

Original Article

GENETIC POLYMORPHISM OF REVERSE TRANSCRIPTASE AND PROTEASE ASSOCIATED WITH THE ANTIRETROVIRAL (ARV) INEFFICIENCY IN PEOPLE LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS TYPE-2 (HIV-2) IN ABIDJAN, CÔTE D'IVOIRE

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ABSTRACT

Objectives: To analyse the genetic polymorphism of reverse transcriptase and protease genes in patients living with HIV-2, to search for possible mutations preventing the effectiveness of antiretroviral drugs (ARVs).

Methods: RNA Viral was extracted after lysis of the virus particles and purified on filter columns (Qiagen). After their extraction, DNA fragments were amplified by the method of reverse transcription/polymerase chain reaction (RT/PCR) on one hand and by the PCR method enchase on the other hand. The study of genetic polymorphisms in reverse transcriptase and protease was performed after sequencing of amplicons.

Results: A significant polymorphism was observed in positions involved in resistance to antiretrovirals. The most frequent was mutations M 46 I, M 36 I/V, V82I, L 89 I on protease and Y 181 I, Y 188 L, G 190A on reverse transcriptase.

Conclusion: Polymorphic mutations were observed in the genes of the protease and reverse transcriptase and could be involved in the HIV-2 resistance to antiretrovirals (ARVs). It would be desirable to do the quantification of DNA provirus of HIV-2 in the other for a durable therapeutic monitoring of patients having HIV-2.

Keywords: Human Immunodeficiency Virus (HIV-2), Reverse transcriptase, Protease, Abidjan, Côte d'Ivoire.

INTRODUCTION

HIV Infection is a pandemic that affects more than 2.3 million people in the world with over 68% in sub-Saharan Africa. In West Africa, Côte d'Ivoire is one of the most affected countries with a prevalence of 3.4 % in the general population [1]. Discovered in 1983, HIV-1 is the first type of HIV identified and as the main agent responsible for the epidemic [2]. In 1986 a second type of HIV, HIV-2 was discovered in West Africa [3]. Epidemiological wise the latter is a minority, but still have a significant presence in West Africa, where it affects 1 to 2 million people [4, 5]. Its outbreak areas are located in Senegal, Guinea, Sierra Leone, Liberia and Côte D'Ivoire, are also found in India and Europe [2].

The HIV-1 virus is very variable classified into four groups (M, O, N and P), the group M (Major) alone is responsible for over 95% of the pandemic [5]. HIV-2, also have several subtypes (A, B, C, D, E, F, G, H) Only subtypes A and B have an epidemic spread [6]. The progression of this HIV-2 type reveals the treatment management problems, encountered in the developing countries, in this case the monitoring of viral load [7, 8]. The treatment regimens prescribed in cases of HIV-1 are not always effective for HIV-2 [9]. There is no anti retroviral (ARV) treatment for patients infected with HIV-2 for now. Several observational studies in developing countries have shown poor results on ARV therapy for HIV-2 [10, 11].

Therefore the epidemiological surveillance of HIV-2 strains is needed in order to improve the health care of people living with HIV-2. The aim of our study is to analyse the genetic polymorphism of reverse transcriptase and protease in patients with HIV-2, to search for possible mutations that

MATERIALS AND METHODS

Study site

The study was initiated by the Laboratory of Biochemistry of the Faculty of Medical Sciences of the University of Felix Houphouët-

Boigny Abidjan. Our study was carried out in the molecular biology unit of the Laboratory of Virology of the Integrated Centre for Bioclinical research in Abidjan (ICBRA).

Study population

Were included in this study, patients with positive serology to HIV-2, naïve to major ARV, who gave their consent to participate in the study and signed a consent form. This study was approved by the National Ethics and Research Committee (NERC). Our study population consisted of a sample of 77 patients recruited between March 2009 and February 2010.

Specimen collection and conservation

After collecting information from patients, venous blood samples were collected in EDTA tubes. The tubes were centrifuged at 3500 revolutions/min for 5 min. Four aliquots of 1 ml of plasma and two pellets of whole blood were stored at -80 °C.

Calculation of phylogenetic trees

Phylogenetic trees were calculated for each fragment using the methods of the Neighbor-Joining and Kimura of two-parameters [12].

Gene amplification

A polymorphism was observed in positions involved in the resistance to anti retrovirals. The most popular was the mutations in codons M 46, M 36, V 82 and L 89 on the protease gene and at the codons Y 181, Y 188 and G 190 for the reverse transcriptase gene.

Molecular study

RNA extraction

RNA Viral was extracted after lysis of the virus particles and purified on filter columns (Qiagen), according to the method described by [13].

Amplification of the HIV-2 polymerase gene (protease and reverse transcriptase) by PCR

Reverse transcription/polymerase chain reaction (RT/PCR)

The final volume of the first PCR was 50 µl and the amount of RNA in patients was 10 µl. The reaction mixture consisted of: 1 µl out of mother stock 20 µmol solutions of a pair of primers, 4 µl of 10 mmol of dNTP, 1 µl of RNase Inhibitor 5 U/µl, 1 µl of enzyme MIX, 10 µl buffer 5 X, 2.5 µl of dithiothreitol (DTT) and MilliQ water qs. For these PCR, primer pair used was PR1-5'-GGG AAA GAA GCC CCG CAA CTT C-3' and RT2-5'-GAA GTC GTC TGG GAT CCA CCA CAC TGT TTT CCA-3'. The thermal cycler was programmed to carry out an enzymatic activation and denaturation at 94 °C for 2 min, 40 cycles of amplification at the temperatures of (94 °C, 55 °C and 68 °C) respectively for 30 s x 2 and 3 min and elongation at 68 °C for 7 min.

Nested PCR

For the second PCR, we have used as final volume 50 µl and the amount of DNA was 5 µl. The reaction mixture consisted of: 1 µl out of mother stock of 30 µmol solutions of a pair of primers, 1 µl of dNTP of 10 mmol, 0.4 µl of enzyme polymerase of 3.5 U/µl, 5 µl of 10 X buffer of 2.5 µl and MilliQ water qs. The second PCR was made with the pair of primers PR3-5'-GCT GCA CAA TTC CTC CTT TCT-3' and RT4-5'-TCC CCA AAT GAC TAG TGC TTC TTT TTC CTA T-3'. The thermocycler was programmed to perform: a reverse transcription at 94 °C for 3 min, 10 cycles of amplification at temperatures of (94 °C, 60 °C and 72 °C) respectively, for 15s, 30 s and 2 min, 20 amplification cycles at temperatures of (94 °C, 60 °C and 72 °C) respectively for 30 s x 2 and 2 min and finally elongation at 72 °C for 7 min.

Statistical analysis

Sequence analysis of HIV-2 PCR has been aligned with the reference sequences of HIV-2 ROD. The wild type of protease has been defined by the alleles M 46, M 36, V 82 and L 89 and those of reverse transcriptase by alleles Y 181, Y 188 and G 190. The calculation of phylogenetic trees was performed with the REGA HIV sub typing Tool.

RESULTS AND DISCUSSION

Seventy-seven PCR were performed in this study. We got a 92% success rate (n = 71) and a failure rate of 8% (n = 6) (fig. 1). Seventy-seven patients were included in the study. Thirty-nine percent (39%) (n = 30) were women and 61% (n = 47) men (fig. 2). The synthesis of different phylogenetic analysis showed that 53% (n = 41) were strains of subtype A and 47% (n = 36) strains of subtype B of HIV-2, circulating in Abidjan (fig. 3).

Phylogenetic analysis showed that 53 % (n = 41) were strains of subtype A, 47 % (n = 36) were subtype B strains. These results are consistent with similar work carried out by several authors, these results confirmed the circulating subtypes A and B of HIV-2 in Côte d'Ivoire [14, 15].

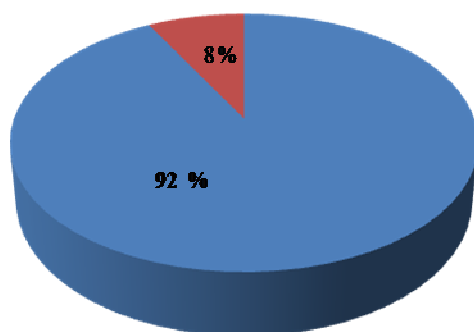


Fig. 1: Percentage of success of PCR

A polymorphism was observed in positions involved in the resistance to anti retrovirals. Most popular were the mutations M 46

I, M V 36 I/V, V 82 I, L 89 I on protease and Y 181 I, Y 188 L, G 190 A on reverse transcriptase (fig 4, 5).

A comparison of our sequences to that of reference sequences showed a polymorphism of the protease and reverse transcriptase genes which may be involved in HIV-2 resistance to anti retroviral (protease inhibitors and reverse transcriptase inhibitors). On the protease gene, the most common were M 46 I/V (methionine converted to Isoleucine or Valine at protease position 46), M 36 I (methionine converted to Isoleucine at protease position 36), V 82 I (valine converted to isoleucine at position 82) and L 89 I (leucine converted to Isoleucine at position 89). On the reverse transcriptase, polymorphism mutations Y 181 I (tyrosine converted to Isoleucine at position 181), Y 188 I (tyrosine converted to lysine at position 188) et and G 190 A (Glycine converted to alanine at position 190) were the most represented.

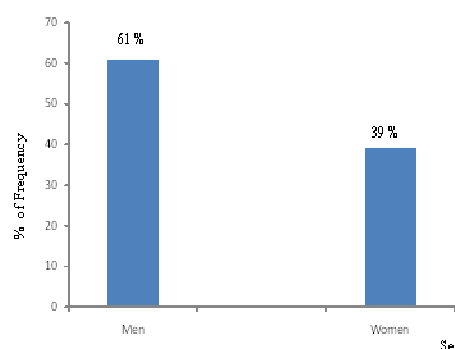


Fig. 2: Description of sex in the observational cohort

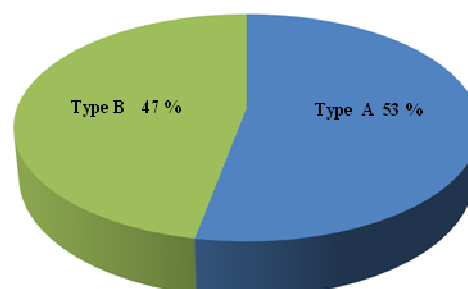


Fig. 3: Distribution of subtypes of HIV-2 obtained from phylogenetic trees in Abidjan, Cote d'Ivoire

Regarding mutations of the polymorphism of the protease gene, studies done by Sabelle and Kevin in 2009 and 2011 respectively have confirmed our results. These results have shown that the mutation at codon M 46 I would be one of the main mutations that induce resistance to protease inhibitors including indinavir (IDV) [16, 17]. The results of the study carried out by Weinberg in 2010, described mutations on codons M 46 I, M 36 I and V 82 I as mutations that could have an effect on the emergence of resistance to protease inhibitors [18]. These mutations observed in this gene have been cited in the study conducted by Ana in 2011, namely mutations at codons M 46 I, M 36 I, V 82 I and L 89 I. These results have shown that these mutations found in HIV-2 antiretroviral-naïve patients, are also known to induce resistance to protease inhibitors in HIV-1 patients. Conversion of methionine at position 46 to Isoleucine or Valine induces resistance to protease inhibitors [19].

Regarding the polymorphism mutations in the reverse transcriptase gene, they provide a natural resistance to non-nucleotide reverse transcriptase inhibitors. This indication is consistent with the work of authors like Alcaro in 2011 which stated that the mutation on the codon Y 181 I confers natural resistance to non-nucleotide reverse transcriptase inhibitors [20]. A study conducted by Ntemgwa in 2009 has shown that the mutation on the cordon Y 188 L also

induces this resistance thus confirming our results [15]. These results were also observed for the codons Y 181 I, Y 188 L and G 190 A which would be one of the mutations which induce resistance to non-nucleotide reverse transcriptase inhibitors [18].

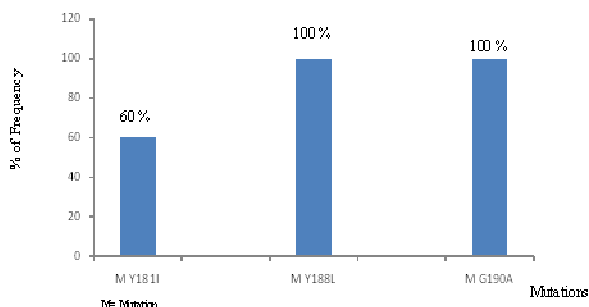


Fig. 4: Polymorphism of reverse transcriptase gene

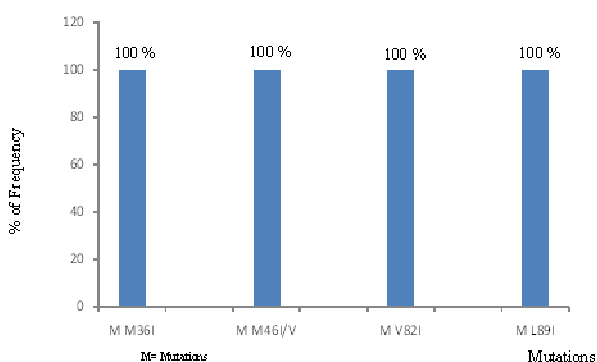


Fig. 5: Polymorphism of protease gene

CONCLUSION

These studies have enabled us to understand the genetic variability of HIV-2 and resistance to anti retroviral in patients naïve to all anti retroviral treatments in Côte D'Ivoire. Phylogenetic analysis confirmed the circulation of HIV-2 subtypes A and B in Côte d'Ivoire. Polymorphism of mutations was observed in the genes of the protease and reverse transcriptase and may be involved in the resistance of HIV-2. It would therefore be necessary to quantify the HIV-2 proviral DNA in order for a durable therapeutic monitoring of HIV-2 patients.

CONFLICT OF INTERESTS

The authors wish to declare that there is no conflict of interest.

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