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Emerging Problems in Infectious Diseases

Neuropathy among drug resistant HIV Patients treated in Jakarta

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Abstract

Introduction: Some people living with HIV (PLWH) receiving ART in Indonesia display poor clearance of replicating virus. This has been associated with HIV-associated sensory neuropathy. Here we assess whether treatment failure reflects the presence of drug resistance mutations.
Methodology: PLWH were stratified by HIV RNA levels using a ≥ 1000 copies/mL cut-off after 5.3 (2-7.5) years on ART. Drug-resistance mutations were analyzed in seven of ten cases with a detectable viral load. The HIV *pol* gene was screened for mutations affecting resistance to nucleoside inhibitors (NRTI), non-nucleoside inhibitors (NNRTI) and protease inhibitors (PI). We recorded co-infections, transmission routes, and neuropathy based on the Brief Peripheral Neuropathy Screen Tool.
Results: The primary HIV subtype was HIV-1 CRF01_AE, but one patient had subtype G. Polymorphisms affecting NRTI or NNRTI (6/7 cases) and protease inhibitors (1/7 cases) were identified. Three mutations affecting NRTI (M184V, M4IL, T215F), two for NNRTI (K103N, G190A) and five for protease inhibitors (M46I, I50V, I54V, V82A, N88NDGS) were evident. Subjects with resistance mutations were mostly intra-venous drug users (4/7) and had a higher risk of neuropathy ($p = 0.016$).
Conclusions: Drug resistance mutations were present in most cases of treatment failure examined and were therefore indirectly a risk factor for peripheral neuropathy.

Key words: Drug resistance; HIV-associated peripheral neuropathy; antiretroviral therapy.

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Introduction

Antiretroviral therapy (ART) hinders HIV replication, constrains viral evolution, facilitates immune restoration, and diminishes infection transmission [1]. People living with HIV (PLWH) in Indonesia are currently treated with NRTIs and NNRTIs as their primary medication and PI as the secondary second-line treatment [2]. Meta-analyses conducted in 2015 found that the prevalence of transmitted drug resistance (DR) to HIV in Southeast Asia was approximately 2.8% [3,4]. Studies comparing African populations from 2009-2013 to 2014-2019 indicated a rise from 3.6% to 6.0% [3].

DR mutations were found in 24% of Indonesian people living with HIV who injected drugs and were included in a study conducted in 2015-2016 [5]. Additionally, ten out of eleven HIV patients in Jakarta experienced virological failure after six months of antiretroviral therapy in 2013-2014 [6]. In Surabaya, East Java, drug resistance mutations in the reverse transcriptase HIV pol region were detected in 37.5% (20 out of 53) of PLWH who had been on ART for over two years [7]. A 2020 study in Pontianak, Kalimantan, found DR mutations in two out of nineteen HIV patients who had been on ART for at least one year [8]. Another Indonesian study revealed that 20% (8 out of 40) of HIV patients had drug-resistance mutations impacting reverse transcriptase inhibitors [9].

Clinical implications associated with HIV replication have been documented in HIV patients with DR mutations [10,11], but no studies have investigated HIV-associated sensory neuropathy (HIV-SN) in this

scenario. HIV-SN is linked to negative health consequences due to decreased mobility and work capacity in patients and can be reliably assessed using the Brief Peripheral Neuropathy Screening Tool (BPNST), which records both signs and symptoms of HIV-SN [12]. We identified HIV-SN in 14.2% of HIV patients receiving treatment at a tertiary hospital in Jakarta and associated it with neuropathic pain [1,13]. This study links DR mutations with HIV-SN in the same cohort.

Methodology

Study subjects

In 2015-17, we recruited 145 HIV patients who had used ART for at least 12 months. According to international guidelines, they received NRTI, NNRTI, and PI [2]. Seven patients who had ≥ 1000 copies of HIV RNA/mL were studied for DR. The study was approved by the Universitas Indonesia Ethical Committee (579/UN2.F1/ETIK/2014). Demographic data and evidence of tuberculosis were collected from medical records. CD4 T-cells were enumerated by flow cytometry (Becton Dickinson, New Jersey, USA). HIV RNA was quantitated using TaqMan COBAS Amplicon (Roche Diagnostics, Mannheim, Germany), with a 50 copies/mL detection limit. Plasma was stored at -80°C for sequencing at the Virology and Cancer Pathobiology Research Centre (Universitas Indonesia. Neuropathy was assessed using the Brief Peripheral Neuropathy Screen Tool (BPNST) [13,14].

Investigation of mutations associated with drug resistance

Plasma was centrifuged (20,000 ×g, 75 minutes, 4 °C) and RNA was extracted using QIAmp Viral Mini Kits (Qiagen, Germany). Amplicons of the *pol gene* were analyzed by agarose gel electrophoresis and EcoDye™ DNA staining, purified using QIAquick PCR purification kits (Qiagen, Germany), and subjected to cycle sequencing using ABI PRISM® BigDye® Terminator v3.1 cycle sequencing kits with the following reactions: 1 cycle of 96 °C for 1 minute, 25 cycles of 96°C for 10 seconds, 53°C for 5 seconds and 60 °C for 4 minutes, one cycle of 4 °C for 5 minutes. Excess dye terminators were removed by precipitation in ethanol/EDTA/sodium acetate. The nucleotide sequences of the *pol* gene were analyzed on an automated DNA sequencer (ABI PRISM 3730 Genetic Analyzer, Applied Biosystems, USA) using seven in-house primers (four forward and three reverse primers overlapping the reverse transcriptase and protease regions), as described previously [6].

Sequence analysis

The *pol* gene nucleotide sequences were assembled and aligned to the CDC-WHO_HIVDR reference using RECall (v2.28.3-21) web-based sequence software. DR mutations were identified using the Stanford HIV Resistance Database (http://hivdb.stanford.edu/).

Table 1. Clinical characteristics and DRM of participating patients.

Clinical Characteristics	HIV RNA ≥ 1000 copies/mL	HIV RNA < 1000 copies/mL	*p*
N	7	138	-
Male/Female	04-Mar	101/37	0.39 [b]
Age (years)	35 (28-48) [a]	36 (23-60) [a]	0.99 [c]
Nadir CD4 T-cells/mL	38 (6-257) [a]	122(1-599) [a]	0.22 [c]
Current CD4 T-cells/mL)	170 (21-170) [a]	445 (128-1166) [a]	0.0002 [c]
Time on ART (years)	4.5 (2-7.5) [a]	4.5 (1-11.5) [a]	0.99 [c]
TB infection (Positive/Negative)	05-Feb	60/78	0.14 [b]
BPNST (Positive/Negative)	04-Mar	23/115	0.002 [b]
Route of transmission			
IDU	4	42	0.68 [b]
Heterosexual	3	65	
Heterosexual, IDU	0	9	
MSM	0	15	
MSM, IDU	0	1	
others	0	6	
ART regimens:			
3TC, EVF, TDF	1	40	
3TC, TDF, ABC	-	1	
3TC, TDF, LPV/r	1	3	
TDF, LPV/r, FTC	-	15	
AZT, 3TC, EVF	4	39	
AZT, 3TC, LPV/r	-	5	
AZT, 3TC, NVP	1	76	

[a]median (range); [b]Chi^2 test; [c]Mann-whitney test. IDU: intravenous drug user; MSM: men sex men; BPNST: Brief neuropathy peripheral test.

Table 2. Clinical characteristics and DRM of PLWH with assessed for DR mutations.

Code/ Transmission route	ART regimens	HIV RNA[d]	Subtype	NRTI	NNRTI	PI
IDR1 / BPNST POSITIVE	3TC, TDF, LPV/r	5.72	CRF01_AE	M41L, V75M, M184V, L210W, T215F	A98G, G190A	M46I, I50V, I54V, V82A, N88NDGS, L33F, T74P, L89MV
IDR3 / BPNST NEGATIVE	AZT, 3TC, NVP	3.45	CRF01_AE	M184V, K219KQ	K103N, H221Y, F227FL, M230L	
IDR5 / BPNST POSITIVE	AZT, 3TC, EFV	3.9	G	M41L, D67N, K70KR, V75I, M184V, T215F	K103N, V108I, P225PH	-
IDR6 / BPNST POSITIVE	AZT,3TC, NVP	3.99	CRF01_AE	-	-	-
IDR7 / BPNST POSITIVE	AZT, 3TC, EFV	4.34	CRF01_AE	M41ML, M184V, T215Y	K101E, K103N	M46ML, V32VL
IDR9 / BPNST NEGATIVE	AZT, 3TC, EFV	4.92	CRF01_AE	M41L, E44D, V75VIM, M184V, L210W, T215Y	A98G, K101H, V108VI, Y181C, G190A	-
IDR10 / BPNST NEGATIVE	3TC, EFV, TDF	5.16	CRF01_AE	K65R, L74I, M184V	L100I, K103N	-

[a] Log_{10} copies/mL. 3TC: Lamivudine; AZT: Zidovudine; NVP: Nevirapine; TDF: Tenofovir; EVF: Evafirenz; LPV/r: Lopinavir/Ritonavir; ABC: Abacavir.

Statistical analyses

Results are presented as median (range). Mann-Whitney unpaired tests were used to compare groups, and categorical group analyses used Chi-square tests (GraphPad Prism 8, San Diego, CA, USA), with $p < 0.05$ accepted as a significant difference.

Results

Out of 145 PLWH screened, ten had ≥ 1000 copies HIV RNA /mL on the day of sample collection. The sequence was acquired from seven instances. No significant variations were observed in gender, age, and duration on antiretroviral therapy among these seven PLWHs and those with < 1000 copies of HIV RNA/mL (n = 138). PLWH with > 1000 copies of HIV RNA/mL exhibited low CD4 T-cell counts, indicating ongoing virological failure despite undergoing antiretroviral therapy. There was no disparity in tuberculosis prevalence or HIV transmission route between PLWH with ≥ 1000 copies of HIV RNA/mL and those with fewer copies (Table 1). Nevertheless, HIV-SN occurred more frequently in PLWH with ≥ 1000 copies of HIV RNA/mL (4/7 vs 11/145; $p = 0.002$). These findings were clear in the comparison between PLWH carrying DR mutations and those with less than 1000 copies of HIV RNA/mL (3/6 vs 11/145; $p = 0.016$). 50% of people living with HIV who have drug resistance also experienced HIV-SN.

Four patients exhibited mutations impacting NRTI and NNRTI, two patients had mutations affecting NRTI, NNRTI, and PI, and one had no mutations (Table 2). The predominant NRTI mutation was M184V, with M41L, L210W, and T215F also found. The NNRTI mutation K103N had the highest occurrence, with G190A and A98G also found. M184V, M4IL, T215F, K103N, and G190A have been described previously in Indonesia [6,9,15].

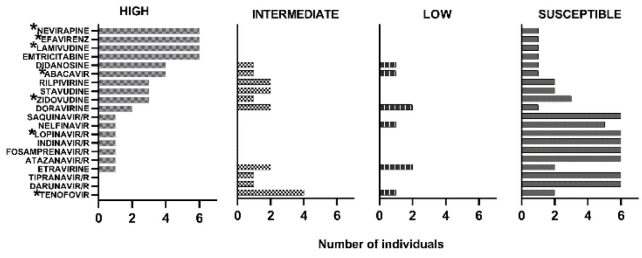
DR mutations were assessed with the HIVdb resistance algorithm [16]. Resistance was detected against multiple antiretroviral drugs in most patients. One PLWH exhibited significant resistance to six PI regimens: atazanafir/ritonavir, fosamprenavir/ritonavir, indinavir/ritonavir, lopinavir/ritonavir, nelfinavir/ritonavir, and saquinavir/ritonavir (Figure 1).

Discussion

We detailed mutations in PLWH on prolonged ART with detectable HIV RNA. This included two cases that appear to be resistant to NRTI, NNRTI, and PI. These patients would benefit from integrase strand transfer inhibitors (INSTi) such as dolutegravir. This has been incorporated into national guidelines across most Southeast Asian countries [11].

The prevalent subtype identified in 6 out of 7 patients was HIV CRF_AE [6]. In Indonesia in 2020,

Figure 1. Levels of drug resistance associated with DRM identified in PLWH with >1000 copies HIV RNA/mL, based on the Stanford HIV drug resistance database algorithm.



*ART drugs used in this study.

CRF_AE was the predominant HIV-1 strain, with subtype B and CRF02_AG following behind [9]. The dominant strains in the TREAT ASIA HIV Observational Database study of 101 patients with virologic failure in the Asia Pacific region, including Indonesia, were HIV subtype C, A1, and CRF01_AE [5]. Here, we identified one patient with HIV-1 subtype G. HIV-1 subtype G was identified in the West Kalimantan, Indonesia region [10]. Subtype G is genetically different from CRF_AE, but its presence here did not correlate with particular drug-resistance mutations. IDR1, an IDU, received treatment with a protease inhibitor (LPV/r) and exhibited eight mutations impacting this drug class, with four major PI mutations: M46I, I50V, I54V, V82A, and N88NDGS. Major mutations were infrequent in Indonesia, typically involving NRTI and PI mutations. Patient IDR7, also an IDU, harbored two mutations impacting PI without ever being treated with PI.

A limitation of our study is the small sample size and the lack of phenotypic drug resistance testing. However, mutations are the most likely cause of treatment failure, and the association with HIV-SN is novel and clinically important.

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