Primary resistance to antiretroviral drugs of HIV strains in Chad: a retrospective investigation by analysis of frozen dried blood spot samples

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Brief report

Primary Resistance to Anti-Retroviral drugs of HIV strains in Chad: a retrospective investigation by analysis of frozen Dried Blood Spot samples

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Abstract: (98 words)

Purpose: No data concerning Anti-Retroviral drugs (ARV) primary resistance mutation rates in Chad are available.

Methods: We retrospectively analysed frozen-stored dried blood spot samples that were collected from 48 Chadian Human Immunodeficiency Virus (HIV)-1 seropositive patients naïve of ARV.

Results: HIV-1 protease and reverse transcriptase genes were successfully sequenced for 24 (60.0%) of the 40 patients displaying a viral load >1000copies/ml. Seven (29.2%) displayed mutations conferring resistance against one or more classes of ARV.

Conclusion: We evidenced high levels of primary ARV resistance mutations in Chad, but lower than those observed in patients with failure to first-line ARV.

Keywords: HIV; Chad; Non-Nucleoside Reverse Transcriptase Inhibitor; primary resistance.

Introduction:

Access to Highly Active Antiretroviral drugs (ARV) has significantly reduced Human Immunodeficiency Virus (HIV) transmission in sub-Saharan African countries which still support the highest burden of the pandemic [1]. However in these countries, access to free but discontinuous first-line antiretroviral regimens consisting almost exclusively of Nucleoside Reverse Transcriptase Inhibitors (NRTI) combined with a Non Nucleoside Reverse Transcriptase Inhibitors (NNRTI) with low genetic barrier to resistance could rapidly lead to the emergence of antiretroviral drugs resistance among ARV-treated patients. In low-income countries of sub-Saharan Africa, monitoring of HIV plasma viral load that is recommended by World Health Organization (WHO) since 2013 and genotyping tests allowing the detection of HIV strains harbouring resistance mutations to antiretroviral drugs are not continuously available. Horizontal or vertical transmission levels of such HIV strains harbouring major resistance mutations to NNRTIs or NRTIs represent a major public health problem and may significantly impact subsequent choices of antiretroviral drugs regimen for newly HIV infected patients in sub-Saharan African countries.

Located in Central Africa, Chad had an estimated HIV seroprevalence of 1.3% among the sexually active population (15-49 years) and ranked fifth in terms of HIV mortality rate (71 per 1000) [2,3]. Moreover, Chad is partly bordered by Cameroon, where a wide diversity of HIV strains has been evidenced [4]. Thus, Chadian HIV strains could present a great viral genetic diversity including non M variants of HIV-1, group M non B subtypes or recombinant strains potentially carrying mutations conferring resistance to antiretroviral drugs used as first or second lines of treatment [5,6]. Only two previous investigations reported high rates of viral mutations conferring resistance to antiretroviral drugs in Chadian patients with virological failure [5,6]. To date, no published data concerning ARV primary resistance

mutation rates in Chad are available. In the present report, we retrospectively investigated frozen dried blood spot (DBS) samples from HIV-1 Chadian patients who were naïve of ARV.

Patients and methods

From August to November 2012, 48 successive newly HIV-1 seropositive adult patients who were naïve of any ARV treatment (30 F/18 M, median age 32years [18-52]) and who were attending to the chronic diseases centre of the hospital "le Bon Samaritain" (N'Djamena, Chad) were enrolled in the present investigation. World Health Organization HIV disease Clinical stage has been defined elsewhere [7]. Median Lymphocytes T-CD4 cells count performed on FACS® count system was 273 cells per mm3 [13 - 1049] for these 48 patients. An oral informed consent for medical research investigations was obtained from each study patient and if needed from their relative family members. This study was approved by local ethics committee. For each patient, blood samples were collected before ARV and sent abroad ("Université" Jean Monnet, Saint-Etienne, and "Université" Reims Champagne Ardennes, France) as dried blood spots (DBS) after approximately one month of storage at room temperature [8,9]. Each DBS sample was then stored at -80°C until performing HIV-RNA viral load and genotyping assays in France according to previously described protocols [8,9]. Protease gene and reverse transcriptase gene region were fully covered by sequencing analysis and obtained sequences were compared against referenced subtype B HBX-2 strain (Genbank accession number: K03455.1). All of our HIV-1 sequences were submitted to the Genbank and obtained an original accession number (Table 1).Concerning drug resistance mutations, interpretation of sequencing results was performed according to the French National Agency for AIDS Research resistance algorithm [10]. HIV-1 subtypes were established using the Stanford HIV database and Sierra version 1.1 HIVdb 8.1.1 [11]. Quantitative variables were compared using the Mann Whitney U-test and qualitative

variables were compared using Pearson's Chi-square test. Statistical analyses were performed using Stat view 5.0 software (SAS institute).

Results

Following RNA extraction from DBS [8], median HIV-1 viral load was estimated to 4.72 log copies/ml [3.24- 5.95] for 40 (83.3%) out of the 48 study patients with a detected viral load upper than 1000 HIV-RNA copies per ml. HIV-1 protease and reverse transcriptase genes were successfully amplified and sequenced for only 24 (60.0%) of the 40 patients with a detectable viral load (Table 1). Median HIV-1 viral load and median CD4 cells counts were respectively statistically higher and lower in patients who had successful sequences than in those that did not (Table 2).

Interestingly, 7 (29.2%) of the 24 successfully sequenced DBS samples exhibited referenced nucleotide mutations conferring HIV-1-resistance against at least one antiretroviral drug. The most frequently detected mutation was the V106I (20.8%) described as a natural polymorphism in HIV-1 non-B subtypes and conferring resistance to etravirine when it is associated to at least one other mutation, according to French National Agency for AIDS Research resistance algorithm [9]. The K103N mutation that confers complete resistance to efavirenz and nevirapine was found in 2 subjects (8.3%) out of the 24. One K65E and one V179D were also detected in two distinct individuals conferring resistance to tenofovir and rilpivirine, respectively. The L90M major protease inhibitor resistance mutation was found in one patient (4.1%). Polymorphic mutations (L10I, G16E, K20I, M36I, I62V, V77I, L89M) were evidenced in 21 sequences (87.5%). HIV-1 protease sequences predicting phenotypic resistance to saquinavir and a combined resistance to nelfinavir, indinavir and atazanavir were evidenced in 3 patients (12.5%).

The most prevalent HIV-1 subtype was Circulating Recombinant Form (CRF) CRF11_CPX subtype and was identified in 7 of the 24 samples (29,2%). Others previously described HIV recombinant forms accounted for 8 out of the 24 samples (33.3%) (Table 1).

All patients were treated by NRTI plus first generation NNRTI (such as efavirenz or nevirapine). Among these 24 patients, 4 died (16.6%) (None among those with mutations leading to resistance or possible resistance to NNRTI), and 14 (58.3%) were lost to follow-up which is consistent with the high attrition rates previously observed in Chad [12].

Discussion

In the present report we retrospectively analysed frozen-stored DBS sampled from a series of HIV-1 seropositive patients who were naïve of Highly Active Antiretroviral drugs (ARV) and were living in Chad (Ndjamena), 2012. All of these patients were newly HIV1-diagnosed and were sampled before initiation of ARV consisting of NRTI and NNRTI combination. We evidenced a CRF11_CPX HIV subtype predominance in accordance with a previously published investigation on Chadian HIV-1 infected patients [5]. Because this previous study focused only patients with detectable viral loads after 6 months of ARV, the reported rates of mutations conferring resistance to at least one antiretroviral drug was high and estimated to 64% [5]. In 2018 Keita et al. observed in plasma samples from newly HIV-diagnosed Malian patients a significant increase of the ARV primary resistance mutation rates evolving from 7.8% in 2010 to 17.5% in 2014, especially mutations conferring resistance to NNRTI like K103N mutation or other natural polymorphic mutations conferring resistance or potential resistance to etravirine, another NNRTI drug not yet currently used in Mali [8]. In the present retrospective monocentric study including a limited series of patients, we showed for the first time high rates of similar ARV primary resistance mutations (29.2%) in Chad,

whose levels were lower than those previously observed in Chadian patients (36-64%) displaying a failure to first-line ARV regimen [5,6].

Our HIV genotyping results could be due to the combined presence of viral RNA and DNA in DBS and the detection of archived mutations currently not detectable by classical genotypic test on plasma RNA. It has been shown that high levels of HIV-1 DNA can induce falsely positive results for the detection of HIV-RNA resistance mutations in about 35% of cases on DBS [13]. However, even if genotyping assays efficiency declined after DBS storage at room temperature [14], the use of DBS offers the advantage of a stable and easy to be transported samples with a minimal biohazard risk. Moreover, sending collected blood samples abroad as DBS for further genotyping analysis could be the most reliable way to monitor resistance to antiretroviral drugs because of recurrent blackouts and material/reagent stockouts in low-income countries such as Chad [5,6]. Samples to send abroad could be randomly selected in case of global monitoring of circulation of strains harbouring resistance or individually selected in suspected virological failure cases. In the absence of available HIV-1 RNA load monitoring for each patient, absence of rise of lymphocyte T-CD4 count under ARV is commonly used as a proxy for virological failure in central Africa. In this situation, first line ARV using NRTI plus NNRTI combination is empirically switched to a second line treatment with Protease Inhibitor without waiting for of HIV RNA load results or genotyping analysis performed abroad.

Taking into account the few previous published Sahelian reports and our present original results, we suggest that free-access to new cheap antiretroviral drugs with high genetic barrier to resistance should be prioritized over any implementation strategy of HIV genotyping assays in low-income central Africa countries such as Mali or Chad. Dolutegravir containing regimen could be a good candidate for new first-line ARV regimen in these low-

income countries, it could be used in association with rifampin and first line anti-tuberculous agents as well as in pregnant women [15]. Dolutegravir containing regimen is now recommended by WHO [15], but to date this ARV drug is not yet in full free-access for the population of Chad.

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Conflicts of interest/Competing interests: none to declare

Ethics approval: not required in this non interventional study

Consent to participate: all patients were informed of the study and gave oral consent for the analysis of their samples abroad.

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Table caption:

Table 1: **Virological data obtained from frozen-stored dried blood spot samples of 24 Chadian HIV-1 seropositive patients naïve of Highly Active Antiretroviral Drugs (ARV).**

1

Patient number	Viral load (log/mL)	HIV-1 subtype	Protease gene mutations	Reverse transcriptase gene mutations	Drug resistance	Possible Drug resistance	Genbank reference
1	4.57	**CRF11_CPX**	I62V, V77I				MW250374
2	4.44	**B**		K65E, V75E, I135V, M164L, I178M		**TDF**	MW250369
3	3.82	**CRF11_CPX**	G16E, V77I				MW250377
4	4.71	**G**		K73EQ, K122P, I142V, K173T, Q174K, D177E, E174D, V292I, D324E, I329V			MW250370
5	4.28	**CRF45_CPX**	K20I, L24F, M36I, L89M	L74H, V179D, K238N, V179D		**RPV, EFV, NVP**	MW250372 (P) MW250373 (RT)
6	4.37	**A1**	L90M, L10I, M36I, L89M		**NFV**	**SQV, IDV, ATV**	MW250375
7	4.02	**CRF13_CPX**	K20I, M36I, V77I, I50N				MW250376
8	5.26	**CRF11_CPX**	L10I, M36I, V77I, L89M				MW250371
9	4.78	**CRF11_CPX**	L10I, M36I, V77I, L89M				MW250353
10	3.63	**D**	K20R, M36I	V106I			MW250354
11	4.61	**CRF45_CPX**	K20X,				MW250356

			M36I, L89M				
12	5.36	**D**	K20I, M36I, V77I, L89M	V106I			MW250355
13	4.73	**CRF45_CPX**	L10I, K20I, M36I, L89M			**SQV**	MW250357
14	4.99	**CRF13_CPX**	K20I, M36I, L89M	V106I			MW250358
15	4.97	**D**	M36I, I62V	V106I			MW250359
16	5.79	**D**	K20R, M61	V106I			MW250360
17	5.70	**D.G**	K20I, M36I, I62V, L89M			**SQV**	MW250361
18	5.43	**G**					MW250368
19	5.75	**CRF11_CPX**	I62V, V77I, L89I				MW250362
20	5.56	**CRF02_AG**	K20I, M36I, L89M	K103N	**EFV, NVP**		MW250363
21	5.47	**CRF11_CPX**	I62V, V77I				MW250364
22	4.38	**CRF13_CPX**	K20I, M36I, V77I				MW250365
23	5.49	**CRF02_AG**	K20I, M36I, L63P, L89M				MW250366
24	5.95	**CRF11_CPX**	I62V, V77I	K103N	**EFV, NVP**		MW250367

Table 2 : **Differences between patients with successfully amplified sequences than those without. *** The 8 patients with indetectable viral load were considered as missing data among the viral loads of patients without successfully amplified sequences.

	Patients with successfully amplified sequences (n=24)	Patients without successfully amplified sequences (n=24)	P
Male sex (n%)	11 (45.80)	7 (29.10)	0.23
Median Age [range] (years)	33 [20-45]	30 [18-52]	0.23
Median World Health Organization HIV disease Clinical stage [range]	3 [1-4]	2 [1-4]	0.15
Median CD4 Cells counts [range] (/mm3)	230 [22-1049]	415 [13-918]	0.01
Median viral load [range] (log copies/ml)	4.87 [3.63-5.95]	4.03 [3.24-4.99]*	0.003