Predominance of CRF06_cpx and Transmitted HIV Resistance in Algeria: Update 2013-2014

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Abstract

Since 2008, no data on HIV diversity or the transmission rate of HIV resistance mutations in naive patients have been presented for Algeria, a country of MENA region. Between 2013 and 2014, we studied 152 samples including 89 naive patients. The current study describes the change in HIV diversity in Algeria with the predominance of CRF06_cpx and the huge increase of transmitted HIV resistance, which now reaches 15%.

Introduction

Algeria, together with 22 countries, belongs to the Middle East and North Africa (MENA) region. Data on the HIV epidemic are sparse in this region, especially for drug users, but recently some countries have increased their HIV surveillance; nevertheless, in others, HIV prevalence data are still not available.[1,2]

According to UNAIDS in 2013[3] there were 230,000 (160,000–330,000) people living with HIV in the MENA region, with an estimated 25,000 (14,000–41,000) new HIV infections, including 2,300 (1,500–3,400) among children. In this area in 2013, 15,000 (10,000–21,000) people died of AIDS-related causes. Treatment coverage is 11% (8–16%) for people living with HIV.

For Algeria,[3] country reports indicated in 2013 that 25,000 people were living with HIV (13,000–43,000) with an adult prevalence rate of 0.1% for 36.5 million inhabitants. According to the Algerian Ministry of Health and Hospital Reform, 8,258 cases were reported in 2013 with 1,460 AIDS cases and 6,798 HIV-positive cases.[4] The number of new infections has been stable for years with 700–800 cases per year. Only 19% of infected people have received antiretroviral (ARV) drugs in national reference centers.

In previous studies[5,6] we observed a high diversity of HIV type 1 in Algeria, with a complicated pattern of subtypes and circulating recombinant forms (CRFs), particularly in the southern part of the country. Although the B subtype was predominant, CRF06_cpx and CRF02_AG were widely represented. Concerning drug resistance mutations, some transmitted mutations to nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs) occurred in untreated patients. In 2008, we did not observe any resistance mutation to nonnucleoside reverse transcriptase inhibitors (NNRTIs) because of the recent introduction of this class of drugs in Algeria. Since 2008, no study has pointed out the diversity of HIV in Algeria or the transmission rate of resistance mutations in naive patients.

Materials and Methods

We studied 152 samples collected between 2013 and 2014 from either untreated or ARV-treated patients under clinical failure of therapy from El Hadi Flici hospital in Algiers. Blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes and plasma was separated and stored at -80°C. One hundred and ten plasma samples were spotted using Whatman 903 paper (Dried Plasma Spot: DPS). Viral RNA was extracted from plasma or DPS. From plasma, RNA extraction was carried out using the High Pure Viral RNA Kit (Roche Diagnostics Systems) and from DPS, RNA extraction was carried out using the Nuclisens kit (BioMe´rieux).

The viral RNA was used in reverse transcription polymerase chain reaction (RT-PCR) of RT and Prot genes followed by a nested PCR, using two sets of primers in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) thermal cycler. The outer and inner primers for RT are described in a previous publication.[7] The fragments obtained were sequenced on both strands using the Big Dye Terminator v 3.1 Cycle Sequencing Kit with a DXL 3500 DX

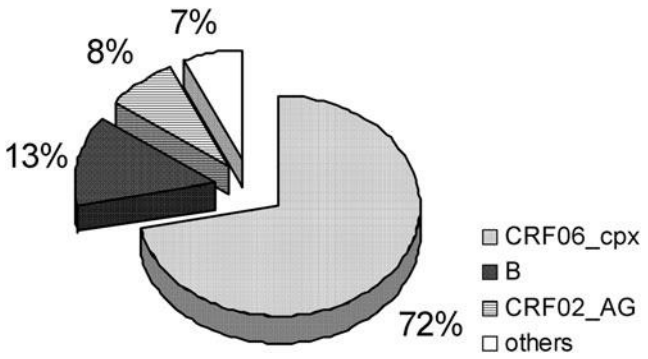

FIG. 1. Subtype distribution of HIV-1-infected patients in Algeria in 2013–2014. ''Others'' represented one A1 subtype, two CRF22_01A1, one CRF07_BC, two recombinant forms BF, one recombinant form BG, and one recombinant form B-CRF02.

sequencer (Applied Biosystem, Courtaboeuf, France). The primers of the nested PCR were used for sequencing the RT and Prot regions. The derived nucleotide sequences of the RT and Prot regions were aligned with Clustal W2 alignment program with known reference strains of M and N pooled from the HIV-1 gene databank (http://hiv-web-lanl.gov/).

Phylogenetic trees were inferred using the neighbor-joining method from matrix distances calculated after gapstripping of alignments, with a Kimura two-parameter algorithm. Phylogenetic trees were visualized using ITOL (Interactive Tree of LIFE) software.[8] The mutations involved in antiretroviral resistance were recorded according to the algorithm of the Agence Nationale de Recherche sur le SIDA (ANRS/France) (http://hivfrenchresistance.org, 2014, version 24). GenBank accession numbers for the sequences reported in this study are KT339064 to KT339174 for RT and KT338945 to KT339063 for Prot.

Results

Among the 152 collected samples, 89 were obtained from naive patients. Their mean age was 38 years (5–81) and the M/W sex ratio was 1.4. The median viral load was 5,017,844 copies/ml (102–100 million). From the 152 samples studied, 124 were amplified, with 119 sequenced on the Prot gene and 110 on the RT gene.

The molecular characterization of the viruses, based on genomic sequence analysis, is presented in Fig. 1. The predominant subtype was largely CRF06_cpx, followed by the B subtype, CRF02_AG, and other non-B strains (A1, recombinant B-CRF02, CRF07_BC, CRF22_01A1, recombinant BF, and recombinant BG).

Figure 2 indicates the proportion of resistance mutations in naive patients. Viruses harboring mutations were present in 28 of 89 samples from naive patients. Some were major

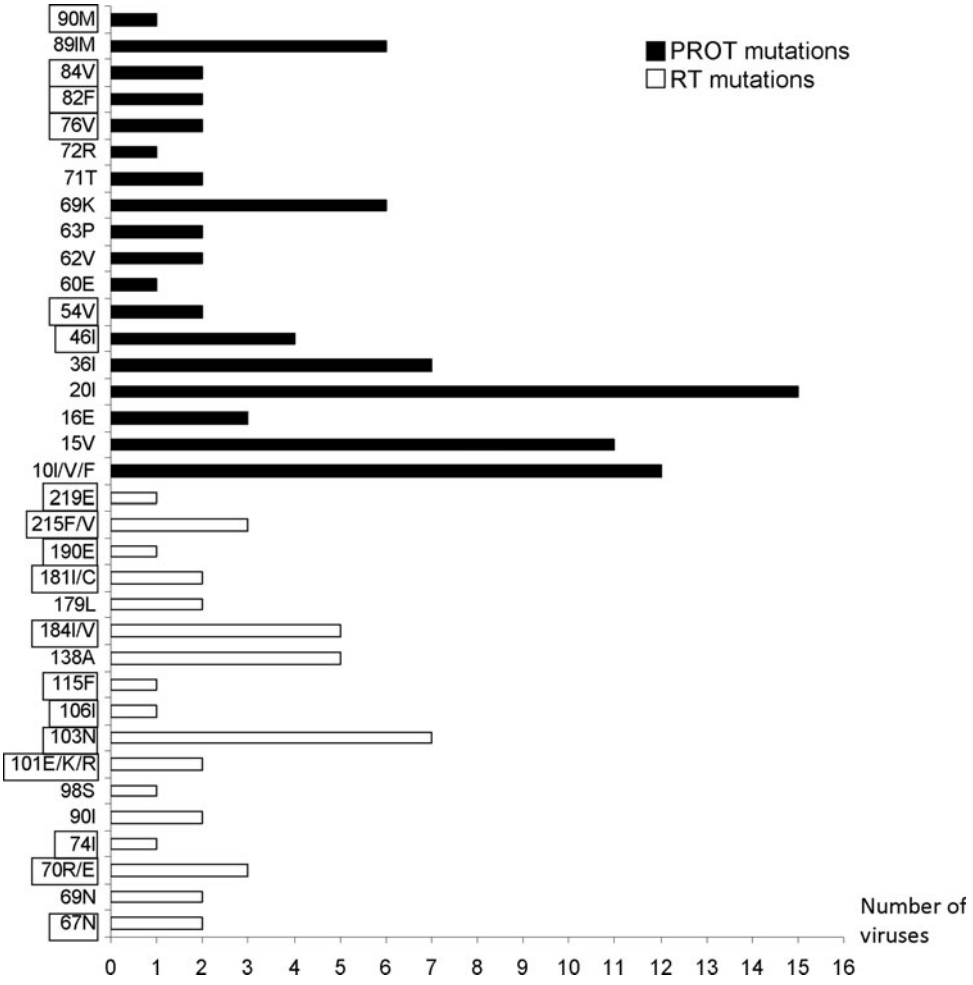

FIG. 2. Number of viruses bearing reverse transcriptase (RT) and protease (PROT) mutations in the 89 naive patients analyzed (Drug Resistance Algorithms, Stanford HIVDB 7.0.1 and ANRS 2014.v24). *Boxed mutations* are listed on the drug resistance mutations for surveillance of transmitted HIV-1 drug resistance.[12]

resistance mutations such as M184V for NRTI, K103N for NNRTI, and V82F for PI. The prevalence of HIV-1 resistance-associated mutations in this study was found to be 31% (according to Drug Resistance Algorithms, Stanford HIVDB 7.0.1 and ANRS 2014.v24). This significant prevalence is similar to that reported in a recent update on HIV-1 transmitted drug resistance in Africa or in other countries.[8–11] However, if we consider the list established by Bennett *et al.*,[12] the percentage of transmitted mutations was 15%.

Discussion

In previous studies published in 2006 and 2008, we observed high HIV-1 diversity in Algeria with a predominance of the B subtype followed by CRF02_AG and CRF06_cpx.[5,6] In the current study, the diversity was maintained, but CRF06_cpx became widely predominant. The evolution of the HIV infection, previously originating primarily in Europe and now from others neighboring countries, surely explains this epidemiological change.

In this study, we observed a prevalence of 31% of HIV-1 resistance-associated mutations, 15% considering the list established by Bennett *et al.*[12]: this was a huge increase compared to the previous analysis.[6] Nevertheless, this result is not surprising and has already been observed elsewhere.[9–11] In Africa, the prevalence of drug resistance mutations varies across countries, reaching 54.9% in Central Africa.[9] In other countries where access to antiretroviral therapy increased for years, such as Portugal or China, the same number of mutations is noted.[10,11] However, these mutations are perfectly correlated with the HIV treatments proposed in Algeria and in all the countries of the MENA region.

These results support the recommendation for epidemiological monitoring of the entire national territory; also recommended is routine resistance testing before the initiation of antiretroviral therapy in Algeria to investigate transmitted drug resistance in naive populations represented by recently infected individuals and/or pregnant women.

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Author Disclosure Statement

No competing financial interests exist.

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