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Evolution of genetic diversity in HIV-1 among infected adult's patients from Côte d'ivoire between 2019 and 2023

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Previous studies of molecular epidemiology for HIV-1 have shown that the genetic diversity of circulating recombinant forms of the virus evolves over time, being influenced by the virus's high capacity for replication and genetic recombination, as well as by population mobility, which is responsible for the spread of new variants in different countries. The aim of this study is to describe HIV-1 variants in Côte d'Ivoire by using sequencing of tree targets; reverse transcriptase, protease and integrase genes of 139 isolates. Participants were recruited in six care centers in Abidjan. Subtypes and recombinant forms were generated using HIVGRAD software (https://www.hiv-grade.de/cms/grade/). Phylogenetic analyses were carried out by the program (https://www.hiv.Lanl.gov/content/sequence/HIV/HIVTools.html). The results showed that CRF02_AG is the majority variant, with a frequency of 84% (61/73), followed by subtypes A, A1 and G, which represented 3% (2/73), 1% (1/73) and 1% (1/73) respectively. 7% (5/73) of the viruses have complex genetic profiles: CRF01_AE/CRF02_AG/A1 (1%); CRF01_AE/A/A1 (1%); CRF01_AE/K (1%); CRF02_AG/A1 (1%); CRF02_AG/F2 (1%) and 4% of strains are unidentified. Although CRF02_AG was the majority variant in the circulating strains, this study revealed a high proportion of complex recombinant forms (7%) and unidentified strains (4%) compared with the results of the study conducted in 2019. The identification of complex strains and unidentified isolates highlights the need to identify HIV-1 subtypes based on whole viral genomes.

Key words: HIV-1, diversity, complex recombinants, variants, Côte d'Ivoire.

INTRODUCTION

HIV-1 has high genetic diversity due to the low fidelity of the reverse transcriptase, the rapid kinetics for replication, genetic recombination and selection pressure (Hu and Temin, 1990). To date, 4 major groups of viruses have

been reported: groups M, N, O and P. Group M is mainly responsible for the global HIV-1 epidemic and is subdivided into 10 different subtypes (A-D, F-H, J-L), with 118 circulating recombinant forms (CRF) and 100 unique recombinant forms (URF) (Yamaguchi et al., 2020). The molecular epidemiology of HIV-1 is evolving over time due to the appearance and spread of mosaic genomes of the virus resulting from genetic recombination of the virus and population migrations that have allowed new variants of the virus to spread to different countries around the world (Hemelaar et al., 2020; Zhang et al., 2010; Bbosa, et al., 2019; Sivay et al., 2021). In West Africa, and particularly in Côte d'Ivoire, the first circulating HIV-1 variant described is the subtype A (Louwagie et al, 1993). Subsequent studies revealed that the majority of circulating strains were CRF02_AG recombinants (Adjé-Touré et al 2003), followed by CRF01_AE recombinants and CRF06_cpx complex recombinants (Toni, 2003). More studies have revealed the appearance of strains B and C, and the CRF02/CRF09, CRF02/A1 and CRF09_cpx recombinants (Déchi, 2019). This evolving dynamic in the molecular epidemiology of HIV-1 in Côte d'Ivoire requires close monitoring through molecular characterization of a larger number of viral strains. The aim of this study was to determine the genetic diversity of HIV-1 in Côte d'Ivoire in 2023 and compare it with the data from the study carried out in 2019 in order to determine the evolution of the genetic diversity of HIV-1 in Côte d'Ivoire.

MATERIALS AND METHODS

Study population

A cross-sectional and prospective study was carried out from February 2022 to October 2023 in three sites of Abidjan, the university hospital centers Cocody, the university hospital centers Treichville and the Pasteur Institute Cote d'Ivoire. Eligible participants were adult subjects infected with HIV-1 confirmed by serological tests and PCR.

A total of 139 individuals were recruited in this study. The study was approved by the National Health and Life Sciences Ethics Committee (CNESVS) (reference number: 197-20/MSHP/CNESVS-kp) and all participants have a consent for the study.

RNA extraction and molecular amplifications

Six milliliters of whole blood were collected from each participant and the samples were centrifuged at 2200 rpm for 10 min to obtain plasma.

Viral RNA was extracted from 200 µl of plasmas using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's recommendations.

One step RT-PCR

One-step RT-PCR System with SuperscriptIII DNA kit (Invitrogen, USA) was used to synthesize the cDNA and followed by an initial PCR with oligonucleotide for three targets (reverse transcriptase, protease, integrase) (Table 1).

The RT-PCR of 50 µl contains 25 µl of Master mix (2X), 1 µl of 0.5 nM of each primer, µl of Taq-RT Superscript III and 20 µl of extracted RNA. The Eppendorff thermocycler (VAPO Protect) was used for amplification with the following thermal amplification conditions: 55°C for 30 min, 94°C for 2 min for 1 cycle, 94°C for 30 s, 55°C for 30 s, 68°C for 1 min, 30 s for 45 cycles and a final extension at 68 ◦C for 5 min.

Nested PCR

Nested PCRs were perform using the FIREPol Master (SOLIS BIODYNE, Estonia) with primers for the three targets of HIV-1 (Table 1). A 50 µl reaction contains 10 µl of Master mix (5X), 1µl of 0.5 nM of each primer, and 10 µl of amplicons of RT-PCR. The followings parameters were applied in Eppendorf thermocycler: 95°C for 5 min for 1 cycle, 95°C for 30 s, 61°C for 60 s, 72°C for 4 min for 45 cycles and a final extension at 72°C for 10 min.

Sanger sequencing

Nested-PCR products were migrated into a 1% agarose gel for identification and selection of positive fragments of viral targets, which were purified using the ChargeSwitch-ProPCR Cleanup Kit (Invitrogen). Sequence reaction of the fragments obtained was carried out using the BigDye Terminator v3.1 kit (Applied biosystems, USA). The products of the sequence reaction were purified using an ethanolic purification process with Agencourt CleanSEQ (BECKMAN) and migrated to an Analyzer 3500 XL Genetic Analyzer (Applied biosystems, USA) to determine the nucleotide sequences of amplified targets.

Bioinformatics analysis

The chromatograms obtained were analyzed using MEGA7.0.14 software. Sequence analysis to determine HIV-1 subtypes was performed using HIVGRAD viral genotyping software (https://www.hiv-grade.de/cms/grade/) from the ANRS-MIE algorithm. Phylogenetic analyses were performed using the sequence alignment program (https://www.hiv.Lanl.gov/content/sequence/HIV/HIVTools.html). Microsoft Excel 2013 was used for data processing and SPSS Statistics 17.0.1 for statistical analysis.

RESULTS

Characteristics of study participants

From 139 patients recruited in this study, (66%) 92/139 were female and 47 were male (34%). The median age of the study population was 44 years, ranging from 18 to 77 years in female and 17 to 70 years in male. The median

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Table 1. PCR and sequencing primers.

Name	Sequences (5'-3')	Target
MJ3	AGT AGG ACC TAC ACC TGT CA	Reverse Transcriptase
MJ4	CTG TTA GTG CTT TGG TTC CTC T	
A35	TTG GTT GCA CTT TAA ATT TTC CCA TTA GTC CTA TT	
NE135	CCT ACT AAC TTC TGT ATG TCA TTG ACA GTC CAG CT	
5'PROT1	TAA TTT TTT AGG GAA GAT CTG GCC TTC C	Protease
3'PROT1	GCA AAT ACT GGA GTA TTG TAT GGA TTT TCA GG	
5'PROT2	TCA GAG CAG ACC AGA GCC AAC AGC CCC A	
3'PROT2	AAT GCT TTT ATT TTT TCT TCT GTC AAT GGC	
INPS1	TAG TAG CCA GCT GTG ATA AAT GTC	Integrase
INPR8	TTC CAT GTT CTA ATC CTC ATC CTG	
INPS3	GAA GCC ATG CAT GGA CAA G	
INPR9	ATC CTC ATC CTG TCT ACT TGC C	

Source: https://hivhfrenchresistance.org/protocols/.

viral load was 4.8 Log10 copies/ml.

Molecular analysis

Three targets of viral genome of HIV-1 were amplified in PCR methods. The positivity rate of molecular tests is 52% (73/139). 15% (11/73) were positive of the reverse transcriptase, 93% (68/73) for the protease, and 25% (18/73) of the integrase with bands of 798 bp, 507 bp and 750 bp respectively (Figure 1).

Distribution of viral subtypes in the study population

The analysis of nucleotides sequences of amplified targets showed that the majority of circulating HIV-1 subtypes were CRF02_AG, by 84% (61/73), and the subtypes A, A1, and G, were 3% (2/73), 1% (1/73) and 1% (1/73) in this study. 7% (5/73) of viruses were complex recombinant strains and 4% (3/73) of strains cannot be identified by the HIV data base and were classified unidentified strains (Figure 2).

Phylogenetic analyses of genes of interest

Phylogenetic analyses of the genes of interest using the PHILM sequence alignment program (https://www.hiv.Lanl.gov/content/sequence/HIV/HIVTools.html) confirmed the predominance of CRF02_AG at the reverse transcriptase, protease and integrase gene levels at frequencies of 64% (7/11), 90% (61/68), and 55% (10/18) respectively Figure 3.

Complex recombinants

Complex recombinants were 7% (5/73) of HIV-1 strains and have divergent subtype designations among reverse transcriptase, protease and integrase with the involvement of CRF01_AE, A, A1, G, CRF02_AG and K. The following genetic profiles were generated for the five recombinants strains. (Table 2).

DISCUSSION

The phylogenetic analyses of three targets of reverse transcriptase, protease, and integrase genes have demonstrated the circulating of recombinant form CRF02_AG as dominant variant by 84%.

The study has high predominance of this variant in West African countries like specifically Ghana in 2023 by 53.9% (Appah et al., 2023) and Gabon in 2022 by 37.95% (Bivigou-Mboumba et al., 2022). The differences observed could be due to the increase in secondary and complex recombinants of CRF02_AG in Ghana (31.5%) and subtypes A (22.3%) and G (7.83%) in Gabon.

The study showed a slight decrease in the prevalence of CRF02_AG and subtype A with previous study in Côte d'Ivoire in 2019. Dechi et al. have estimated prevalence by 85% for CRF02_AG and 10% for subtype A (Dechi et al., 2019). The decrease in proportions is due to the advantage of complex HIV-1 recombinants (7%) in our study.

The high replication capacity of the CRF02_AG recombinant and its high prevalence are thought to be at the origin of its secondary recombinants (Zhang et al., 2010). In addition, co-infections and superinfections are

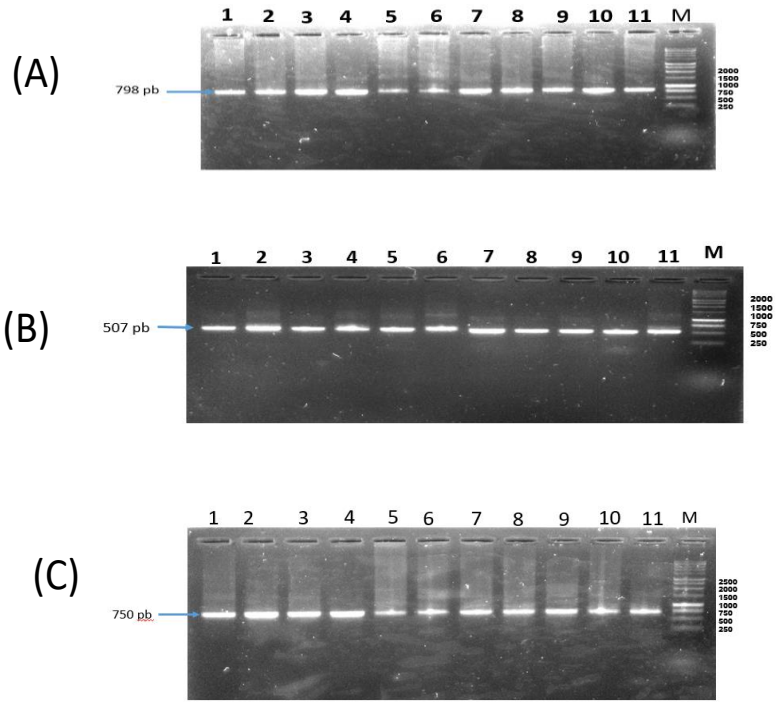

Figure 1. Electrophoretic profile on 1% agarose gel for amplification of HIV-1 targets : reverse transcriptase (A), Protease (B) and integrase (C). Lane 1-11: HIV DNA extracts after Nestded-PCR, M : Molecular Weight marker.

Figure 2. Distribution of viral sub-types.

frequent phenomena that have been described as factors favoring the emergence of complex recombinants (Fang et al., 2004; Fultz, 2004). Thus, the circulation of CRF02_AG, CRF01_AE and subtypes A, A1, F2 and K would have favored the emergence of complex recombinants CRF01_AE/CRF02_AG/A1 (1%); CRF01_AE/A/A1 (1%); CRF01_AE/K (1%); CRF02_AG/A1 (1%); CRF02_AG/F2 (1%) in our study. These prevalences are low compared with those found in the study by Wagner and colleagues on circulating recombinant forms (CRFs) of HIV-1 and single recombinant forms (URFs) in Israel. They identified secondary HIV-1 recombinants in 26% of the cases and these forms were CRF01_AE/CRF02_AG/A3 (10.8%),

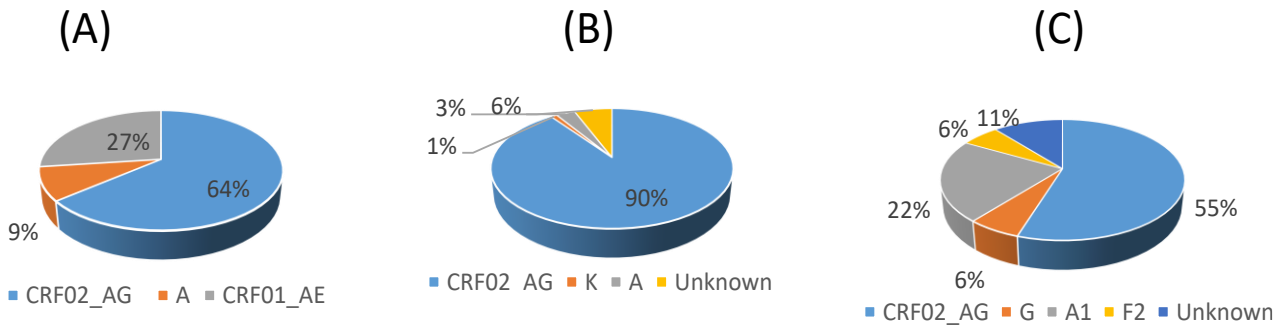

Figure 3. Phylogenetic analyses with HIV-1 targets: Reverse transcriptase (A); Protease (B); Integrase (C).

Table 2. Genotypic profile of complex recombinant HIV-1 strains.

Code	Targets			Genetic profile
	Reverse transcriptase	Protease	Integrase	
TGR010	CRF01_AE	CRF02_AG	A1	CRF01_AE/ CRF02_AG/A1
TGR015	CRF01_AE	A	A1	CRF01_AE/A/A1
TGR024	CRF01_AE	K		CRF01_AE/K
TGR027	ND*	CRF02_AG	A1	CRF02_AG/A1
TGR009	ND*	CRF02_AG	F2	CRF02_AG/F2

ND*: Not determined.

CRF02_AG/A4 (6.6%), CRF02_AG/B (2.8%), CRF02_AG/G (2.8%), CRF01_AE/B (1.4%), and CRF02_AG/A6 (1.4%) (Wagner et al.,2022). The differences observed in the diversity and prevalences of the strains identified could be explained by the large size of their sample.

In our study, phylogenetic analyses of HIV-1 reverse transcriptase, protease, and integrase genes confirmed the predominance of CRF02_AG in the 03 genes, with frequencies of 64, 90, and 55% respectively. These prevalences are similar with the study in Nigeria in 2022 with prevalence of 58.8% for reverse transcriptase, 69.7% for protease and 66.7% for integrase (Oluniyi et al., 2022).

However, 4% of the nucleotides sequences in our study have no correspondence to unknown HIV-1 subtypes. This frequency is high and not similar to the findings in 2022 in Gabon of 1.8% of unknown HIV-1 subtypes according to the Stanford algorithm (Bivigou-Mboumba et al. 2022). The difference could be explained by the difference in the used algorithms in other HIV-1 strains in the two studies.

Conclusion

This study has shown that the circulating recombinant form CRF02_AG remains the predominant form of circulating HIV-1 subtypes in Côte d'Ivoire, but it has revealed the emergence of complex recombinant viruses which are favored by the A and G strains. The circulation of these strains suggests high genetic variability and heterogeneity of HIV-1 in Côte d'Ivoire. The structures of these strains need to be clarified by subsequent sequencing studies of their entire genomes, which will make it possible to identify the strains at the origin of these complex recombinants, as well as unidentified strains that could be new strains. Finally, the factors favoring the emergence of new variants or affecting the pathophysiology of the infection, such as superinfection, need to be studied in this country with a high prevalence of HIV-1 infection. Our results propose the establishment of longitudinal surveillance of isolates HIV-1 circulating in Côte d'Ivoire for monitoring the evolution and prevalence of the different HIV-1 subtypes in the country.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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