The Emergence of HIV-1 Transmitted Drug Resistance Mutations Among Antiretroviral Therapy-naive Individuals in Buleleng, Bali, Indonesia

Ni Luh A. Megasari[1,2], Devi Oktafiani[1], Elsa Fitriana[1], Siti Q. Khairunisa[2], Shuhei Ueda[2,3], Tomohiro Kotaki[3], Nasronudin[1,2], Soetjipto[1,2], Masanori Kameoka[3,4]

[1] Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.
[2] Indonesia-Japan Collaborative Research Centre for Emerging and Re-emerging Infectious Diseases, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia.
[3] Department of Public Health, Kobe University Graduate School of Health Sciences, Hyogo, Japan.
[4] Center for Infectious Diseases, Kobe University Graduate School of Medicine, Hyogo, Japan.

Corresponding Author:
Masanori Kameoka, MD. Department of Public Health, Kobe University Graduate School of Health Sciences, 7-10-2 Tomogaoka, Suma-ku, Kobe, Hyogo 654-0142, Japan. email: mkameoka@port.kobe-u.ac.jp.

ABSTRAK

Latar belakang: *peningkatan cakupan terapi antiretroviral (ART) merupakan penyebab utama penurunan mortalitas akibat faktor terkait acquired immune deficiency syndrome (AIDS). Akan tetapi, timbulnya transmitted drug resistance (TDR) mempersempit pilihan ART yang efektif bagi individu yang belum pernah memperoleh terapi (ART-naive), yang mana dapat menghambat kesuksesan terapi. Bali menempati peringkat ke-enam sebagai provinsi di Indonesia dengan jumlah kasus kumulatif infeksi HIV tertinggi. Tujuan penelitian ini untuk mengidentifikasi adanya TDR pada individu ART-naive di Kabupaten Buleleng, Bali.* ***Metode:*** *tiga puluh sembilan individu ART-naive berpartisipasi dalam studi ini. Analisis genotipik dilakukan terhadap sampel darah yang diperoleh dari partisipan.* ***Hasil:*** *28 gen protease (PR) dan 30 gen reverse transcriptase (RT) dari 37 sampel berhasil dianalisis. Subtyping menunjukkan CRF01_AE sebagai circulating recombinant yang paling dominan di Kabupaten Buleleng, Bali. TDR terhadap inhibitor PR tidak ditemukan, akan tetapi TDR terhadap inhibitor RT teridentifikasi pada lima dari 30 individu (16,7%).* ***Kesimpulan:*** *adanya TDR pada individu ART-naive di Kabupaten Buleleng, Bali, perlu diperhatikan karena dapat menghambat keberhasilan terapi dan mempersempit pilihan terapi yang efektif. Surveilans berkesinambungan perlu dilakukan untuk monitoring TDR pada individu ART-naive.*

Kata kunci: *HIV-1, CRF01_AE, Bali, antiretroviral therapy (ART), transmitted drug resistance (TDR).*

ABSTRACT

Background: *the global scale-up of antiretroviral therapy (ART) is the primary factor contributing to the decline in deaths from acquired immune deficiency syndrome (AIDS)-related illnesses. However, the emergence of transmitted drug resistance (TDR) compromises the effects of ART in treatment-naïve individuals, which may hinder treatment success. The present study aimed to identify the presence of TDR among treatment-naive individuals in Buleleng, Bali, which is currently ranked sixth among Indonesian provinces with the highest cumulative human immunodeficiency virus type 1 (HIV-1) infection cases.* ***Methods:*** *thirty-nine ART-naive individuals in Buleleng Regency General Hospital were enrolled in the present study. Blood samples from participants were subjected to a genotypic analysis.* ***Results:*** *28 protease (PR) and 30 reverse transcriptase (RT) genes were successfully amplified*

and sequenced from 37 samples. HIV-1 subtyping revealed CRF01_AE as the dominant circulating recombinant form in the region. No TDR for PR inhibitors was detected; however, TDR for RT inhibitors was identified in five out of 30 samples (16.7%). ***Conclusion:*** *these results indicate the emergence of TDR among ART-naive individuals in Buleleng, Bali. This issue warrants serious consideration because TDR may hamper treatment success and reduce ART efficacy among newly diagnosed individuals. Continuous surveillance with a larger sample size is necessary to monitor TDR among ART-naive individuals.*

Keywords: *HIV-1, CRF01_AE, Bali, antiretroviral therapy (ART), transmitted drug resistance (TDR).*

INTRODUCTION

The Joint United Nations Programme on human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) (UNAIDS) reported a marked reduction in deaths from AIDS-related illnesses, from a peak of 1.9 million in 2004 to 940,000 in 2017. The global scale-up of antiretroviral therapy (ART) is the primary factor contributing to the decline in deaths.[1] Combination ART decreases the replication of HIV type 1 (HIV-1), thereby improving the survival of infected individuals and lowering the risk of transmission.[2,3]

UNAIDS estimated a decline in new HIV infections in Indonesia, from 62,000 cases in 2005 to 49,000 cases in 2017.[1] The Indonesian Ministry of Health reported 280,623 cumulative HIV infection cases up to December 2017, with 102,667 cumulative AIDS cases. Provinces with the highest cumulative HIV infection and AIDS cases include DKI Jakarta, East Java, Papua, West Java, Central Java, and Bali.[4]

HIV-1, which is responsible for most of the global HIV pandemic, has been subdivided into group M (major), group O (outlier), group N (non-major, non-outlier), and new group P (pending). Group M, the pandemic group of HIV-1, has been further divided into subtypes A to K. Besides these subtypes, circulating recombinant forms (CRFs) and unique recombinant forms (URFs), as a result of recombination among 2 or more subtypes and/or CRFs, have also been identified in group M. CRF01_AE, the second predominant circulating CRF accounting for 5% of infection cases worldwide in 2004-2007, is responsible for the vast majority of infections in Southeast Asia, including several regions in Indonesia.[5–10]

In December 2017, 91,369 Indonesians were receiving ART; 88,386 among them received a first-line regimen, and 2,983 a second-line regimen.[4] First-line ART regimens comprise two nucleoside reverse transcriptase (RT) inhibitors (NRTIs) and a non-nucleoside RT inhibitor (nNRTI), while two NRTIs plus a ritonavir-boosted protease (PR) inhibitor (PI) are adopted for second-line regimens.[11]

Although ART is successful in Indonesia in reducing AIDS case fatality rate since 2004, the emergence of drug resistance has been reported not only among ART-experienced individuals, but also among newly diagnosed, ART-naive individuals.[6–10] The emergence of drug resistance-associated mutations (DRMs) compromises the effectiveness of ART, resulting in lower viral suppression and hinders treatment success.[12] Several DRMs in the HIV-1 pol gene are known to reduce viral susceptibility towards treatment, particularly a first-line regimens combining a NRTI and nNRTI.[13] Thus, the presence of DRMs may be a cause for concern in various countries including Indonesia, where 88,386 out of 91,369 individuals on ART (96.7%) receive a first-line regimen[11], and thus rely heavily on ART with first-line regimens for the treatment of HIV-1.[4]

The emergence of transmitted drug resistance (TDR) is attributed to the transmission of a drug-resistant virus.[14] TDR compromises the effectiveness of ART for treatment-naïve individuals.[15] Our previous studies revealed the appearance of TDR for RT inhibitors among 4.3% (2/47) and 12.9% (4/31) of ART-naive individuals residing in Surabaya and West Papua, respectively.[9,10] Bali, a popular tourism destination,[16] is now ranked sixth among Indonesian provinces with the highest cumulative HIV cases, and ranks fifth for cumulative AIDS cases.[4] Acquired drug resistance were previously reported to be found in 10% of ART-experienced

individuals in Buleleng, a regency located in the northern of Bali.[17] The present study aimed to identify the presence of TDR among ART-naive individuals in Buleleng, Bali.

METHODS

We determined the necessary sample size by consulting with a statistic lecturer at the Faculty of Medicine, Universitas Airlangga, and recruited 39 individuals recently diagnosed with HIV-1 infection at the Voluntary Counselling and Testing Clinic of Buleleng Regency General Hospital, Bali. Five milliliters of ethylenediaminetetraacetic acid (EDTA)-anticoagulated peripheral blood samples were collected from study participants in February 2018. DNA was then extracted from whole blood samples using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). Demographic and clinical data on study participants were retrieved from medical records.

Amplification of HIV-1 Genomic Fragments

Viral pol gene encoding full-length PR (PR gene) and RT (RT gene) was amplified from DNA extracted from peripheral blood samples by the nested polymerase chain reaction (PCR) using GoTaq Green Master Mix (Promega, Madison, WI, USA) and the following primers. The primers DRPRO5, 5′-AGACAGGYTAATTTTTTAGGGA-3′ [corresponding to nucleotides (nt) 2074-2095 of the HIV-1 reference strain, HXB2 (GenBank accession no. K03455)] and DRPRO2L, 5′-TATGGATTTTCAGGCCCAATTTTTGA-3′, (nt 2716 to 2691) were used in first PCR for the amplification of the PR gene, and the primers DRPRO1M, 5′-AGAGCCAACAGCCCCACCAG-3′ (nt 2148 to 2167) and DRPRO6, 5′-ACTTTT GGGCCATCCATTCC-3′ (nt 2611 to 2592) were used for nested PCR. The primers RT1L, 5’-ATGATAGGGGGAATTGGAGGTTT-3’ (nt 2388 to 2410) and GPR2M, 5’-GGACTA CAGTCYACTTGTCCATG-3’ (nt 4402 to 4380) were used in first PCR for the amplification of the RT gene, while RT7L, 5’-GACCTACACCTGTCAACATAATTGG-3’ (nt 2485 to 2509) and GPR3L, 5’-TTAAAA TCACTARCCATTGYTCTCC-3’ (nt 4309 to 4285) were used for nested PCR.

Sequencing Analysis, HIV-1 Subtyping, and Detection of Drug Resistance-associated Mutations

Successfully amplified viral PR and RT genes were subjected to a sequence analysis performed by Macrogen South Korea (http://dna.macrogen.com). Sequencing data were assembled and aligned using Genetyx version 10 software (Genetyx, Tokyo, Japan). HIV-1 subtyping was then conducted using the Recombinant Identification Program (RIP) available on the HIV sequence database website (www.hiv.lanl.gov)[17] and jumping profile Hidden Markov Model (jpHMM) (http://jphmm.gobics.de/submission_hiv).[18] In addition, neighbor-joining (NJ) trees with a Kimura two-parameter model were constructed using MEGA 6.2 software with bootstrap values (1,000 replicates) for relevant nodes being reported on a representative tree. The appearance of DRMs in successfully sequenced PR and RT genes was analyzed according to the International Antiviral Society-USA (IAS-USA) panel.[13] The nucleotide sequences of viral gene fragments have been deposited in the GenBank database under accession numbers MK656030-MK656087.

Ethics Statement

Ethical approval for this research was obtained from the Ethics and Law Committee of Universitas Airlangga Hospital (Ethical approval no. 033/KEH/2016) and the Institutional Ethics Committee of Kobe University Graduate School of Medicine (approval no.: 784). Written informed consent was obtained from all study participants prior to sample collection.

RESULTS

Demographic and Clinical Information on Study Participants

All participants were confirmed to be ART-naive from medical records. Twenty-four (66.7%) participants were male. The youngest participants were 16 years old and the oldest was 47 years old, with the most predominant age group being 30-34 years old. Fifteen individuals (38.5%) were co-infected with tuberculosis (TB). Regarding the clinical stage of HIV

infection, 9 (23.1%), 18 (46.2%), and 12 (30.8%) individuals were classified as stages 2, 3, and 4, respectively. Among participants, 11 (28.2%) were underweight (body mass index <18.5). The demographic and clinical data of 39 study participants are shown in **Table 1**.

Table 1. Demographic and clinical data of ART-naïve individuals in Buleleng regency, Bali

Variables	n (%)
Sex	
- Male	24 (66.7)
- Female	15 (41.7)
Age (years)	
- 15-19	4 (10.3)
- 20-24	6 (15.4)
- 25-29	8 (20.5)
- 30-34	9 (23.1)
- 35-39	8 (20.5)
- 40-44	3 (7.7)
- 45-49	1 (2.6)
Clinical stage	
- II	9 (23.1)
- III	18 (46.2)
- IV	12 (30.8)
TB co-infection	
- Yes	15 (38.5)
- No	24 (61.5)
Underweight	
- Yes (BMI <18.5)	11 (28.2)
- No (BMI ≥18.5)	28 (71.8)

TB, tuberculosis; BMI, body mass index

HIV-1 Subtyping

Sequencing data were successfully obtained from 37 out of 39 samples, comprising 28 PR genes (297-bp; nt 2253 to 2549) and 30 RT genes (1680-bp; nt 2550 to 4229). HIV-1 subtyping by RIP and jpHMM was consistent with that by NJ trees (data not shown). Thirty-six samples (97.3%) were classified as CRF01_AE, while a sample (2.7%) was classified as CRF02_AG. The NJ trees of the PR and RT genes are shown in **Figure 1**.

Appearance of DRMs

TDR was defined as the presence of at least one major DRM listed in the International AIDS Society United States (IAS-USA) panel database.[13] Regarding the results obtained, no TDR was detected in PR genes; however, several minor mutations were detected. Among 28 PR genes, 10 (35.7%) contained L10I/V [amino acid substitution from leucine (L) to isoleucine (I) or valine (V) at position 10 in the PR gene], 12 (42.9%) G16E, 11 (39.3%) K20R/I, 28 (100%) M36I, one (3.6%) D60E, eight (28.6%) L63P, 26 (92.9%) H69K, one (3.6%) A71V, one (3.6%) V77I, six (21.4%) V82I, 28 (100%) L89I, and 10 (35.7%) I93L. M36I, H69K, and L89I, which presented in more than 90% samples, are common polymorphisms in non-B subtypes, including CRF01_AE.[19]

Five out of 30 samples (16.7%) possessed TDR in the RT genes. The demographic characteristics of individuals with major DRMs in RT genes are shown in **Table 2**. The E138G/A mutation was detected in 2 samples (6.24%), while other major mutations, including K103N, G190A, and K219Q, were each found in one sample (3.12%). Besides the major DRMs, minor mutations were detected in 4 samples (13.3%), including V90I (3.12%), V106I (3.12%), and V179D/F (6.24%).

DISCUSSION

We herein report the circulating HIV-1 subtype and prevalence of TDR among HIV-1-infected, ART-naive individuals residing in Buleleng, Bali, Indonesia. Among 37 successfully sequenced samples, 36 (97.3%) were classified as CRF01_AE, and the remainder (2.7%) as CRF02_AG. These results are consistent with previous findings for the predominance of CRF01_AE in various regions in Indonesia.[6–10] However, the HIV-1 gene fragments analyzed in the present study were insufficient to identify the actual CRF since recombinant forms of HIV-1 possibly contain various sequences derived from more than 2 different subtypes and/or CRFs. Therefore, full-genomic sequencing analyses of the HIV-1 genome must be carried out in a future study.

A genotypic drug resistance study revealed no evidence of circulating PI-related TDR in Buleleng. This may have been due to the limited usage of PIs in this region. Among ART-experienced individuals in Indonesia, only 3.3% (2,983 of 91,369) were receiving a PI-

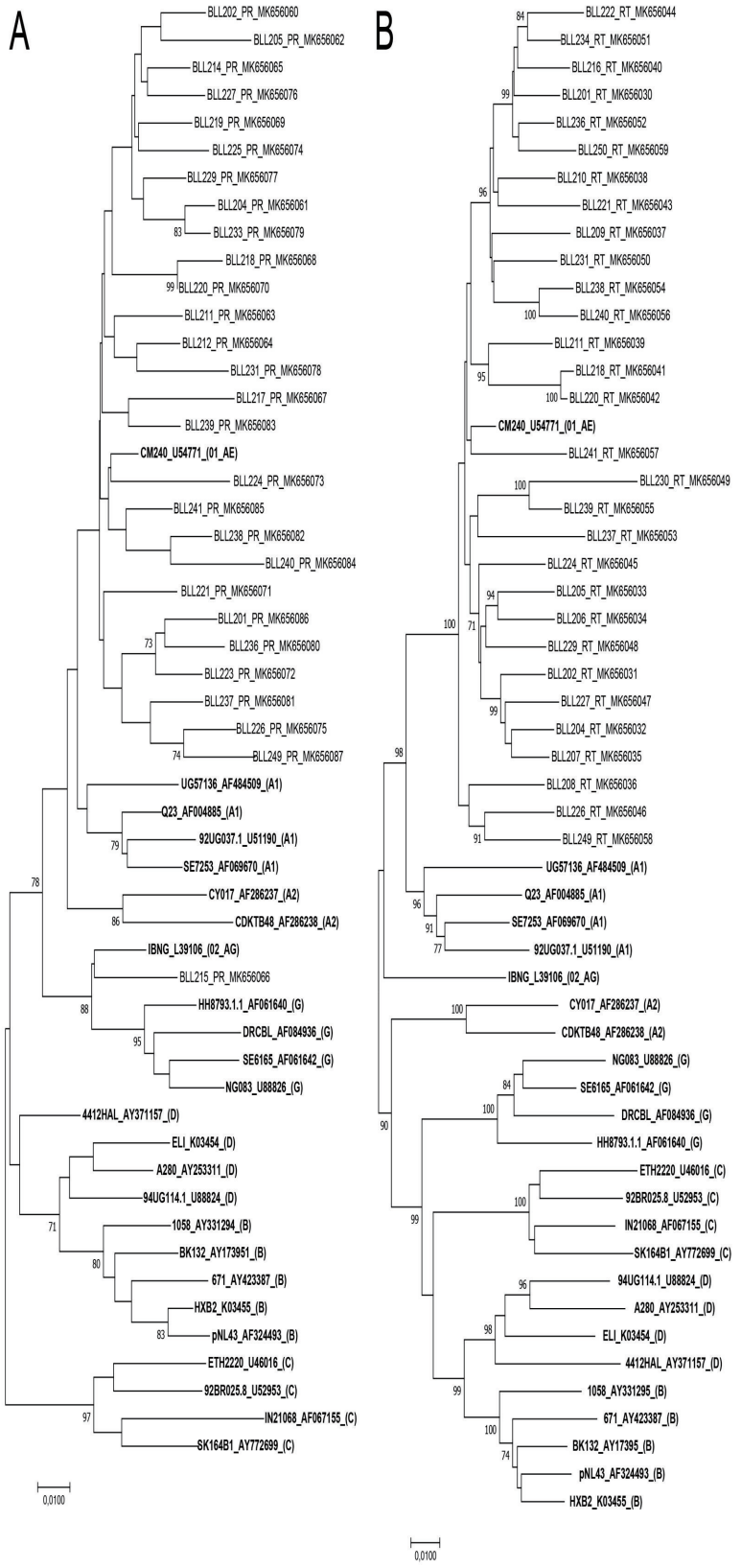

Figure 1. Phylogenetic tree analysis of HIV-1 PR and RT gene sequences collected from ART-naive individuals in Buleleng, Bali, Indonesia. Phylogenetic trees were constructed for the HIV-1 PR (A) and RT (B) genes newly sequenced in the present study. The corresponding viral genes of reference HIV-1 strains representing subtypes A1, A2, B, C, D, and G, as well as CRF01_AE (01_AE) and CRF02_AG (02_AG) were included in the analyses (shown in bold). Sequence IDs are presented as a sample ID or the ID of the reference HIV-1 strain, a GenBank accession number, and the subtype or CRF of the reference strain (shown in parentheses) in that order. Bootstrap values were shown if they were >70.

Table 2. Demographic characteristics and drug resistance-associated major mutations in RT genes derived from ART-naïve individuals in Buleleng Regency, Bali.

ID	Age (years)	Sex	Subtype[b]	Drug Resistance Mutations[a]		
				NRTI	nNRTI	Resistance
BLL202	38	Male	CRF01_AE		K103N	Efavirenz, nevirapine
BLL222	29	Female	CRF01_AE		E138A	Etravirine, rilpivirine
BLL227	29	Female	CRF01_AE		G190A	Efavirenz, etravirine, nevirapine
BLL229	47	Male	CRF01_AE	K209Q		Multi-NRTI, stavudine, zidovudine
BLL240	23	Female	CRF01_AE		E138G	Etravirine, rilpivirine

[a] Drug resistance mutations were based on guidelines published by the International Antiviral Society-USA (IAS-USA).
[b] The subtype of the RT gene was assigned based on RIP and phylogenic analyses.
RT, reverse transcriptase; NRTI, nucleoside reverse transcriptase inhibitor; nNRTI, non-nucleoside reverse transcriptase inhibitor; RIP, recombinant identification program.

based regimen in 2017. Although no TDR was detected in PR genes, minor mutations, including L10I/V, G16E, K20R/I, M36I, D60E, L63P, H69K, A71V, V77I, V82I, L89I, and I93L, were identified in the present study. These mutations potentially affect viral susceptibility to ritonavir-boosted atazanavir (ATV/r), ritonavir-boosted fosamprenavir (FPV/r), ritonavir-boosted indinavir (IDV/r), ritonavir-boosted lopinavir (LPV/r), nelfinavir (NFV), ritonavir-boosted saquinavir (SQV/r), and ritonavir-boosted tipranavir (TPV/r).[13] The presence of these minor mutations needs to be taken into cosideration because LVP/r, which is recommended by the Indonesian Ministry of Health as a second-line regimen of ART[11], is potentially affected.

In contrast, the prevalence of TDR against RT inhibitors was 16.7% (5/30), which is higher than that reported in previous studies in Surabaya (4.3%; 2/47) and West Papua (12,9%; 5/31).[9,10] Identified TDR, including E138G/A, K103N, G190A, and K219Q, may affect nNRTIs, such as rilpivirine (RPV), efavirenz (EFV), nevirapine (NPV), and etravirine (ETV), and NRTIs, including zidovudine (AZT) and stavudine (d4T). K219Q, which is also known as a thymidine analogue-associated mutation (TAM), is associated with multi-NRTI resistance, excluding emtricitabine (FTC) and lamivudine (3TC). AZT, EFV, and NPV are included as recommended options for individuals starting first-line ART in Indonesia;[11] thus, the presence of TDR affecting these inhibitors need to be considered. Minor mutations, including V90I, V106I, and V179D/F, were also detected. These minor mutations may affect ETV.[13]

Based on clinical data, 38.5% ART-naive individuals were co-infected with TB. TB is regarded as the leading opportunistic disease and cause of death in individuals with HIV infection.[20,21] Regarding the nutritional status, 28.2% individuals were underweight. Low BMI is correlated with mortality in HIV-infected individuals,[21–23] as higher BMI and fat mass among ART-naïve individuals were reported to be associated with slower disease progression.[24] A previous study reported that women who were underweight prior to ART died from AIDS more than twice as rapidly than normal weight women.[25] As for the clinical stage of HIV infection, 46.2 and 30.8% individuals were classified as stages 3 and stage 4, respectively. Higher clinical stages were correlated with higher mortality in HIV-infected individuals.[21,22]

The results of the present study indicate the emergence of TDR among ART-naive individuals in Buleleng, Bali. In the WHO TDR surveillance guidelines, the prevalence of TDR is categorized into three groups: low level (<5%), moderate level (5–15%), and high level (>15%).[26] According to this guideline, the prevalence of TDR in Buleleng is considered to be high level, indicating inadequate first-line regimens. However, the present results may have overestimated the prevalence of TDR because there were several limitations in the design of the study. The WHO guidelines aim to recruit recently infected individuals (younger than 25 years of age and CD4+ T-cell counts higher than 500 cells/mm^3);[27] however, these criteria were not

applied in the present study for practical reasons. The number of samples collected was also limited. During sample collection in February 2018, less than 50 individuals were newly diagnosed with HIV infection at the Voluntary Counselling and Testing Clinic of Buleleng Regency General Hospital, Bali. Among those individuals, only 39 individuals were agreed to be enrolled in this study. We believe the 39 samples are a minimum necessary number for this study. Therefore, in order to clarify and monitor TDR among ART-naive individuals, continuous surveillance with a larger sample size and compliance with the WHO selection criteria for TDR surveillance are necessary. Besides TDR, the presence of individuals who were co-infected with TB, underweight, and diagnosed with a higher clinical stage also need proper consideration, as these conditions were correlated with HIV-related mortality.[20–25]

CONCLUSION

There is the emergence of TDR was found among ART-naive individuals in Buleleng, Bali. This issue warrants serious consideration because TDR may hamper treatment success and reduce ART efficacy among newly diagnosed individuals. Continuous surveillance with a larger sample size is necessary to monitor TDR among ART-naive individuals.

ACKNOWLEDGMENTS

This work was supported by Kementerian Riset, Teknologi, dan Pendidikan Tinggi Republik Indonesia through the Pendidikan Magister menuju Doktor untuk Sarjana Unggul (PMDSU) scholarship. This work was also supported by the Japan Initiative for the Global Research Network on Infectious Diseases (J-GRID) from the Ministry of Education, Culture, Sport, Science and Technology in Japan, and the Japan Agency for Medical Research and Development (AMED); and the Institute of Tropical Disease as the Center of Excellence (COE) program by Kementerian Riset, Teknologi, dan Pendidikan Tinggi Republik Indonesia. We sincerely thank the staff at the Voluntary Counselling and Testing Clinic of Buleleng Regency General Hospital, Bali, for their kind cooperation with this study.

REFERENCES

1. Joint United Nations Programme on HIV/AIDS (UNAIDS). UNAIDS Data 2018 [Internet]. 2018. Available from: www.unaids.org/sites/default/files/media_asset/unaids-data-2018_en.pdf.
2. HIV-CAUSAL Collaboration. Ray M, Logan R, Sterne JAC, et al. The effect of combined antiretroviral therapy on the overall mortality of HIV-infected individuals. AIDS [Internet]. 2010 Jan 2;24(1):123–37.
3. Cohen MS, Chen YQ, McCauley M, et al. Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med [Internet]. 2011;365(6):493–505.
4. Direktorat Jenderal Pencegahan dan Pengendalian Penyakit Kementerian Kesehatan RI (Ditjen P2P Kemenkes RI). Laporan Situasi Perkembangan HIV-AIDS & PIMS di Indonesia Januari-Desember 2017 [Internet]. 2018. Available from: http://siha.depkes.go.id/portal/files_upload/Laporan_HIV_AIDS_TW_4_Tahun_2017__1_.pdf.
5. Hemelaar J. The origin and diversity of the HIV-1 pandemic. Trends Mol Med [Internet]. 2012;18(3):182–92.
6. Khairunisa SQ, Kotaki T, Witaningrum AM, et al. Appearance of drug resistance-associated mutations in human immunodeficiency virus type 1 protease and reverse transcriptase derived from drug-treated Indonesian patients. AIDS Res Hum Retroviruses [Internet]. 2015;31(2):255–9.
7. Yunifiar MQ, Kotaki T, Witaningrum AM, et al. Sero- and molecular epidemiology of HIV-1 in Papua Province, Indonesia. Acta Med Indones [Internet]. 2017;49(3):205–14.
8. Khairunisa SQ, Ueda S, Witaningrum AM, et al. Genotypic characterization of human immunodeficiency virus type 1 Prevalent in Kepulauan Riau, Indonesia. AIDS Res Hum Retroviruses [Internet]. 2018;34(6):555–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29589465.
9. Kotaki T, Khairunisa SQ, Witaningrum AM, et al. HIV-1 transmitted drug resistance mutations among antiretroviral therapy-Naïve individuals in Surabaya, Indonesia. AIDS Res Ther [Internet]. 2015;12:5.
10. Witaningrum AM, Kotaki T, Khairunisa SQ, et al. Genotypic characterization of human immunodeficiency virus type 1 derived from antiretroviral therapy-naive individuals residing in Sorong, West Papua. AIDS Res Hum Retroviruses [Internet]. 2016;32(8):812–7.
11. Kementerian Kesehatan Republik Indonesia. Peraturan Menteri Kesehatan Republik Indonesia Nomor 87 Tahun 2014 tentang Pedoman Pengobatan Antiretroviral [Internet]. 2014. Available from: siha.depkes.go.id/portal/files_upload/Buku_Permenkes_ARV_Cetak.pdf.

12. Wittkop L, Günthard HF, de Wolf F, et al. Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV (EuroCoord-CHAIN joint project): a European multicohort study. Lancet Infect Dis [Internet]. 2011;11(5):363–71.
13. Wensing AM, Calvez V, Günthard HF, et al. 2017 update of the drug resistance mutations in HIV-1. Top Antivir Med [Internet]. 24(4):132–3.
14. Yang W-L, Kouyos R, Scherrer AU, et al. Assessing the paradox between transmitted and acquired HIV type 1 drug resistance mutations in the Swiss HIV cohort study from 1998 to 2012. J Infect Dis [Internet]. 2015;212(1):28–38.
15. Bertagnolio S, Perno CF, Vella S, Pillay D. The impact of HIV drug resistance on the selection of first- and second-line ART in resource-limited settings. J Infect Dis [Internet]. 2013;207 Suppl:S45-8.
16. Utama IGBR. Strategi menuju pariwisata Bali yang berkualitas. J Kaji Bali. 2013;03(02):69–90.
17. Megasari NLA, Oktafiani D, Ana EF, et al. Genotypic characterization of human immunodeficiency virus type 1 isolated from antiretroviral treatment-experienced individuals in Buleleng Regency, Bali, Indonesia [published online ahead of print May 21, 2019]. AIDS Res Hum Retroviruses. doi:10.1089/aid.2019.0058.
18. Siepel AC, Halpern AL, Macken C, Korber BT. A computer program designed to screen rapidly for HIV type 1 intersubtype recombinant sequences. AIDS Res Hum Retroviruses [Internet]. 1995;11(11):1413–6.
19. Bulla I, Schultz A-K, Meinicke P. Improving hidden Markov models for classification of human immunodeficiency virus-1 subtypes through linear classifier learning. Stat Appl Genet Mol Biol [Internet]. 2012;11(1):Article 1.
20. Iemwimangsa N, Pasomsub E, Sukasem C, Chantratita W. Surveillance of HIV-1 drug-resistance mutations in Thailand from 1999 to 2014. Southeast Asian J Trop Med Public Health [Internet]. 2017;48(2):271–81.
21. Trinh QM, Nguyen HL, Nguyen VN, Nguyen TVA, Sintchenko V, Marais BJ. Tuberculosis and HIV co-infection-focus on the Asia-Pacific region. Int J Infect Dis [Internet]. 2015;32:170–8.
22. Tadege M. Time to death predictors of HIV/AIDS infected patients on antiretroviral therapy in Ethiopia. BMC Res Notes [Internet]. 2018;11(1):761.
23. Masiira B, Baisley K, Mayanja BN, Kazooba P, Maher D, Kaleebu P. Mortality and its predictors among antiretroviral therapy naïve HIV-infected individuals with CD4 cell count ≥350 cells/mm^3 compared to the general population: data from a population-based prospective HIV cohort in Uganda. Glob Health Action [Internet]. 2014;7:21843.
24. Naidoo K, Yende-Zuma N, Augustine S. A retrospective cohort study of body mass index and survival in HIV infected patients with and without TB co-infection. Infect Dis poverty [Internet]. 2018;7(1):35.
25. Martinez SS, Campa A, Bussmann H, et al. Effect of BMI and fat mass on HIV disease progression in HIV-infected, antiretroviral treatment-naïve adults in Botswana. Br J Nutr [Internet]. 2016;115(12):2114–21.
26. Sharma A, Hoover DR, Shi Q, et al. Relationship between body mass index and mortality in HIV-infected HAART users in the women's interagency HIV study. PLoS One [Internet]. 2015;10(12):e0143740.
27. World Health Organization. World Health Organization Global Strategy for the Surveillance and Monitoring of HIV Drug Resistance [Internet]. 2012. Available from: https://apps.who.int/iris/bitstream/handle/10665/77349/9789241504768_eng?sequence=1.