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HIV Drug Resistance among Pre-treatment Cases in Thailand: Four Rounds of Surveys during 2006-2013

Sombat Thanprasertsuk[1,*], **Kunjanakorn Phokhasawad**[2], **Achara Teeraratkul**[2], **Sanchai Chasombat**[3], **Naparat Pattarapayoon**[4], **Siriphan Saeng-aroon**[5], **Porntip Yuktanon**[4], **Surapol Kohreanudom**[4], and **Cheewanan Lertpiriyasuwat**[6]

[1]Department of Disease Control, Ministry of Public Health, Thailand [2]Thailand MOPH-US CDC collaboration, Division of Global HIV and Tuberculosis, Thailand [3]Bureau of Vector Borne Diseases, Department of Disease Control, Ministry of Public Health, Thailand [4]Bureau of AIDS, TB and STIs, Department of Disease Control, Ministry of Public Health, Thailand [5]National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand [6]Institute of Research, Knowledge Management and Standards for Disease Control, Department of Disease Control, Ministry of Public Health, Thailand

Abstract

In Thailand, antiretroviral therapy (ART) was initiated to treat human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) cases using the empirical regimen with no prior genotypic test to determine drug resistance. In order to assess prevalence rate of HIV drug resistance (HIVDR) among pre-treatment cases, four rounds of survey were carried out in ART clinics, including six, eight, 33 and four ART clinics in each round during 2006–2013. For which, HIVDR testing results were available in 310, 350, 797, and 413 cases in four rounds. It was revealed that HIVDR rates among naive cases were 2.0%, 2.8%, 4.0% and 4.8%, while in experienced cases, the rates were 0, 3.3%, 11.4% and 13.9%. The rates among all cases were 1.9%, 2.9%, 4.4% and 5.6%. Resistant drugs with the highest rates among all cases in the survey round 4 were nevirapine (3.6%) and efavirenz (3.1%). The results indicated the need to continue surveillance for pre-treatment HIVDR, and posed challenges to implement activities for protecting efficacy and prolong the use of empirical first-line regimen. A strategy to apply genotyping test, in a cost-effective approach, should be considered to prepare for situation when HIVDR increases beyond a critical level.

Keywords

antiretroviral therapy; HIV; resistance; pre-treatment; Thailand

[*]Corresponding author, sombat.than@yahoo.com.

Introduction

The antiretroviral therapy (ART) has been scaled up in Thailand for all eligible human immunodeficiency virus infection (HIV) infected cases and acquired immune deficiency syndrome (AIDS) since 2002.[1] As of September 2014, 271,652 people living with HIV/AIDS (PLHIV) were treated with ART in nearly 1,000 ART clinics nationwide.[2] The first national HIV/AIDS treatment guideline was published in 2002, and the enrollment criteria were revised in 2010 and 2014. Highly active ART, consisting of two nucleoside reverse transcriptase inhibitors (NRTI) and one non- nucleoside reverse transcriptase inhibitor (NNRTI), is recommended as an empirical first-line regimen with no prior genotyping. Criteria for enrollment to ART in earlier guidelines were symptomatic cases with CD4 count at 200 cells/μl or less. However, the recruitment criteria using CD4 level has been shifted to 350 cells/μl or less in 2010.[3] Since 2014, PLHIV are eligible for ART, regardless of CD4 level.[4]

Monitoring of treatment includes regular testing of CD4 and viral load (VL). Cases with good drug adherence and VL of more than 1,000 copies/ml after a year of treatment are tested with genotypic analysis to identify possible antiretroviral drug resistance. Reports of genotyping are used for deciding to switch to a second-line regimen. All recommended treatment and laboratory testing costs are subsidized by health insurance schemes.

The objective of this study was to assess the prevalence of HIV drug resistance (HIVDR) in ART pre-treatment PLHIV. The Bureaus of AIDS, tuberculosis and sexually transmitted infections, with technical support from the Thailand MOPH – US CDC Collaboration, launched the survey projects among newly enrolled PLHIV initiating ART in selected clinics since 2005. Up through 2013, four rounds of surveys were conducted. Monitoring of HIVDR prevalence rates among ART pre-treatment cases overtime enables the national program to review the efficacy of empirical first-line treatment regimen.

Methods

The survey was designed to describe characteristics of pre-ART cases and assess prevalence of HIVDR. The first round was carried out in six clinics in 2006, and subsequently in eight clinics in 2007, 33 in 2008–2009 and four clinics in 2013. To collect sufficient specimens, duration of each survey ranged between 6–15 months (Table 1).

Sample Size Estimation

Sample size was calculated using the standard normal approximation set for expected proportion of treatment failure and/or observed genotypic mutation between 8– 25%. Distance from proportion to limit was ±2–5%. Sample size of each survey was at least 300 naive cases.

Survey Site Selection Criteria

The sites were selected purposively in each round. Selected criteria included ability to provide ART for HIV cases, having on site laboratory facilities or being connected to

another laboratory to monitor treatment results, possessing the required data set, and being forecasted to have sufficient cases for the survey.

Population Frame and Data Collection

The study population was PLHIV aged 18 years old or above. Cases eligible for the first-line ART initiation at the sites were those who were naive to ART, or who were experienced to ART and had stopped using ART (ART prophylaxis) or mother to child prevention. Consecutive sampling of every patient presented at the clinic was used until the enrollment period ended.

Data were extracted from the routinely collected data, including demographic data (gender, age, marital status, education and occupation), clinical findings (asymptomatic or symptomatic), history of previous exposure (naive or experienced) and CD4 results.

Specimen Collection and HIV Genotypic Test

Plasma for VL and genotyping were separated on site. Samples were shipped in cold chain using frozen cold packs. Duration from blood drawn to reach the laboratory was warranty processed within 72 hours without temperature monitoring.

The key laboratory tests were HIV VL and genotyping. In all rounds, VL was performed for all cases at the pre-treatment stage in the regular laboratory connected to each ART clinic. Genotypic test was performed in subjects with VL more than 1,000 copies/ml as recommended[3]. In the first round, genotyping was performed at Chiang Mai University using the TRUGENE HIV-1 genotyping Kit. In the second and third rounds, tests were carried out at the regular laboratories using the same commercial kit. In the fourth round, the in-house test was conducted at the National Institute of Health, World Health Organization (WHO) and a designated laboratory for HIVDR testing for surveillance using both reverse transcriptase (RT) and protease inhibitor (PI) primers. The methodology followed as previously described[5,6] and sequences were then interpreted using the Stanford HIV drug resistance database[7].

In this study, major drug resistance mutation interpreted by the genotypic test with the most updated version at the time of each survey was reported as resistance. Resistance to PIs was not analyzed since PI was not used in the first-line regimen and to avoid misleading factors from naturally occurring polymorphism[8].

Data Analysis

Demographic and other collected data were analyzed to observe frequency distribution of each variable. Survey statistics adjusted for clusters and Kruskal-Wallis test were used to test significant differential of each characteristic between the surveys. Likelihood- ratio chi-square for trend was applied to test HIVDR prevalence by rounds.

Trends of HIVDR prevalence rate among naive and experienced cases were determined with the likelihood-ratio chi-square test for trend analysis using Stata statistical software version

13 (College station, Tx stataCorpLP). Frequency of resistance to each drug was also analyzed.

Ethical Consideration

Cases were fully informed of the objectives and benefits of the survey. Data were collected after an informed consent was obtained. Participant's confidentiality was maintained using anonymous testing protocol. For subjects found to have HIVDR, the treatment was switched to second-line regimen according to the national guideline.

The Ethical Review Committee for Research in Human Subjects in the Ministry of Public Health, Thailand, approved Survey 1 as endorsed by document number 60/2007. The ethical approval was extended for Surveys 2 and 3 in the official letter with reference number 0327/2534 dated 11 Dec 2009. Survey 4 was approved by the same committee in document number 6/2013.

Results

The number of cases treated with ART for the first time at the sites during the survey rounds 1 to 4 were 311, 362, 969, and 431 respectively. HIV genotyping was conducted on 310, 351, 823 and 415 cases, and results were available in 310, 350, 797 and 413 cases respectively. The distribution of cases by occupation and type of hospital in four rounds showed no significant difference (Table 2). However, other demographic variables, including gender, age, marital status and education, were statistically different. In round 4, 61.5% of cases were male when compared with 48.4–53.2% in rounds 1–3 (p-value 0.006).

Among cases in round 4, 26.9% were less than 30 years old while participants in this age group in the earlier three rounds ranged between 9.7 and 19.4% (p-value 0.002). Proportion of cases with single marital status was higher (31.7%) in round 4 compared to 13.4–17.4% in rounds 1–3 (p-value 0.0002). In rounds 1–3, proportion of cases who held a bachelor degree or higher were 7.3–13.2% while proportion in round 4 (21.6%) was higher (p-value <0.001).

In terms of clinical condition, cases in round 4 tended to be more asymptomatic (59.9%) than in rounds 1–3 (15.7–48.1%, p-value <0.001). Median CD4 count increased from 38 cells/μl in round 1 to 167 cells/μl in round 4 (p-value <0.001). Median VL observed in round 1 was 212,000 copies/ml while it was 158,099 copies/ml in round 4. However, the trend did not reach the significant level (p-value 0.063).

Among cases with HIVDR results, the majority was ART naive. In rounds 1–4, numbers of naive cases were 304, 320, 753 and 377; and experienced cases were seven, 30, 44 and 36. Overall HIVDR prevalence rates among naive cases by rounds using aggregated computing were 2.0%, 2.8%, 4.0% and 4.8% (p-value 0.046), and in experienced cases, the rates were 0, 3.3%, 11.4% and 13.9% (p-value 0.277) (Figure 1). Prevalence rates among total subjects in rounds 1–4 were 1.9%, 2.9%, 4.4% and 5.6% (p-value 0.182).

Among naive cases, the highest rate of resistance (3.3%) was observed in nevirapine (NVP) in round 3. Resistance to etravirine (ETR) and rilpivirine (RPV) in round 4 were equal (2.7%). In addition, HIVDR was also found with NRTI group such as lamivudine (3TC) at 1.9% in round 3. In experienced cases, the highest rates of resistance were to NVP and efavirenz (EFV) in round 4, with a rate of 13.9% to each drug. In total, NVP (3.6%) and EFV (3.1%) were the highest in round 4 (Figure 2).

Discussion

In current ART practice in resource-limited countries, empirical regimen is used without prior genotypic testing[4]. This practice is based on the assumption of low HIVDR rates and the genotyping of each PLHIV before initiating ART would not be cost-effective. However, when large number of HIV cases received ART, HIVDR can emerge and be transmitted[9]. Therefore, periodical surveys to monitor the prevalence of HIVDR in pre-ART cases were essential to assess program effectiveness. Such surveys were also recommended by WHO[10,11].

In this article, a series of four consecutive surveys during 2006–2013 to assess HIVDR rates among pre- ART cases was reported. The selected demographic factors and certain laboratory results in survey round 4 were found to be different from rounds 1–3. This difference might be caused by change in enrollment criteria. The eligibility in 2010 was a CD4 of 350 cell/μl or less[3] while the cutoff for initiation in the earlier was a CD4 at 200 cell/μl or less.

Our study found an upward trend of HIVDR prevalence, with the highest rates of 4.8% among ART naive cases and 13.9% among experienced cases in round 4. Among all ART naive cases, the rates were still low, yet rising with significant trend over time. This finding indicated the necessity to continue monitoring HIVDR for evaluating the use of the currently recommended ART regimens without prior individual genotyping. The experienced cases, such as those receiving ART prophylaxis or prevention mother- to-child transmission, or those who have defaulted from previous ART should be closely monitored since the observed rates in these individuals were relatively high.

Resistance was the most common for NNRTIs while resistance to NVP and EFV were observed in round 4 as well. Resistance to other antiretroviral was lower in all rounds.

Other studies in Thailand revealed that HIVDR prevalence rates among pre-treatment cases varied from 2–17.6%[12–16]. However, these surveys aimed to measure single-period prevalence rate and some were performed in tertiary care settings. As participants were enrolled from regional, provincial and community hospital settings in this study, characteristics of participants in the pre-treatment HIVDR prevalence study might be different, which reflected variation of HIVDR rates.

Pre-treatment HIVDR rates from other countries varied widely. The prevalence rate during 2009–2010 in Vietnam was 3.5%[17]. In Zimbabwe, the overall HIVDR rate during 2008–2010 was 6.3%[18], with the prevalence in experienced cases being 12.1% and naive cases 5.7%. During 2013–2014, a survey in South Africa showed a prevalence of 9.0%[19]. Data

from Latin America country revealed higher prevalence. In Honduras, the prevalence in 2013–2015 was observed to be 11.5%[20] while the prevalence during 2011–2015 was 13.4% in Nicaragua[21]. An alarming prevalence of 15.5% was reported from Mexico in 2015[22].

In this study, there were three major limitations. Firstly, survey sites were varied, not randomly chosen, and sample sizes differed in each round, effecting data representativeness. Variation existed for reagent kits and interpretation of resistance among laboratories used, noting that genotyping test in the first three rounds was commercial assay based. The other limitation was that small samples in ART experienced cases were included in the study. Therefore, prevalence of HIVDR in this group must be interpreted with caution. To overcome these limitations, the fifth survey following the WHO recommended method[23] has been planned for 2017. Findings from the upcoming survey would be essential to assess HIVDR among pre- treatment cases.

Public Health Actions and Recommendations

Results from this study as well as from the other surveys, locally and globally, indicated a need to continue surveillance for pre-treatment HIVDR and serious challenges to ART programs in resource- limited countries. Activities in developing practical guidelines to protect efficacy and prolong the use of empirical first-line ART regimens, such as HIV treatment literacy and strengthening of adherence to medication, should be implemented. The manager of national ART program together with partners should consider stewardship strategy on the use of empirical ART regimen as well as a strategy to apply genotyping test when HIVDR has increased beyond a critical level. In addition, since pre-exposure prophylaxis for HIV using selected ARV was promoted, particular attention should be given to monitor the circulating HIVDR.

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Disclaimer

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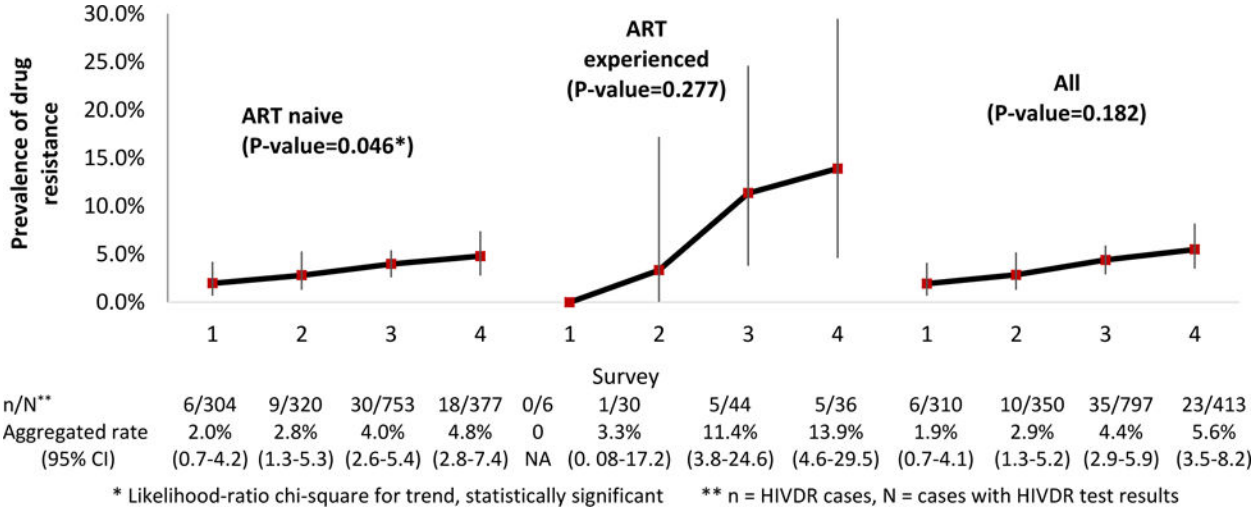


Figure 1.

Trend of HIV drug resistance prevalence rates among antiretroviral therapy (ART) naive, experienced and all cases from 4 rounds of survey in Thailand, 2006–2013

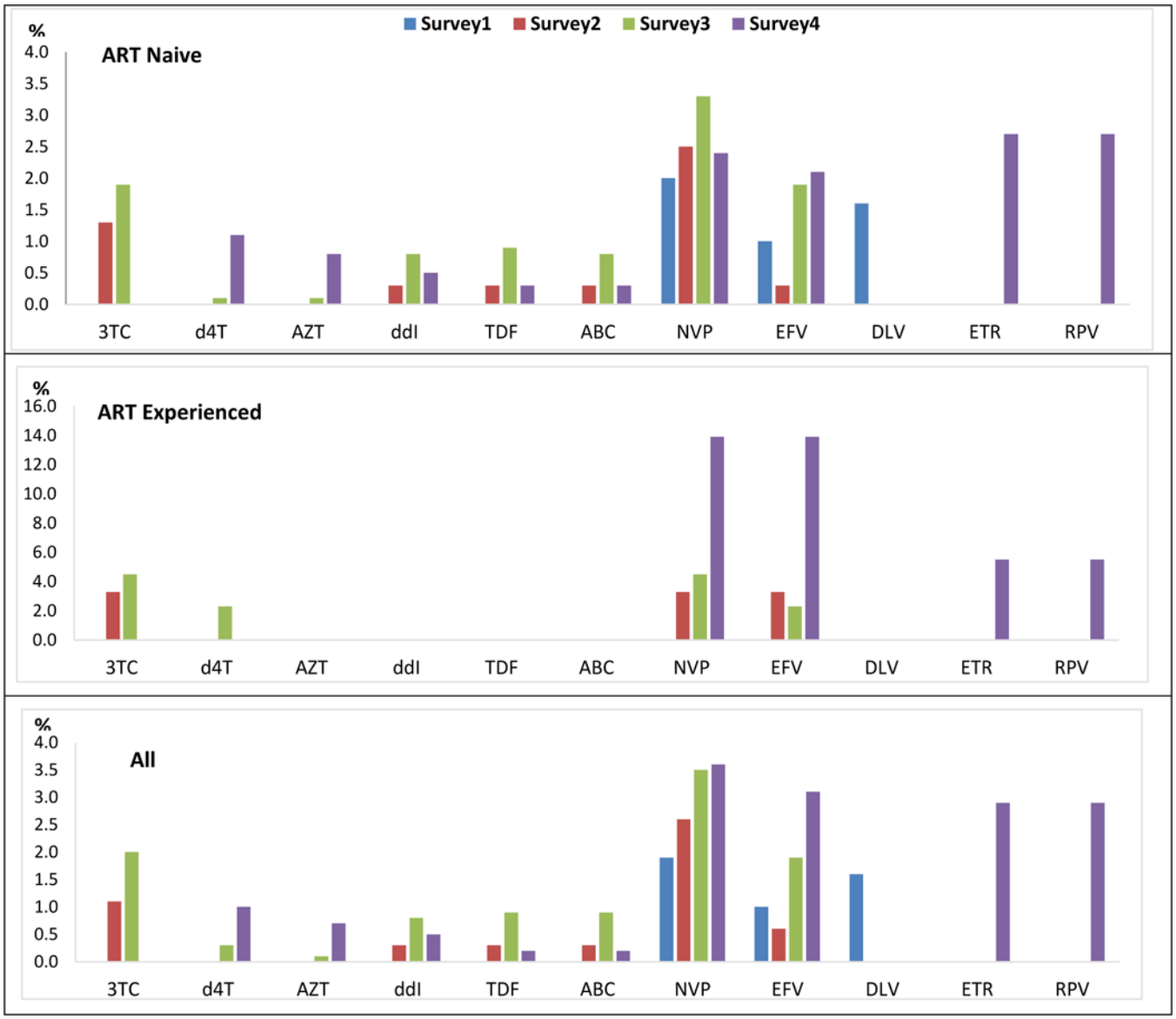
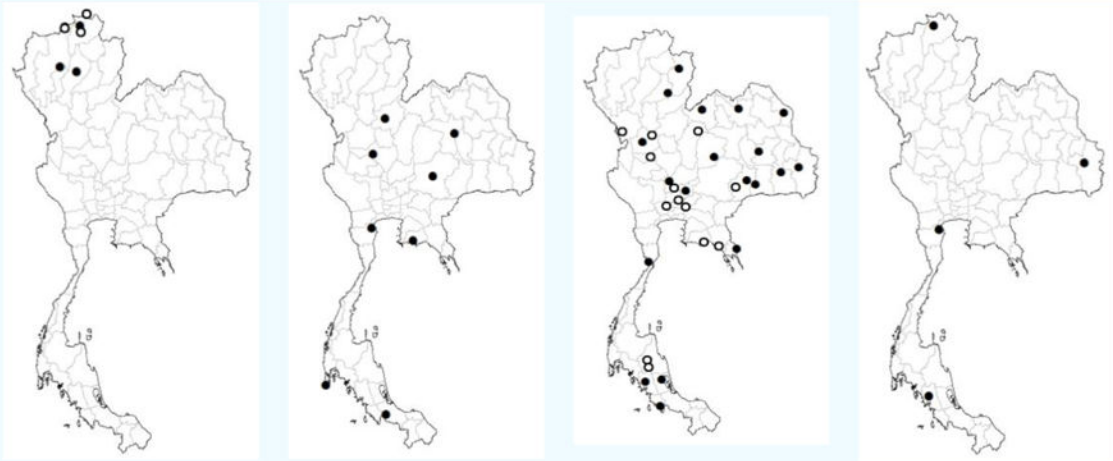


Figure 2. HIV drug resistance rates in each antiretroviral drug classified by antiretroviral therapy naive, experienced and all cases from 4 rounds of survey in Thailand, 2006–2013

Abbreviations: antiretroviral therapy (ART), lamivudine (3TC), stavudine (d4T), zidovudine (AZT), didanosine (ddI), tenofovir (TDF), abacavir (ABC), nevirapine (NVP), efavirenz (EFV), delavirdine (DLV), etravirine (ETR), etravirine (RPV)

Table 1.

Pre-treatment HIV drug resistant (HIVDR) surveys information in Thailand, 2006–2013

Survey information	Round 1	Round 2	Round 3	Round 4
Survey sites: antiretroviral therapy (ART) clinics	6 (3 regional/provincial and 3 community hospitals in 3 northern provinces)	8 (8 regional/provincial hospitals in 8 provinces)	33 (19 regional/provincial and 14 community hospitals in 12 provinces)	4 (4 regional/provincial hospitals from 4 provinces)
Enrollment period (months)	9 (Feb-Oct 2006)	6 (Jul-Dec 2007)	15 (Jul 2008-Oct 2009)	12 (2013)
HIVDR laboratory	Chiang Mai University	Multi-sites, depending on the existing systems		National Institute of Health
HIVDR test	Commercial	Commercial		In-house
Location of participating clinics				

● Regional/provincial hospital ○ Community hospital

Table 2.

Distribution of cases by demographic characteristics, types of hospital, symptoms and laboratory results from 4 rounds of surveys in Thailand, 2006–2013

Variable	Number (Percent)				P-value
	Round 1	Round 2	Round 3	Round 4	
Gender	310	351	823	413	
Male	164 (52.9)	170 (48.4)	438 (53.2)	254 (61.5)	0.006 *
Female	146 (47.1)	181 (51.6)	385 (46.8)	159 (38.5)	
Age (year)	308	351	805	413	
Median age (min-max)	38 (21–65)	35 (18–62)	36 (18–67)	37 (18–70)	0.002 #
<30	30 (9.7)	68 (19.4)	143 (17.8)	111 (26.9)	<0.001 *
30–39	151 (49.0)	183 (52.1)	388 (48.2)	123 (29.8)	
40–49	96 (31.2)	77 (21.9)	204 (25.3)	122 (29.5)	
≥50	31 (10.1)	23 (6.6)	70 (8.7)	57 (13.8)	
Marital Status	310	351	823	401	
Single	54 (17.4)	47 (13.4)	133 (16.2)	127 (31.7)	<0.001 *
Married/widowed/ divorced	256 (82.6)	304 (86.6)	690 (83.8)	274 (68.3)	
Education	299	349	799	408	
Grade 6 and below	210 (70.2)	160 (45.8)	480 (60.1)	159 (39)	<0.001 *
Grade 7–12	67 (22.4)	143 (41.0)	261 (32.7)	161(39.5)	
Bachelor degree and higher	22 (7.4)	46 (13.2)	58 (7.3)	88 (21.6)	
Occupation	263	338	726	407	
Commercial and business owner	32 (12.2)	60 (17.8)	79 (10.9)	77 (18.9)	0.279 *
Government/private sector	6 (2.3)	53 (15.7)	62 (8.5)	51 (12.5)	
Farmer and laborer	185 (70.3)	164 (48.5)	455 (62.7)	167 (41.0)	
Unemployed	40 (15.2)	61 (18.0)	130 (17.9)	91 (22.4)	
Student				21 (5.2)	
Hospital Type	310	351	823	415	
Community	44 (14.2)	0	272 (33.0)	0	0.112 *
Regional and provincial	266 (85.8)	351 (100.0)	551 (67.0)	415 (100)	
Symptom	310	351	823	401	

Variable	Number (Percent)				P-value
	Round 1	Round 2	Round 3	Round 4	
Asymptomatic	149 (48.1)	55 (15.7)	159 (19.3)	240 (59.9)	<0.001*
Symptomatic	161 (51.9)	296 (84.3)	664 (80.7)	161 (40.1)	
CD4	310	350	815	408	
Median (cells/μl) (IQR)	38 (15–96.5)	58 (20–139.5)	55 (20–136)	167 (47–278.7)	<0.001#
Viral Load	310	351	810	400	
Median (copies/ml) (IQR)	212,000 (87,775–494,000)	194,000 (55,200–568,000)	209,767 (75,075–537,500)	158,099 (48,675–455,860)	0.063#

*Survey statistic adjusted for Clusters

#Kruskal-Wallis test