HIV Drug Resistance after Failure of 6 Month First-line Therapy in a Hospital: A Case Series

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ABSTRAK

Laporan ini merupakan laporan pertama mengenai kejadian resistensi obat HIV di RSUPN Dr. Cipto Mangunkusumo. Kami melakukan uji deteksi resistensi obat HIV pada 11 pasien HIV kasus baru yang mengalami gagal virologi setelah dilakukan pengobatan antiretrovirus lini pertama selama 6 bulan. Dengan metode sekuensing, dilakukan analisis mutasi gen penyandi resistensi obat HIV. Subtipe HIV-1 serta hasil uji resistensi secara genotip diinterpretasi menggunakan database Stanford DR. Dari sepuluh sampel plasma yang berhasil diamplifikasi dan disekuensing, ditemukan resistensi secara genotipik. Berdasarkan rejimen antiretrovirus yang diterima, ditemukan manifestasi resisten terhadap rejimen lamivudine (90%), tenofovir (83%), nevirapine (100%) dan efavirenz (100%). Menarik untuk diperhatikan bahwa tidak ditemukan manifestasi resistensi terhadap zidovudine, termasuk pada empat pasien HIV/AIDS yang mendapatkan zidovudine dalam rejimen terapinya. Mutasi NRTI yang banyak ditemukan adalah M184VI dan K65R, sedangkan mutasi NNRTI adalah Y181CFGVY, K103N, A98AG, E138GQ dan G190AGS. Tidak ditemukan mutasi mayor terhadap PI. Berdasarkan temua tersebut, memperkuat urgensi monitoring virologi, survey resistensi obat HIV serta akses pilihan terapi yang sesuai pada kasus gagal terapi.

Kata kunci: *resistensi obat HIV, gagal virologi, HIV-1.*

ABSTRACT

This is the first report of HIV drug resistance in RSUPN Dr. Cipto Mangunkusumo. We reviewed eleven new cases of HIV patients who had virologic failure after 6 months first-line antiretroviral therapy. With the sequencing method, analysis of gene mutations encoded HIV drug resistance. Genotypic resistance results and HIV-1 subtype were interpreted by Stanford DR database. Of ten plasma samples that were successfully amplified and sequenced, all samples were resistant to at least one antiretroviral drug. Genotypic resistance towards the antiretroviral drugs being used was observed in lamivudine (90%), tenofovir (83%), nevirapine (100%) dan efavirenz (100%). It is interesting that no zidovudine resistance were found, including in four patients receiving zidovudine in their HAART. The common NRTI mutations were M184VI and K65R, while NNRTI mutations were Y181CFGVY, K103N, A98AG, E138GQ and G190AGS. No mayor PI mutations were found. Based on these findings, we supports the need for appropriate virology monitoring and HIV drug resistance survey in clinical practice and access to drug options in case of virology failure.

Keywords: *HIV drug resistance, virologic failure, HIV-1.*

INTRODUCTION

The first case of HIV infection in Indonesia was detected in 1987 and by the end of 2013, about 120.000 people were reported living with HIV.[1] Several programs were introduced to combat HIV/AIDS, including providing highly active antiretroviral therapy by the Ministry of Health. The coverage of antiretroviral therapy was estimated to be around 30% (39.418/12.000).[1]

The increasing number of people with access to ART has resulted in substantial declines in HIV related incidence, morbidity and mortality. However, the widespread of antiretroviral therapy have consequences in mutation and selection process. The large mutation number of HIV-1 was also affected by the absence of a proofreading mechanism and the high rate of replication virus.[2] Drug resistant mutations can lead to decreasing susceptibility to some anti-retroviral drugs, poor treatment response and cause failure therapy.[2]

In well-resourced settings, clinical guidelines recommend that resistant testing be done before administration of antiretroviral therapy and evaluated every 6 months.[3] However, in Indonesia, routine virological monitoring and resistant testing were not recommended yet. The lack of examination access was associated with accumulation of resistance mutations, limiting future therapeutic choices. Moreover, these resistant viruses could be a source of new resistant cases transmission.

Currently, only a few studies have described the epidemiology of drug resistant HIV among treatment-exposed patients. There is no study reporting the mutations patterns of HIV among failing patients. The present study assessed HIV drug resistance in patients with virologic failure to obtain an overview of resistance condition, as well as raise awareness of the transmission of HIV-1 strains resistant.

CASE ILLUSTRATION

Eleven new cases of HIV in the period from September 2013 to March 2014, were found to have failed therapy. Virologic failure was defined as a viral load >200 copies/ml after receiving 6 months first-line antiretroviral therapy. Most patients were male (54,6%) and heterosexual contact (54,6%) were the predominant route of transmission. All patients were receiving two nucleoside reverse transcriptase inhibitors (NRTIs) plus one non-nucleoside reverse transcriptase inhibitor (NNRTI). Combination of 3TC/TDF/EFV was the common regimen being used (36,3%). (**Table 1**)

Resistance testing of plasma virus was conducted at the Institute of Human Virology and Cancer Biology laboratory. Genotyping of HIV-1 pol region, especially the region coding protease and reverse transcriptase, was undertaken in plasma specimens using in house sequencing methods.

Table 1. Demographical and clinical data of patients

Pts No.	Sex/Age	Trans Route	OI	ARV	CD4 M-0	CD4 M-6	VL
1	M/30	MSM	TB	3TC, AZT, NVP	52	164	43,500
2	M/31	IVDU	TB; Hep B	3TC, TDF, EVF	25	11	195,000
3	M/29	IVDU	TB	3TC, TDF, EFV	4	20	232,000
4	F/31	HS	None	3TC, AZT, NVP	27	118	106,000
5	M/32	IVDU	Hep C	3TC, TDF, NVP	21	6	104,000
6	F/27	HS	TB	3TC, TDF, EVF	67	157	33,200
7	F/30	HS	Hep C	3TC, TDF, NVP	33	38	33,900
8	F/32	HS	None	3TC, TDF, EFV	100	127	326,595
9	M/35	Unclear	None	3TC, AZT, NVP	166	274	385
10	F/25	HS	TB	3TC, AZT, EFV	63	114	6,180
11	M/32	HS	TB	3TC, TDF, EFV	47	150	126,000

OI: Opportunistic infection; ARV: Antiretroviral Regimens; M-0: month 0; M-6: month 6; VL: viral load; MSM: Male sex with male; IVDU: Intravenous drugs user; HS: heterosexual contact; 3TC: Lamivudine; AZT: Zidovudine; NVP: Nevirapine; TDF: Tenofovir; EVF: Evafirenz

Antiretroviral drug resistance mutations (DRM) were identified and interpreted using the Standford HIVdb genotypic resistance algorithm. HIV-1 drug susceptibility was categorized as susceptible, potential low-level resistance, low-level resistance, intermediate resistance, or high-level resistance.

Genotypic Resistance Test

Of the 11 participants with HIV RNA >200 copies/ml, ten samples were successfully amplified and sequenced. The major HIV subtype in this study was CRF01_AE. All samples had at least one major drug resistant mutation (DRM), especially major mutation related with NNRTI resistance. One patient who has no major NRTI mutation was infected by HIV-1 subtype C. Overall, the number of NNRTI-associated resistance mutations were higher than NRTI-associated resistance mutations. Three viral strains from patients were resistant to all received drugs, six viral strains were resistant to two of the drugs constituting HAART, whereas one viral strain resistant only resistant to one regimen that was accepted.

The most prevalent NRTI mutations were M184V (n=9), followed by K65R (n=4) and K70EK (n=2). One patient had HIV-1 strain with reverse thymidine analog mutation (TAMs). The major NNRTI mutations were Y181C (n=5) and K103N (n=4), followed by G190AG/S (n=3) and K101E (n=2). No major PI associated resistance mutations were found. (**Table 2**)

Antiretroviral Resistance Levels

Based on Standford HIVdb genotypic resistance algorithm, high-level resistance was seen in all NRTI and NNRTI regimens, except zidovudine. The level of resistance was more varied to the NRTIs than NNRTIs. Observed profiles included not only to regimens consumed and available in Indonesia but also other NRTIs and NNRTIs that were not included in prescribed regimens as didanosine and etravirine. The distribution of high-level resistance among the study participants were resistance to nevirapine (10/10), lamivudine (9/10), abacavir (9/10), efavirenz (8/10), stavudine (5/10), emtricitabine (5/10), tenovofir (4/10), rilpivirine (3/10), didanosine (1/10), and etravirine (1/10). (**Figure 1**)

DISCUSSION

The population of our study is probably quite representative of the global population of HIV-infected patients in Indonesia. The data clearly demonstrate the predominance of HIV-1 CRF01_AE. In similar with other studies, this result indicates that CRF01-AE has stabilized its predominance in Indonesia.[4-6]

The predominance of the M184V, Y181C and K103N mutations was expected to occur due to the use of standard first-line regimens which are lamivudine for the NRTIs and nevirapine or efavirenz as NNRTI regimens. The M184V mutation selected by the use of 3TC has been

Table 2. Distribution of ARV associated resistance mutations

Patients No	ARV Regimens	Subtype	NRTI mutation	NNRTI mutation	PI mutation
1	3TC, AZT, NVP	CRF01_AE	M184V	A98AG, Y181CY, G190AG, H221HY	-
2	3TC, TDF, EFV	CRF01_AE	D67N, K70E, M184V	K101E, E138G, G190S	-
3	3TC, TDF, EFV	CRF01_AE	L74LV, Y115FY, M184V	K101E, E138Q, G190S	L10V
4	3TC, AZT, NVP	CRF01_AE	M184V	Y181C	-
5	3TC, TDF, NVP	CRF01_AE	K65R, M184V	A98AG, K103N, Y181C	L10I
6	3TC, TDF, EFV	CRF01_AE	K65R, M184I	V90I, K103N, Y181C	-
7	3TC, TDF, NVP	CRF01_AE	A62AV, K65KR, M184I	Y181CFGV	-
8	3TC, TDF, EFV	C	K70EK	K103N, P225HP	-
9	3TC, AZT, NVP		Amplification failure		
10	3TC, AZT, EFV	CRF01_AE	M184I	K103N, P225HP	-
11	3TC, AZT, EFV	CRF01_AE	K65R, M184V, T215S	A98G, L100I, V108I	-

found in similar setting in which 3TC included in the first-line antiretroviral regimens.[7-9] Y181C mutation occurred in nevirapine containing therapy, while K103N mutation occurred more frequently in efavirenz containing therapy. These mutations were also reported by several studies in another country.[8,10-12]

The K65R mutation was commonly observed in patients who failed a tenofovir-containing regimens. The relatively high proportion of K65R mutation exceeds the estimated rate in accordance with other reports.[13-15] It was probably caused by shifting in therapeutic regimen selection since tenofovir is expected to be a substitute of thymidine analog regimens. This result need to be criticized and studied further to ensure the efficacy of tenofovir and its potency in causing resistance.

Limited TAMs were observed in this study and no zidovudine associated TAMs were found. This is in contrast with data reported by Marconi (2008), Gupta (2009) and Pham (2013).[11,16,17] This event may be explained by the fact that most of HIV-1 subtype in the present study was subtype CRF01_AE, whereas other studies were dominated by subtype B as well as mutations encoding resistance standards used today are based on mutations that occur in subtype B.[18,19] Some studies have reported that non-B HIV-1 have natural polymorphism that affect its susceptibility, but there is no recent studies on the incidence of HIV-1 subtype CRF01_AE polymorphism and its association with resistance to zidovudine. Other causes are thought to have a role is because of the high level of the M184V mutation that gives the opposite effect to the workings of thymidine analogues. Similar to other studies, this finding suggested that TAMs takes a longer time to develop compared with M184V and NNRTI-associated mutations.

The combination of K101E, E138G, G190S mutations were found in two patients, while one case of G190S mutations were found in other combination. The G190S mutation was non-polymorphic mutation that selected by nevirapine and efavirenz and is one of NNRTI resistance mutation most commonly found.[8,11,12] The K101E mutation is minor mutation encoding all regimens of NNRTI resistance.[20] This mutation was selected by nevirapine and efavirenz, in appropriate with the available regimens. The E138Q/G mutation is generally selected by etrivirine and rilpivirine, regimens that are not used in this study. The finding of this mutation was suggested that mutations could occur spontaneously, not only due to the pressure of the treatment.

In our study, no PI associated resistance mutations were identified. This finding affirms that PI is still viable as second-line therapy in Indonesia.

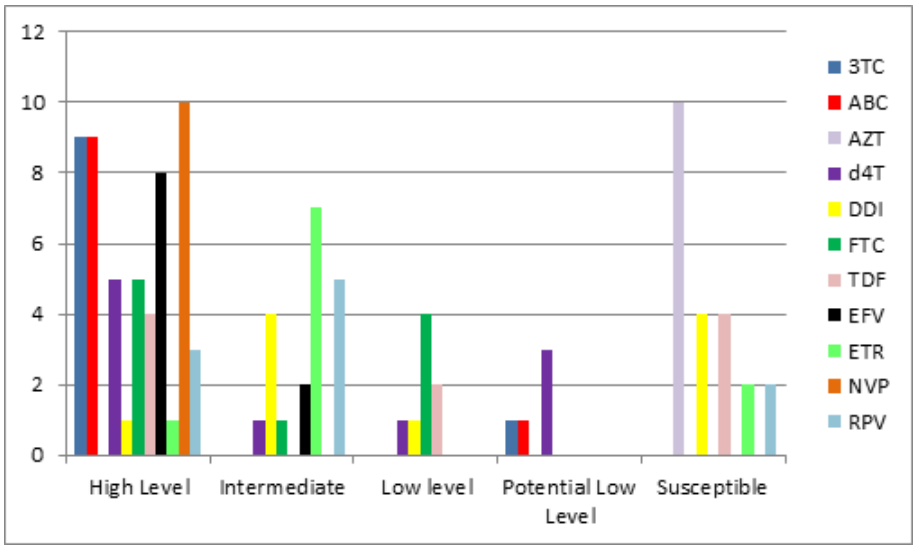

Figure 1. Distribution of antiretroviral resistance level

CONCLUSION

This case series demonstrates the potential of HIV drug resistance in patients with virologic failure after 6 months therapy. The most common mutations were associated with NRTI and NNRTI resistance, respectively. HIVDR testing should be considered in all virologic failure cases to prevent unnecessary/premature switches.

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