisms that contribute to HIV persistence during ART, including HIV latency, immune dysfunction and persistent low-level spread of the virus to uninfected cells could lead to detectable viremia.[24] Otherwise limited drug penetration within tissues and the presence of immune sanctuaries have been argued as potential mechanism allowing HIV to spread during ART.[24–26] Amongst 4 patients with detectable viremia, 2 harbored virus with drug-resistance mutations which compromising triple therapy in progress. In one patient, after 7 years on ART, appearance of drug resistance mutations such as M184V, V111I, Q151M and I54M indicates difficulties in the future therapeutic choice. The same drug-mutations are reported in HIV-2-treated patients in Abidjan (Ivory Coast).[9] As reported by Jallow et al.,[23] these findings justify the need to offer the viral load for patient follow-up in order to detect virological escapes early.[23] Thus, it seems that keeping the viral load to undetectable or very low level will be a good marker to predict the good evolution of the infection, which remain stable for many years. However our number of study patients is very low to draw a solid conclusion regarding virological issue.

Conclusion

Viral load and $CD4^+$ T-cells count are the markers commonly used to monitor patients Infected with HIV, in that they can be considered predictors of patient status and used to make the response to antiretroviral therapy. In lack of VL testing, as is the case with HIV-2 infection, patients monitoring in our currents conditions could lead to dramatic situations characterized by the onset and accumulation of drug-resistance mutations and in the future the absence of therapeutic options.

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Conflicts of interest

The authors declare no conflicts of interest concerning this article.

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