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ABSTRACT

Background: Little is known about the HIV-1 integrase resistance in CNS. This study aimed to evaluate integrase resistance in CSF, as a marker of CNS, and compare it to the HIV resistance in plasma.

Methods: The HIV integrase was sequenced both in plasma and CSF for 59 HIV-1 patients. The clinical and biological data were collected from clinical routine care.

Results: Among the 59 HIV-1 patients, 32 (54.2%) were under antiretroviral (ARV) treatment. The median (IQR) HIV-1 RNA in viremic patients was 5.32 (3.85-5.80) and 3.59 (2.16-4.50) versus 4.79 (3.56-5.25) and 3.80 (2.68-4.33) in CSF log_{10} copies/mL for ARV naïve and treated patients, respectively. The patients were mainly infected with non-B subtypes (72.2%) with the most prevalent recombinant form CRF02_AG (42.4%). The HIV-1 integrase sequences presented resistance mutations for 9/27 (33.3%) and 8/32 (25.0%) in CSF for ARV naïve (L74I n=3, L74I/M n=1, T97A n=1, E157Q n=4) and treated (L74I n=6, L74M n=1, 1 T97A n=1, 1 N155H n=1) patients, respectively. Integrase resistance mutations in CSF were similar to that in plasma, except for 1/59 patients.

Conclusions: This work shows similar integrase resistance profiles in CNS and plasma in a population of HIV-1 viremic patients.

INTRODUCTION

The introduction of highly active antiretroviral (ARV) therapy has decreased the opportunistic infections and mortality of people living with HIV infection, and then enhanced their health and quality of life. The integrase strand transfer inhibitors (INSTIs) are one of the latest class of ARV drug approved for use as a part of ARV therapy to control the HIV infection. Current HIV guidelines recommend INSTIs in the treatment of naïve or previous ARV treated HIV-1 patients in a 3 or 2 drug regimens (US Food and Drug Administration, February 2018, and the European Medicines Agency, June 2018), and their use is growing worldwide.

However, despite simplifying and/or well tolerated regimens, suboptimal treatment adherence, possible drug-drug interactions, ARV sub-penetration in compartments could lead to virological failure and ARV resistance. [1–3] For example, the HIV infection of the CNS could be established during primary infection and be persistent in untreated patients. [4,5] For ARV treated patients, an HIV positive viral load in the CNS could be detected in case of viral replication in peripheral and viral crossing into the blood brain barrier, or if the drug penetration may be suboptimal in this compartment. [6–8]

Until now, little is known about INSTIs resistance in the CNS. We aimed to describe the INSTIs resistance in the CSF, as a representation of the CNS, in comparison with the circulating HIV-1 in plasma.

METHODS

Patients

All CSF–plasma pairs with HIV-1 integrase sequences between 2008 and 2020 in Pitié-Salpêtrière Hospital (Paris, France) were studied. The lumbar puncture is reserved for people affected by severe cognitive impairment without etiological orientation and were part of routine clinical care. Socio-demographic and clinical data as well as treatment regimen were collected for all studied patients using the computerized medical chart NADIS® for which all patients provided signed consent.

Genotyping resistance testing

The integrase resistance mutations were determined using the ANRS consensus technique (http://www.hivfrenchresistance.org) and were interpreted with SmartGene® according to the last ANRS (Agence Nationale de Recherche sur le Sida et les hépatites virales- maladies émergentes - version 30) or Stanford HIVDB 8.9.1 algorithm.

Phylogenetic analysis

The HIV-1 integrase sequences were aligned with seaview software (http://doua.prabi.fr/software/seaview). The PhyML software was used for maximum-likelihood phylogenetic reconstruction (Generalized time-reversible model). The reliability of tree topologies was assessed by bootstrapping using 500 replications. Maximum-likelihood trees were rooted on an outgroup: HIV subtype B consensus (HxB2: K03455; www.hiv.lanl.gov). Tree figures were viewed and modified with FigTree software (http://tree.bio.ed.ac.uk/software/figtree/).

Statistical methods

Quantitative variables were summarized by medians and IQR and qualitative variables by percent.

RESULTS

Overall, 59 HIV-1 patients with a median age of 44 (IQR 36-52) (27 ARV non- treated including 21 naïve; 32 ARV treated) were analyzed (table 1). Among the treated patients, 22/32 received an ARV treatment containing an INSTI. The median (IQR) HIV-1 RNA in viremic patients was 5.32 (3.85-5.80) and 3.59 (2.16-4.50) versus 4.79 (3.56-5.25) in plasma and 3.80 (2.68-4.33) in CSF log_{10} copies/mL for ARV non-treated and treated patients, respectively (table 1). The patients were then mainly infected with non-B subtypes (72.2%) with the most prevalent recombinant form CRF02_AG (42.4%). The nadir and CD4 cell counts were in median (IQR) 46 (16-20) and 113 (29-274) cells/mm^3, respectively (table 1).

For ARV-non treated patients, 9/27 (33.3%) have HIV-1 with integrase resistance mutations in both plasma and CSF: L74I (n=3), L74I/M (n=1), T97A (n=1), E157Q (n=4) (table 2). For ARV-treated patients, 8/32 (25.0%) and 9/32 (28.1%) presented HIV-1 with integrase resistance mutations in CSF and plasma, respectively. The mutations were as follows: L74I (n=6), L74M (n=1), 1 T97A (n=1), 1 N155H (n=1) both in plasma and CSF. Only 1 E138K, associated to L74I, was present only in plasma for one patient (table 2).

Overall, the resistance for integrase inhibitors represented 11.8% and 13.5% in CSF and plasma according to the ANRS algorithm, respectively. In details, the integrase inhibitor's resistance was 18.5% for non-treated patients and 6.2% and 9.4% in ARV treated patients in CSF and plasma, respectively. According to the 8.9-1 version of Stanford University drug resistance database, only the two ARV-treated patients with HIV-1 major integrase inhibitor resistance mutations were considered resistant to INSTI, in plasma (E138K) and both in CSF and plasma (N155H).

Phylogenetic analysis was processed in all patients for the paired CSF-plasma integrase genes. The median of the genetic distance was 0.0 (IQR 0.0-0.54) and below 5.1% for all patients except for one (13.8%). This patient had HIV-1 integrase sequences without resistance mutations.

DISCUSSION

This study compared for the first time the integrase resistance profile for HIV patients in plasma and in CSF, and evidenced no supplementary integrase resistance mutation in CSF in comparison with plasma, except for 1/59 patients.

Overall, the presence of integrase resistance mutations and integrase inhibitor's resistance were similar in this study between the CNS and plasma, despite the CNS could be a sanctuary for HIV replication. [6] Our results suggested that it was probably the same HIV due to a cross of the virus through the drug-resistant blood brain barrier as all patients had a detectable HIV plasma viral load. The phylogenetic analysis concurred to this hypothesis as no noticeable difference was evidenced between the HIV-1 integrase of plasma and CSF. These patients had probably adherence issue, possible drug-drug interactions that limited the treatment efficacy. It should be also interesting to study integrase resistance in some HIV patients that have discordant HIV viral load, with an undetectable HIV viral load in plasma and a detectable HIV viral load in CSF.

The most commonly observed integrase resistance mutations were L74I and E157Q. The L74I was reported as natural polymorphism in naïve HIV patients for integrase inhibitors. [9] The E157Q substitution has been previously described as a polymorphism present in around 5% of HIV obtained from ART-naive patients. [10–12] In our study, the presence of E157Q was higher (14.8%) probably linked to the non-B subtype, especially the CRF02_AG. Indeed, this substitution could be present three more times in this subtype than in B subtype. [10] It could be considered that these major resistance substitutions were polymorphism and not acquired resistance mutations.

Resistance to at least one ARV anti-integrase drug was quite similar in this study for HIV ARV treated patients to a 2014 French nationwide study (9.4% versus 12.0%, respectively). [13] On the contrary, the proportion of integrase inhibitor's resistance in non-treated HIV patients was superior compared to the ARV-naïve chronically HIV infected patients in 2015/2016 in France. [12] It could be explained by the difference of non-B subtype's proportion in the two studies (72.2% versus 54.8% in the present and in the previous nationwide study, respectively), involving a possible increase of natural integrase polymorphism linked to non B-subtypes. [14] The higher prevalence of non-B subtypes in our study could be explained by the continuation of the diagnosed non-B subtypes increase already observed between 2010 and 2016 in France. [12] Another hypothesis was that HIV patients evaluated in this study requiring a CSF genotype because of their neurological symptoms were mostly late presenters infected with non-B subtypes.

Our study has some limitations as the small number of the subjects and the heterogeneity of this population, as naïve, no treated and ARV-treated patients, and for this last category, the heterogeneous ARV treatment including or not an INSTI. This is in relation with the observational status, the low practice and need of lumbar punction and the long period of time study. Indeed, the ARV treatment recommendations have been evolved during this time period. Furthermore, it is noticed that the lumbar punction was performed at different time of the disease, at the HIV diagnosis or after several years of the disease.

In conclusion, we showed similar integrase resistance profiles in CNS and plasma in this population of viremic patients. Two predominant integrase substitutions were observed probably due to polymorphism possibly linked to non-B subtypes. This work suggested to perform lumbar punctures only in the presence of events contributing to potential viral rebounds in CSF, therapy failure or ART interruption.

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TRANSPARENCY DECLARATION

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	Total (n=59)	ARV non-treated Patients (n=27)	ARV treated Patients (n=32)
Age, years median (IQR)	44 (36-52)	42 (35-48)	48 (37-53)
Male, n (%)	35 (59.3)	15 (55.5)	20 (62.5)
B subtype, n (%)	17 (28.8)	9 (33.3)	8 (25.0)
Time since HIV diagnosis, months median (IQR)	96.4 (0.0-187.5)	1 (0.3-145.1)	122.0 (40.2-212.6)
CSF HIV-1 RNA, log_{10} copies/mL median (IQR)	4.2 (3.13-4.95)	4.79 (3.56-5.25)	3.80 (2.68-4.33)
Plasma HIV-1 RNA, log_{10} copies/mL median (IQR)	4.71 (3.14-5.44)	5.32 (3.85-5.80)	3.59 (2.16-4.50)
Plasma zenith HIV-1 RNA, log_{10} copies/mL median (IQR)	5.70 (5.09-6.34)	5.72 (5.27-6.20)	5.65 (4.98-6.38)
Nadir CD4, cell count/mm^3 median (IQR)	46 (16-120)	38 (15-117)	66 (18-121)

CD4, cell count/mm^3 median (IQR)	113 (39-274)	45 (21-118)	188 (79-426)
CD8, cell count/mm^3 median (IQR)	664 (406-847)	543 (297-805)	719 (442-917)
Total time under ARV treatment, weeks median (IQR)	423 (89-789)*	461 (220-669)*	423 (51-848)
Time under current ARV treatment, weeks median (IQR)	15 (5-56)		15 (5-56)
Current treatment, %			
- **NRTIs+Pis**	6 (10.2)		6 (18.7)
- **NRTIs+PIs+MVC**	3 (5.1)		3 (9.4)
- **NRTIs+INIs**	16 (27.1)		16 (50.0)
- **PIs + INIs**	3 (5.1)		3 (9.4)
- **NRTIs + Pis + INIs**	2 (3.4)		2 (6.3)
- **Pis**	1 (1.7)		1 (3.1)
- **NRTIs + NNRTIs + INIs**	1 (1.7)		1 (3.1)

Table 1: Characteristics of patients

*6 patients of the ARV non-treated group at time of the HIV genotypes have received ARV treatment in the past.

Patient	Age	Sex	Time since HIV diagnosis	Total time under ARV treatment (duration of the cessation of treatment)	Time under current ARV treatment	Integrase resistance mutation CSF	Integrase resistance mutation Plasma	CSF HIV VL	Plasma HIV VL	Zenith HIV VL	Nadir	CD4+	CD8+	Subtype	ARV
1	31	F	0			L74I/M	L74I/M	5.3	6.0	6.0	46	79	664	CRF02_AG	naive
2	49	F	970	17 (326)		L74I	L74I	4.7	2.5	6.8	31	99	812	A	no
3	42	M	379			L74I	L74I	4.8	5.6	5.3	4	20	142	CFR02_AG	naive
4	24	F	350			L74I	L74I	6.3	5.9	6.1	10	24	244	A	naive
5	54	M	2			T97A	T97A	4.4	3.7	3.7	46	46	692	B	naive
6	47	M	820			E157Q	E157Q	3.4	5.2	5.6	9	9	285	B	naive
7	33	F	791	667 (116)		E157Q	E157Q	4.8	4.7	5.9	263	334	1424	CFR02_AG	no
8	25	F	4			E157Q	E157Q	5.1	5.4	5.4	205	205	228	CFR02_AG	naive
9	45	F	1			E157Q	E157Q	3.9	5.1	5.0	nd	nd	nd	CR02_AG	naive

10	54	M	384	226	46	L74I	L74I	4.5	3.6	5.0	16	195	1104	CRF02_AG	RAL+RTV+DRV+3TC
11	52	F	1263	1055	285	L74I	L74I	5.3	5.8	5.8	122	nd	nd	A	FTC+TDF+LPV+RTV
12	73	M	1137	1059	2	L74I	L74I	2.6	4.0	6.6	25	64	813	CRF02_AG	RTV+DRV+FTC+TDF
13	42	M	420	420	62	L74I	L74I	2.7	1.7	5.4	10	209	407	CRF011_cpx	RTV+ATV+DTG
14	49	M	485	485	15	L74I	L74I	2.8	2.6	2.8	174	174	641	CRF02_AG	LPV+RTV+3TC+ZDV
15	58	M	17	14	8	L74I	L74I, E138K	2.4	3.1	5.9	391	628	466	A	DTG+ABC+3TC
16	39	F	625	601	9	L74M	L74M	2.1	1.9	6.1	237	919	1081	CRF02_AG	RTV+TDF+FTC+ATV
17	52	M	381	202	70	T97A	T97A	4.2	5.5	6.3	7	14	754	B	RAL+ABC+3TC+MVC
18	36	F	577	577	50	N155H	N155H	4.5	6.1	6.1	39	39	347	CRF02_AG	RAL+RTV+ATZ

Table 2 : Characteristics of HIV patients with HIV integrase resistance mutations.

VL : Viral Load; nd: not done

Time since diagnosis, total time under ARV treatment and Time under current ARV treatment are expressed in weeks. The HIV viral loads were expressed as log_{10} copies/mL; The CD4. CD8 and nadir were expressed as cells/mm^3.