Transmitted Antiretroviral Drug Resistance in Newly HIV-Infected and Untreated Patients in Ségou and Bamako, Mali

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Abstract

The WHO recommends regular surveillance for transmitted antiretroviral drug-resistant viruses in HIV anti-retroviral treatment (ART)-naïve patients in resource-limited settings. This study aimed to assess the prevalence of mutations associated with resistance in ART-naïve patients newly diagnosed with HIV in Bamako and Ségou in Mali. HIV-positive patients who never received ART were recruited in Bamako and Ségou, Mali. The reverse transcriptase (RT) and protease (PR) genes of these patients were sequenced by the “ViroSeq” method. Analysis and interpretation of the resistance were made according to the WHO 2009 list of drug resistance mutations. In all, 51/54 (94.4%) sample patients were sequenced. The median age (IQR) of our patients was 24 (22–27) years and the median CD4 count was 380 (340–456) cells/mm³. The predominant subtype was recombinant HIV-1 CRF02_AG (66.7%) followed by CRF06_cpx (12%) and CRF09_cpx (4%). Four patients had mutations associated with resistance, giving an overall prevalence of resistance estimated at 7.9%. There were two (4%) patients with nucleoside reverse transcriptase inhibitor (NRTI) mutations (one M184V and one T215Y), two (4%) with non-NRTI mutations (two K103N), and one (2%) with a protease inhibitor mutation (one I54V). The prevalence of primary resistance in newly infected patients in Mali is moderate (7.9%). This indicates that the standard NNRTI-based first-line regimen used in Mali is suboptimal for some patients. This study should be done regularly to inform clinical practice.

Introduction

THE HIV PREVALENCE in Mali was 1.6 and 1.3% in 2003 and 2006, respectively.¹ In low and middle-income countries (LMIC), only a few people had access to antiretroviral (ARV) drugs until the past few years. The introduction of generic fixed dose drugs (like Triomune, which is largely used in Mali) and other affordable ARV combinations through local and international efforts has led to a significant increase in the number of ARV-treated patients. The most dramatic increase in antiretroviral therapy coverage occurred in sub-Saharan Africa, approximately a 20% increase between 2009 and 2010 alone. In LMIC, 47% of the 14.2 million treat-

ment eligible people living with HIV were on antiretroviral therapy at the end of 2010, compared to 39% at the end of 2009 (World AIDS Day Report 2011).

HIV treatment started in Mali in 1997 with few patients, but by the end of 2011 more than 35,000 patients were on treatment. Many studies performed in LMIC showed that patients who failed first-line and second-line regimen harbored considerable resistance mutations, especially the thymidine analog mutations (TAMs). A recent study from second-line failure patients in Mali showed that 8/94 (8.6%) harbored multiresistant virus to all drugs available in Mali.² Those patients can infect new patients with virus harboring drug resistance mutations.

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The World Health Organization (WHO) recommends HIV drug resistance surveillance among ARV-naïve patients for all countries involved in ARV access programs. Previous studies reporting on ARV primary resistance from chronically infected, ARV-naïve patients in Burkina Faso,³ Côte d'Ivoire,^{4,5} Mali,^{6–8} Mozambique,⁹ Uganda,¹⁰ Rwanda,¹¹ and Zambia¹² showed a low prevalence of ARV drug resistance, about or below 5%. Recent data published from Niger and Guinea-Conakry showed that the prevalence of transmitted virus with resistance mutation was higher: 6% in Niger and 9% in Guinea Conakry.¹³ In 2008, a study in Mali showed a prevalence of 2.5% and 1% for nonnucleoside reverse transcriptase inhibitor (NNRTI) and nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations, respectively.⁶ No protease inhibitor (PI) resistance mutation was found. The last survey was done in 2009 and reported a primary resistance prevalence of 9.9%, but that study considered many minor resistance mutations.⁸ The aim of the current study was to assess the prevalence of antiretroviral drug resistance mutations in HIV-1 from recently diagnosed and untreated patients living in Bamako and Ségou, Mali, 13 years after ARV introduction in the country.

Materials and Methods

The study was performed in two districts in Mali, Bamako and Ségou, using the same survey method in both sites. According to WHO guideline for HIV drug resistance threshold, all newly HIV-1 diagnosed patients at voluntary counseling and testing centers in March 2010 were consecutively included. We modified the criteria of patient selection, and we selected men and women more than 18 years old. The counseling and testing sites were located in CESAC (Centre d'Ecoute, d'Animation et de Conseil) in Bamako, USAC Commune V (Unité de Soins de l'Animation et de Conseils) in Bamako, and the Nianankoro Fomba Hospital (HNF) in Ségou.

Protease (PR) and reverse transcriptase (RT) sequencing was performed using the Viroseq method (Abbott and Celera Diagnostics). Drug resistance mutations were identified according to the 2009 update on surveillance drug resistance mutations list as follows: (1) NRTIs: D67E/G/N, T69D, K70E/R, L74I/V, V75A/M/S/T, F77L, Y115F, F116Y, Q151M, M184I/V, L210W, T215Y/F/I/S/C/D/V/E, K219E/N/Q/R; (2) NNRTIs: L100I, K101E/P, K103N/S, V106A/M, V179F, Y181C/I/V, Y188C/H/L, G190A/E/S, P225H, M230L; and (3) PIs: L23I, L24I, D30N, V32I, M46I/L, I47A/V, G48M/V, I50L/V, F53L/Y, I54A/L/M/S/T/V, G73S73A/C/T, L76V, V82A/C/F/L/M/S/T, N83D, I84A/C/V, I85V, N88D/S, L90M.¹⁴ Phylogenetic analyses were performed by estimating the relationships among RT and PR sequences and reference sequences of HIV-1 genetic subtypes and circulating recombinant forms (CRF) obtained from the Los Alamos Database (<http://hiv-web.lanl.gov>). Phylogenetic reconstruction was performed using a Kimura two-parameter model and the neighbor-joining method.

Results

Study population

We included 54 patients in this study; 22 patients were from CESAC, 21 from HNF, and 11 from USAC commune V. Clinical data were available for all patients. Only samples

from 51 patients were amplifiable. Of these, 32 were women and 19 men. The median (IQR) age was 24 (22–27) years and the median (IQR) CD4 cells count was 380 (340–456) cells/mm³ (Table 1). All analyses were performed on the 51 samples.

Subtype distribution

Three samples did not amplify and hence were not sequenced. Phylogenetic analyses showed that most of the patients, 34 (66.6%), were infected with CRF02_AG recombinant virus. The subtype distribution of the remaining patients was as follows: six (12%) CRF06_cpx, two (4%) CRF09_cpx, one (2%) CRF05_DF, one (2%) subtype F-2, one (2%) recombinant CRF1501_B, and six (12%) were not clustered with any of the known subtypes in the phylogenetic analysis. These viruses displayed *pol* gene mosaics, involving different subtypes. Each of the subtypes CRF01_AE and A-1 were present in five of six isolates. The subtype distribution is shown in Fig. 1A and the unknown subtype is classified in Fig. 1B.

Resistance mutations

Resistance analysis among the 51 samples showed at least one drug resistance mutation in four samples, leading to a primary resistance prevalence of 7.8% [95% confidence interval (CI 95), 2.2–18.9%]. All four patients who harbored resistant viruses were infected with the CRF02_AG subtype. One patient from Ségou (MLRP07) harbored a resistance mutation and the three other patients who were harboring mutations were from Bamako. NRTI resistance mutations were found in two samples (3.9%; CI 95, 0.5–13.5%), T215Y mutation in one patient and M184V in another patient. NNRTI resistance mutations were detected in samples from two patients (3.9%; CI 95, 0.5–13.5%), both exhibiting the K103N mutation. A major PI resistance mutation (I54V) was present in one patient (2%; CI 95, 0.00–10.4%); the same patient had the M184V mutation, implying dual class resistance.

Discussion

Here, we report a 7.8% (2.2–18.9%) prevalence of primary drug resistance mutations in recently diagnosed, ARV-naïve HIV-infected patients from Mali in 2010. This shows that HIV-1 isolates exhibiting ARV resistance mutations are circulating among treatment-naïve patients in Mali. We hypothesize that the level of resistance observed in the current study underestimates primary resistance in the population since minority resistant variants, which are undetectable by conventional genotyping, were not assessed in our study. Studies have shown that some minority resistant variants have clinical implications; in particular, NNRTI-resistant variants in minority proportions may impact virological response to an NNRTI-based regimen.^{15,16}

Our study fulfilled almost all the criteria recommended in the WHO HIV drug resistance (HIVDR) threshold survey method.¹⁴ Specifically, the median age of the patients in our study was 24 years, consistent with the HIVDR recommended threshold of under 25 years. In addition, as recommended by the WHO: (1) patients were consecutively included, (2) the number of samples was >47, and (3) there was no known exposure to ARV drugs, including PMTCT in pregnant women. Furthermore, the survey was conducted in small

TABLE 1. PATIENTS DESCRIPTION WITH SOCIODEMOGRAPHIC AND BIOLOGICAL DATA

Patient ID	Sex	Age (years)	CD4 cells/mm ³	HIV-1 subtypes	Resistance-associated mutation	Drug which mutations confer resistance
MLRP01	M	24	678	CRF 06_cpx	—	—
MLRP02	M	32	529	CRF 02_AG	—	—
MLRP03	F	21	430	CRF 02_AG	—	—
MLRP04	F	22	348	CRF02_AG	—	—
MLRP05	F	23	352	CRF06_cpx	—	—
MLRP06	F	25	406	CRF 02_AG	—	—
MLRP07	F	26	290	CRF 02_AG	K103N	NVP, EFV
MLRP08	M	27	320	CRF 02_AG	—	—
MLRP09	F	26	340	CRF 02_AG	—	—
MLRP10	F	26	360	CRF 02_AG	—	—
MLRP11	F	28	502	CRF09_cpx	—	—
MLRP12	M	30	498	U	—	—
MLRP13	F	29	620	U	—	—
MLRP14	M	27	270	CRF 02_AG	—	—
MLRP15	F	24	380	CRF 02_AG	—	—
MLRP16	M	22	338	U	—	—
MLRP17	F	21	326	CRF 02_AG	—	—
MLRP18	F	29	512	CRF 02_AG	T215Y	d4t, AZT
MLRP19	F	18	456	CRF 02_AG	—	—
MLRP20	M	19	342	CRF 02_AG	—	—
MLRP21	F	19	368	CRF 06_cpx	—	—
MLRP22	M	20	416	CRF 1501_B	—	—
MLRP23	F	28	412	CRF 02_AG	—	—
MLRP24	F	24	431	F2	—	—
MLRP25	F	24	348	CRF 02_AG	—	—
MLRP26	F	26	422	CRF 06_cpx	—	—
MLRP27	M	25	451	U	—	—
MLRP28	F	23	228	CRF 02_AG	—	—
MLRP29	F	22	359	CRF 02_AG	—	—
MLRP30	F	20	273	CRF09_cpx	—	—
MLRP31	M	22	286	CRF 02_AG	M184V/154V	3TC, FTC/IDV, NFV, LPV, FosAPV
MLRP32	F	25	348	CRF 02_AG	—	—
MLRP33	M	28	321	CRF 02_AG	—	—
MLRP34	F	30	356	U	—	—
MLRP35	M	31	376	CRF 02_AG	K103N	NVP, EFV
MLRP36	F	27	495	CRF 02_AG	—	—
MLRP37	F	22	276	CRF 06_cpx	—	—
MLRP38	M	23	508	CRF 02_AG	—	—
MLRP39	F	24	462	CRF 02_AG	—	—
MLRP40	F	25	365	CRF 02_AG	—	—
MLRP41	M	24	297	CRF 02_AG	—	—
MLRP42	F	28	398	CRF 02_AG	—	—
MLRP43	F	24	394	U	—	—
MLRP44	M	22	402	CRF 05_DF	—	—
MLRP45	M	28	298	CRF 02_AG	—	—
MLRP46	F	31	473	CRF 02_AG	—	—
MLRP47	M	18	356	CRF 06_cpx	—	—
MLRP48	F	20	476	CRF 02_AG	—	—
MLRP49	M	22	511	CRF 02_AG	—	—
MLRP50	F	26	428	CRF 02_AG	—	—
MLRP51	M	23	512	CRF 02_AG	—	—

geographic areas, based in the capital cities where ARV drugs have been widely available for more than 3 years, and available to at least 20% of eligible individuals (ARV coverage is approximately 85% in Mali). The WHO HIVDR criteria recommends ensuring that patients have been HIV infected within the past 3 years, but we could not include this in our study because we lacked tools to precisely determine the date of infection.

The level of primary resistance (7.8%) found in our study is higher than 1.5% and 2.5% reported by Derache and others in 2005 and 2006, respectively.⁶ This raises concerns about the routine use of an NNRTI plus two NRTIs in first-line regimens without resistance testing in Mali because detected resistance mutations can compromise the effectiveness of NRTIs (AZT, d4T, 3TC, FTC) and NNRTIs (NVP and EFV) used in first-line therapy. Primary resistance to indinavir was detected in one

FIG. 1. (A) Graphic representation of subtype distribution in the study population. (B) *Pol* gene mosaic viruses ("unknown, U" viruses) with recombination points and subtypes involved.

patient, but a case could be made for considering a ritonavir-boosted PI containing regimen in some first-line patients, provided barriers such as higher costs and limited PI availability can be overcome. Taken together, our findings emphasize the need to optimize virological control in patients on treatment and expand access to viral load monitoring and resistance testing in resource-limited settings. Given that primary resistance has exceeded 5% in the major cities in Mali, surveillance in other parts of the country is warranted to obtain estimates of the level and evolution of HIV-1 primary drug resistance throughout the country.

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Author Disclosure Statement

No competing financial interests to declare exist.

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