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ARTIKEL PENELITIAN

The Relationship of Genetic Diversity of Human Immunodeficiency Virus-1 with Acquired Drug Resistance Mutation in HIV Patients on First-Lines Antiretroviral Therapy

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Abstrak

Tujuan: Penelitian ini bertujuan untuk mengetahui hubungan keragaman genetik HIV-1 dengan mutasi resistensi obat didapat pada pasien HIV-1 yang menerima obat antiretroviral lini pertama di RSUP Dr. M. Djamil Padang. **Metode:** Penelitian dilakukan pada 20 pasien yang didiagnosis berdasarkan tes skrining antibodi HIV-1 menggunakan metode sederhana/cepat di Laboratorium Sentral RSUP Dr . M. Djamil Padang dan telah mendapat terapi ARV lini pertama minimal 6 bulan. **Hasil:** Hasil penelitian menunjukkan bahwa keragaman genetik HIV-1 ditemukannya mutasi resistansi obat. **Kesimpulan:** keragaman genetik HIV-1 yang ditemukan merupakan bentuk rekombinan CRF-AE dan CRF - BC serta subtipe A dan B , didapat resistensi obat yaitu M184L dan T215N (jenis mutasi yang resisten terhadap ARV kelas NRTI) dan G190D (jenis mutasi yang resisten terhadap ARV pada kelompok NNRTI) dan mutasi resistensi obat yang didapat ditemukan pada individu yang terinfeksi HIV-1 subtipe A, tetapi analisis statistik tidak dapat dilakukan untuk menentukan hubungan keragaman genetik yang ditemukan dengan DRM yang didapat.

Kata kunci: Acquired Drug Resistance Mutation (ADRM); Terapi AntiretroviralHIV-1 ; Keragaman Genetik

Abstract

Objective: This study aims to determine the relationship of HIV-1 genetic diversity with acquired drug resistance mutation in HIV-1 patients who receive first-line antiretroviral drugs at RSUP Dr. M. Djamil Padang. **Methods:** The study was conducted on 20 people with HIV who were diagnosed based on HIV-1 antibody screening tests using simple / rapid methods at the Central Laboratory of RSUP Dr. M. Djamil Padang and had received first-line ARV therapy for at least 6 months. **Result:** The results showed that HIV-1 genetic diversity with the discovery of drug resistance mutations. **Conclusion:** The conclusion of this study is that HIV-1 genetic diversity found is a form of recombinant CRF-AE and CRF-BC and subtypes A and B, acquired drug resistance found is M184L and T215N (types of mutations that are resistant to NRTI-class ARVs) and G190D (types of mutations that are resistant to ARVs in the NNRTI group) and acquired drug resistance mutation is found in individuals infected with HIV-1

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subtype A, but statistical analysis cannot be performed to determine the relationship of genetic diversity found with acquired DRM.

Keywords: *Acquired Drug Resistance Mutation (ADRM); Antiretroviral TherapyHIV-1; Genetic Diversity*

INTRODUCTION

Infection by HIV-1 mutant strains causes resistance to antiretrovirals (ARVs) in HIV sufferers who have not received therapy (drug naïve HIV) or who have been treated (acquired drug resistance mutation). This resistance is a major problem that limits the effectiveness of first-line ARV therapy. This results in treatment failure characterized by no clinical improvement, virological failure, and immunological failure in patients with HIV / AIDS (Acquired immune deficiency syndrome) after receiving ARV for at least 6 months.[1,2,3]

Genetic diversity is one of the characteristics of Human immunodeficiency virus-1 (HIV-1). This is caused by high levels of mutation, recombination, and HIV-1 replication so that it has implications for changes in base / amino acid sequences that are targeted for therapy.[4,5]

HIV-1 genetic diversity is based on the combination of highly active antiretroviral treatment (HAART) in HIV patients. Antiretroviral therapy has reduced the rate of morbidity and mortality of HIV infection, but the administration of HAART has caused selective immune pressure in the virus. Virion-virion which escapes from the immune system will continue to replicate causing mutant HIV-1 strains.[6]

The Indonesian government has launched an expansion program for the use of ARVs since 2004, but until 2010 ARV therapy only reached <18% of people with HIV / AIDS in Indonesia. This achievement increased by more than 40% in 2011 and will continue to increase along with the increasing rate of HIV / AIDS in Indonesia. [3]

Expansion of the use of antiretrovirals has reduced the morbidity and mortality rates of people with HIV / AIDS, but on the other hand has increased the incidence of resistance to antiretroviral drugs which hinders the success of HIV infection control programs, especially in countries with limited choice of drugs and genotypic drug resistance / GDR).[7,8,9,3]

Genotypic resistance testing used to detect primary resistance to naïve HIV drugs is a guide to using second-line ARV therapy thereby reducing the likelihood of treatment failure and transmission of mutant HIV strains. [10-13] Such tests are not routinely carried out in countries with limited resources, including Indonesia.

After more than a decade of expansion of ARVs in Indonesia, the emergence of major resistance to first-line antiretroviral drugs on naïve HIV drugs (due to infection with HIV mutant strains) is inevitable, but the data on this are still very limited. So far there has been one study from Surabaya which reported the presence of mutant HIV strains on naive HIV drugs that were resistant to first-line ARVs. This research was conducted by sequencing the reverse transcriptase and protease gene against 58 naïve HIV drug individuals. The prevalence of primary resistance to antiretroviral drugs in this study is still <5% (low prevalence based on the WHO category), but regular monitoring of mutant HIV strains that are resistant to antiretroviral drugs is very important in countries with limited treatment options and a tendency to increase HIV infection / transmission / AIDS.[3]

METHODS

This study was a descriptive observational study. The subjects studied were HIV sufferers who had understood

and agreed to the research ethics form. The total subject is 20 people. The study population was all HIV-1 patients in RSUP Dr. M. Djamil Padang. Careful samples are parts of the population that meet the inclusion and exclusion criteria.

Inclusion criteria:
- willing to be the subject of research
- HIV-1 patients who have received ARV therapy for ≥6 months

Exclusion criteria:
- HIV patients who do not adhere to taking ARVs

The study was conducted at the Clinical Pathology Laboratory of RSUP Dr. M. Djamil Padang, namely taking and storing blood samples before isolation, the Molecular Biology Laboratory of the Microbiology Section of Faculty of Medicine, Andalas University, which carried out proviral isolation of HIV-1 DNA and PCR (nested PCR technique) amplification and 1st Base Singapore to do sequencing.

Blood collection of peripheral veins

Retrieval of venous blood (phlebotomy) is carried out aseptically in the region of the cubital fossa vein by trained personnel. 2 mL of venous blood was included in vacutainer EDTA for proviral isolation of HIV-1 DNA.

Isolation of HIV-1

Proviral DNAHIV-1 DNA is extracted from samples (whole blood EDTA) using the PureLink genomic DNA (Invitrogen) kit. Work procedures are in accordance with the protocol recommended by the manufacturer, including the following stages: blood thinning, DNA binding, DNA washing, and DNA elution.

Amplification with Nested PCR

Amplification using Taq PCR Master Mix (Qiagen). The work procedure is adjusted to the recommended protocol. Optimization of annealing temperature is done to get good results. PCR products were analyzed with 0.8% agarose gel and colored with Gel red.

Sequencing

Sequencing of the reverse transcriptase and protease gene was carried out at 1st Base Singapore.

Analysis *of Sequencing Results*

Sequencing results were analyzed by software to identify genetic diversity of HIV-1. The sequence is analyzed by acquired drug resistance mutation using the Stanford HIV database.

Ethical Approval

The ethics of research in humans is based on the protocol of human use as an object of research and endorsed by the research ethics commission of the Medical Faculty of Andalas University.

RESULT AND DISCUSSION

Analysis of Genetic Diversity

Genetic diversity of reverse transcriptase and protease sequences can be seen in Table 1. below.

Table 1. Genetic Diversity in Reverse Transcriptase and Protease Sequences

Samples Code	Sequences	Genetic Diversity (Type / Subtype / CRF)
1	*Reverse transcriptase*	CRF-BC
2	*Reverse transcriptase*	A
5	*Reverse transcriptase*	CRF-AE
6	*Reverse transcriptase*	CRF-AE
7	*Reverse transcriptase*	CRF-AE
8	*Reverse transcriptase*	B
9	*Reverse transcriptase*	A
10	*Reverse transcriptase*	A
11	Protease	A
12	Protease	A

Analysis of Acquired Drug Resistance Mutation

Analysis of acquired DRM was carried out in 8 reverse transcriptase sequences and 2 protease sequences based on the Standford HIV database. The results of the analysis was can be seen in Table 2 below.

Table 2 Drug Resistance Mutation in Reverse Transcriptase and Protease Sequences

Samples/Sequences	DRM	NRTI / NNRTI resistance
1/*Reverse transcriptase*	-	-
2/*Reverse transcriptase*	M184L, T215N G160D	NRTI NNRTI
5/*Reverse transcriptase*	-	-
6/*Reverse transcriptase*	-	-
7/*Reverse transcriptase*	-	-
8/*Reverse transcriptase*	-	-
11/*Reverse transcriptase*	-	-
16/*Reverse transcriptase*	-	-
11/Protease	-	-
16/Protease	-	-

M = methionine, L = leucine, T = tironin, N = asparagine, G = glycine, D = aspartic acid, NRTI = nucleoside reverse transcriptase inhibitor, NNRTI = non nucleoside reverse transcriptase inhibitor

Amplification of target proviral DNA

Amplification uses the nested PCR method to increase the likelihood of detecting HIV-1 proviral DNA. Amplification was carried out in reverse transcriptase and protease sequences, each through two stages of amplification. The first stage, the DNA template was HIV-1 DNA proviral isolates, while in the second stage, the DNA template was the first PCR product.

The primers used for amplification of reverse transcriptase are:

Stage I : HIV-RT1-R (5'GGA CTA CAG TCY ACT TGT CCA TG-3') and HIV-RT1-F (5' ATG ATA GGG GGA ATT GGA GGT TT-3')

Stages II: HIV-RT2-R (5' TTA AAA TCA CTA RCC ATT GYT CTC C -3') and HIV-RT2-F (5' GAC CTA CAC CTG TCA ACA TAA TTG G-3')

Amplification of reverse transcriptase at both stages was carried out 30 cycles each. Optimization of annealing temperature has been carried out gradually starting at temperatures of 51 ^{0}C to 63 ^{0}C.

Nested PCR for proteases is carried out using primers:

Stage I : HIV-RT21-R (5'CTG TAT TTC TGC TAT TAA GTC TTT TGA TGG G -3') and HIV-MAW26-F (5'TTG GAA ATG TGG AAA GGA AGG AC-3')

Stage II : HIV-RT20-R (5' CTG CCA GTT CTA GCT CTG CTT C-3') and HIV-PRO1-F (5' CAG AGC CAA CAG CCC CAC CA-3')

Amplification of the protease was carried out in 40 cycles in the first stage and 35 cycles in the second stage. Optimization of annealing temperature has been carried out gradually starting at temperatures of 51 ^{0}C to 63 ^{0}C.

Nested-PCR *product electrophoresis*

Nested PCR product electrophoresis was carried out using 0.8% agarose gel. The electrophoresis setting is set at a voltage of 100 volts for 40 minutes. The results are seen using UV lights. A total of 20 proviral DNA isolates were amplified and electrophoresed, but only 16 samples showed sufficient bands (9 samples for reverse transcriptase, 7 samples for proteases).

The PCR products obtained are then sequenced at 1st Base Singapore. Sequencing results showed 10 samples, namely 8 samples of RT and 2 PR sequences which could be further analyzed for genetic diversity and DRM analysis on reverse transcriptase and protease based on the Stanford HIV database.

Analysis of Genetic Diversity

Based on Table 5.1, it can be seen that out of the 8 reverse transcriptase sequences, 3 samples were recombinant CRF-AE, 3 subtype A samples, 1 sample was B subtype and CRF-BC recombinant form, while the two protease sequences were subtype A. Subtype A and the recombinant form of CRF-BC is a new subtype / recombinant found in HIV-1 patients in West Sumatra.

Previous research by Roselinda and Jekti (2012) on HIV-1 patients in West Sumatra, found 1 recombinant form namely CRF_01AE and 1 subtype, namely subtype B [14]. Efrida and Eka Putra (2014**)** found 3 subtypes in HIV-1 patients in RSUP Dr. M. Djamil, namely AE / B subtypes, AE subtypes, and B subtypes [15].

Analysis of Acquired Drug Resistance Mutation

Based on Table 5.2, DRM was found in 1 of 8 reverse transcriptase sequences, whereas in the protease sequence no DRM was found. The three types of DRM are M184L and T215N (a DRM that is resistant to first-line ARVs in the NRTI group) and G190D (a DRM that is resistant to first-line ARVs in the NNRTI).

Relationship between Genetic Diversity and Drug Resistance Mutation

Drug resistance mutation in the 10 sequences analyzed was only found in 1 reverse transcriptase sequence with subtype A so that statistical analysis cannot be performed to see the relationship. Further research with more sequences is

needed to prove the relationship between genetic diversity of HIV-1 and acquired DRM.

Based on the results of the research that has been done, it can be concluded that HIV-1 genetic diversity found is a form of recombinant CRF-AE and CRF-BC and subtypes A and B, acquired drug resistance found is M184L and T215N (types of mutations that are resistant to NRTI-class ARVs) and G190D (types of mutations that are resistant to ARVs in the NNRTI group) and acquired drug resistance mutation is found in individuals infected with HIV-1 subtype A, but statistical analysis cannot be performed to determine the relationship of genetic diversity found with acquired DRM.

CONCLUSION

The conclusion of this study is that HIV-1 genetic diversity found is a form of recombinant CRF-AE and CRF-BC and subtypes A and B, acquired drug resistance found is M184L and T215N (types of mutations that are resistant to NRTI-class ARVs) and G190D (types of mutations that are resistant to ARVs in the NNRTI group) and acquired drug resistance mutation is found in individuals infected with HIV-1 subtype A, but statistical analysis cannot be performed to determine the relationship of genetic diversity found with acquired DRM.

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CONFLICT OF INETEREST

Nothing.

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