ORIGINAL ARTICLE

Factors Affecting HIV Viral Load of Antiretroviral Therapy-Experienced and Naïve Individuals Residing in Bali, Indonesia

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ABSTRACT

Introduction: In 2020, The Joint United Nations Programme on HIV/AIDS (UNAIDS) updated their target to 95-95-95 by 2030, in which signified 95% individuals receiving antiretroviral therapy (ART) to achieve viral suppression. The Ministry of Health of Indonesia in 2020 reported that only 14% were known to be virally suppressed after six months of ART. This mandates further attention in order for the country to achieve the 95-95-95 target. Viral load (VL) testing is crucial to identify whether PLHIV on ART has achieved viral suppression. This study aimed to measure HIV VL and identify associated factors, in PLHIV experienced and naıve to ART, residing in Buleleng, Bali, Indonesia. **Methods:** A hundred and two people living with HIV (PLHIV) were enrolled in this study. Plasma obtained were subjected to ribonucleic acid (RNA) extraction, followed by quantitative reverse transcriptase (RT) polymerase chain reaction (RT-qPCR). Plasma viral load level of 32 and 58 samples from ART-naive and experienced individuals, respectively, were successfully measure. **Results:** The presence of major HIV drug resistance mutations (HIVDRMs) and the number of ARV affected were significantly contributed to higher VL among ART-experienced individuals, while tuberculosis (TB) co-infection, body mass index (BMI), and WHO clinical stage were significantly cause higher VL among ART-naive individuals. **Conclusion:** Different factors affect the VL of ART- experienced and naive individuals; thus, appropriate recommendation needs to be tailored in order to help PLHIV to achieve adequate viral suppression.

Keywords: HIV, viral load, antiretroviral therapy, Bali, Indonesia

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INTRODUCTION

Human immunodeficiency virus (HIV) infection remains a major public health problem worldwide. In 2020, The Joint United Nations Programme on HIV/AIDS (UNAIDS) updated their target from 90-90-90 by 2020 to 95-95-95 by 2030, aiming for 95% infected individuals to know their status, 95% individuals diagnosed with HIV infection to receive sustainable antiretroviral therapy (ART), and 95% individuals receiving ART to achieve viral suppression (1). The UNAIDS report in 2021 showed that even the 2020 90-90-90 target has yet been fully reached. It was estimated that around 85% infected individuals worldwide were diagnosed, 75% of those diagnosed were on ART, and 92% individuals receiving ART were virally suppressed (2). The Ministry of Health of Indonesia in 2020 reported that only 71% HIV infected individuals were diagnosed, 40% among those diagnosed were on ART, and only 14% were known to be virally suppressed after six months of ART (3). This mandates further attention in order for the country to achieve the 95-95-95 target.

Viral load (VL) testing is crucial to identify whether PLHIV on ART has achieved viral suppression. In many developing countries, access towards VL testing is limited (4–6). Among 10,397 health facilities across Indonesia providing HIV screening and testing, only 35 were equipped with VL testing (3).

With the emergence of HIV drug resistance mutations (HIVDRMs) in several Indonesian regions (7–11). it is essential for PLHIV to access viral load testing. Virological failure is associated with HIVDRMs, and linked to an increased risk of disease progression and mortality (12–14). This study aimed to measure HIV VL and identify associated factors, in PLHIV experienced and naıve to ART, residing in Buleleng, Bali, Indonesia.

MATERIALS AND METHODS

Study design, ethics statement and sample collection

This was a cross sectional study, ethically approved by the Ethics and Law Committee of Universitas Airlangga Hospital (Ethical approval no. 033/KEH/2016). Prior to sample collection, all participants who agreed to enrol in this study were required to provide a written informed consent.

Purposive sampling technique was employed. People living with HIV, who regularly visited the Voluntary Counselling and Testing Clinic of a General Hospital in Buleleng, Bali, were recruited. All PLHIV age 18 years or older, both on ART or newly diagnosed and has yet received ART, were eligible to enroll. Five milliliters of peripheral blood samples were collected from each participants using ethylenediaminetetraacetic acid (EDTA)- anticoagulated tube. Blood plasma was then separated using centrifugation. Demographic and clinical data on study participants were retrieved from medical records.

RNA isolation and viral load testing

HIV RNA isolation was performed using QIAamp DSP Virus Spin Kit (QIAGEN, Hilden, Germany), and followed by HIV VL measurement using quantitative real time polymerase chain reaction (qRT-PCR) method and Artus HI Virus-1 RG RT-PCR Kit (QIAGEN, Hilden, Germany), as per manufacturer instruction. Both kits were in-vitro diagnostic (IVD) certified; thus, acceptable not only for research, but also for HIV clinical VL testing.

Statistical analysis

Statistical analysis towards data collected was carried out using Mann-Whitney and Spearman's correlation test. Statistical significance was defined by a $p \leq 0.05$. All data were analysed using SPSS Version 17.

RESULTS

Demographic and clinical information of study participants

One hundred and two PLHIV were enrolled in this study. Among those, 63 were ART-experienced, while 39 were newly diagnosed and ART-naïve. No participants have any history of HIV VL testing. Youngest participant was 18 years old, and the oldest was 65 years old. Forty-three participants (42.15%) were female. The most common ART combination received was zidovudine (AZT) + lamivudine (3TC) + nevirapine (NVP) (36/63; 57.14%). Demographic and clinical information of study participants are shown in Table I.

HIV viral load

HIV VL testing was successfully performed using RNA isolated from 58 ART-experienced and 32 ART-naïve PLHIV. Several samples with low RNA purity (260/280 ratio far below 2.0 or higher than 2.2), were failed to be amplified using the qRT-PCR method. Mann-Whitney test indicated a significant difference between HIV VL of ART-experienced and ART-naïve individuals ($p=0.000$; $p \leq 0.05$), and Spearman's test shown a significant correlation between ART status and VL ($p=0.000$; $p \leq 0.05$). Forty nine of 58 ART-experienced PLHIV (84.5%) have achieved viral suppression (Table II).

Among ART-experienced individuals, the lowest HIV VL was <50 copies/mL (undetectable VL), and the highest was 65,070 copies/mL. Significantly higher VL ($p \leq 0.05$) was observed among ART-experienced PLHIV with major reverse transcriptase (RT)-related HIVDRMs, as shown in Table III. The number of antiretroviral (ARV) drugs affected by HIVDRMs also contributes to higher VL.

In ART-naïve individuals, the lowest HIV VL was 6,577 copies/mL, and the highest was 723,500 copies/mL. ART-naïve PLHIV with history of tuberculosis (TB) co-infection, lower body mass index (BMI), and higher clinical stadium exhibited significantly higher VL ($p \leq 0.05$), as shown in Table IV. However, no difference was found among PLHIV with or without major RT-related HIVDRMs.

Table I: Demographic and clinical information of study participants

Variable n %		ART-experienced		ART-naïve	
		n	%		
Sex	Female	28	44.4	15	38.5
	Male	35	55.6	24	61.5
Age	18-25	10	15.9	10	25.6
	26-35	23	36.5	19	48.7
	36-45	21	33.3	9	23.1
	46-55	7	11.1	1	2.6
	>55	2	3.2	0	0
Length of ART	6-12 months	16	25.4	N/A	N/A
	13-24 months	12	19.0	N/A	N/A
	25-36 months	9	14.3	N/A	N/A
	37-48 months	10	15.9	N/A	N/A
	49-60 months	4	6.3	N/A	N/A
	>60 months	12	19.0	N/A	N/A
ART regimen	AZT + 3TC + NVP	36	57.1	N/A	N/A
	AZT + 3TC + EFV	3	4.8	N/A	N/A
	TDF + 3TC + NVP	3	4.8	N/A	N/A
	TDF + 3TC + EFV	21	33.3	N/A	N/A
Tuberculosis co-infection	Yes	18	28.6	15	38.5
	No	45	71.4	24	61.5
WHO clinical stage	1	N/A	N/A	0	0
	2	N/A	N/A	9	23.1
	3	N/A	N/A	18	46.2
	4	N/A	N/A	12	30.8
BMI	<18.5	N/A	N/A	11	28.2
	≥18.5	N/A	N/A	28	71.8

AZT, zidovudine; 3TC, lamivudine; NVP, nevirapine; EFV, efavirenz; TDF, tenofovir; N/A, not applicable or data not available.

Table II: Correlation between ART status and HIV viral load

Viral load	ART-experienced		ART-naïve		Spearman's p
	n	%	n	%	
Undetectable (<50 copies/mL)	0	0	49	84.5	0.000*
≥50 – 5,000 copies/mL	0	0	7	12	
>5,000 copies/mL	32	100	2	3.5	
Total	32	100	58	100	

*Significant if p≤0.05

Table III: HIV viral load of ART-experienced individuals

Variables		Viral load median (copies/mL)	p
The presence of reverse transcriptase-related HIV drug resistance mutations	No mutation	0	0.000*
	Minor mutation(s)	0	
	Major mutation(s)	26,398	
The number of antiretroviral drugs affected	0	0	0.000*
	1	1,126	
	3	58,122	

*Significant if p≤0.05

Table IV: HIV viral load of ART-naïve individuals

Variables		Viral load median (copies/mL)	p
Tuberculosis co-infection	Yes	1,489,600	0.004*
	No	0	
Body mass index	< 18.5	1,823,500	0.039*
	≥ 18.5	280,850	
WHO clinical stage	Clinical stage 2	32,674	0.01*
	Clinical stage 3	731,920	
	Clinical stage 4	1,191,200	
The presence of reverse transcriptase-related HIV drug resistance mutations	No mutation	595,590	0.489
	Minor mutation(s)	1,614,500	
	Major mutation(s)	746,300	

*Significant if p≤0.05

DISCUSSION

Viral load testing holds a critical role in regards to achieve the 95-95-95 target sets by the UNAIDS to end HIV infection epidemics by 2030 (1,4–6). In this study, no participants have any history of HIV VL testing, which might be correlated with the limited facilities providing HIV VL testing in Indonesia (3). HIV VL of ART-experienced and ART-naıve were significantly different, with 84.5% ART-experienced individuals achieved viral suppression. Despite the first line ART in Indonesia might still be adequate, this mandates further evaluation in order to achieve 95% viral suppression among individuals achieving ART. First line ART in Indonesia includes two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitors (NNRTI) (15). All ART-experienced participants in this study received first line ART regimen. Integration of other ARV drug class, such as boosted-protease inhibitor (bPI) or integrase inhibitor (InSTI) might be beneficial in helping PLHIV to achieve viral suppression (16,17).

Significantly higher VL (p≤0.05) was observed among ART-experienced individuals with major reverse transcriptase-related HIVDRMs, as shown in Table III. Failure in achieving viral suppression is associated with an increased risk of disease progression and mortality (12–14). Several studies in Indonesia have identified the presence of HIVDRMs among ART-experienced individuals, including in Bali, Indonesia (7,8,11). High levels of HIVDRMs towards NRTI, NNRTI, and PI was observed among PLHIV failing antiretroviral therapy (18,19). The number of ARV drugs affected also contributes to VL. Significantly higher VL (p≤0.05) was observed among individuals with HIVDRMs towards all ARV drugs in the regimen. This should be taken into consideration to determine effective antiretroviral drugs combination, in order to achieve viral suppression.

Significantly higher VL (p≤0.05) was observed among ART-naıve PLHIV with history of tuberculosis (TB) co-infection, lower body mass index (BMI), and higher WHO clinical stadium, as shown in Table IV. Previous study reported higher VL was positively associated with TB development in PLHIV (20) Compared to individuals with HIV/hepatitis C virus (HCV) co-infection, PLHIV co-infected with TB were shown to have a higher VL, with average increase of 2.5 folds (21) Tuberculosis infection shown to correlate with lower CD4 cells count among PLHIV, and contributes to higher HIV VL (22,23). Individuals with higher BMI were reported to exhibit lower viral load and higher CD4 cells count prior to the initiation of ART, with increasing likelihood of achieving immunologic reconstitution over time (24,25). WHO clinical stages were significant disease progression predictor in PLHIV (26). Higher VL (>5000 copies/mL) was associated with a nearly doubled risk of developing a WHO stage 3 or 4 (27). These situations indicate ART-naıve PLHIV with either TB co-infection, lower BMI, or higher WHO clinical stadium to receive further attention in order to achieve sufficient viral suppression.

Unlike in ART-experienced individuals, the presence of major RT-related HIVDRMs among ART-naıve individuals did not contribute to higher VL. However, in order to achieve long-term treatment success, therapy using appropriate ARV is needed in order to achieve viral suppression. High rate of pretreatment drug resistance (PDR) among ART-naıve individuals potentially limited the efficacy of standardized first-

and second-line regimens (28,29). Previous studies in several Indonesia regions such as Bali, Jakarta, and Pontianak, shown a moderate to high prevalence of (PDR) (8–10). This mandates routine HIV resistance testing and adequate intervals of viral load surveillance among PLHIV initiating ART and those already receiving ART. Substitution of ARV or regimen switching might be prescribed when HIVDRMs and persistently high VL were observed. HIVDRMs situation might also affect National's Policy regarding first- and second-line regimen (28–30).

CONCLUSION

Different factors affect the VL of ART- experienced and naive individuals residing in Buleleng, Bali, Indonesia. To achieve adequate viral suppression, appropriate recommendation needs to be tailored in accordance to PLHIV's situation.

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