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Cross-sectional study of virological failure and multinucleoside reverse transcriptase inhibitor resistance at 12 months of antiretroviral therapy in Western India

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Abstract

The free antiretroviral therapy (ART) program in India has scaled up to register second largest number of people living with HIV/AIDS across the globe. To assess the effectiveness of current first-line regimen we estimated virological suppression on completion of 1 year of ART. The study describes the correlates of virological failure (VF) and multinucleoside reverse transcriptase inhibitor (NRTI) drug resistance mutations (DRMs).

In this cross-sectional study conducted between June and August 2014, consecutive adults from 4 State sponsored ART clinics of western India were recruited for plasma viral load screening at 12±2 months of ART initiation. Individuals with plasma viral load >1000 copies/mL were selected for HIV drug resistance (HIVDR) genotyping. Logistic regression analyses were performed to assess factors associated with VF and multi-NRTI resistance mutations. Criteria adopted for multi-NRTI resistance mutation were either presence of K65R or 3 or more thymidine analog mutations (TAMs) or presence of M184V along with 2 TAMs.

Of the 844 study participants, virological suppression at 1 year was achieved in 87.7% of individuals. Factors significantly associated with VF ($P < 0.005$) were 12 months CD4 count of ≤100 cells/μL (adjusted OR −7.11), low reported adherence (adjusted OR −4.44), and those living without any partner (adjusted OR −1.98). In patients with VF, the prevalence of non-nucleoside reverse transcriptase inhibitor (NNRTI) DRM (78.75%) were higher as compared to NRTI (58.75%). Multi-NRTI DRMs were present in 32.5% of sequences and were significantly associated with CD4 count of ≤100 cells/μL at baseline (adjusted OR −13.00) and TDF-based failing regimen (adjusted OR −20.43). Additionally, low reported adherence was negatively associated with multi-NRTI resistance (adjusted OR −0.11, $P = 0.015$). K65R mutation was significantly associated with tenofovir (TDF)-based failing regimen ($P < 0.001$).

The study supports early linkage of HIV-infected individuals to the program for ART initiation, adherence improvement, and introduction of viral load monitoring. With recent introduction of TDF-based regimen, the emergence of K65R needs to be monitored closely among HIV-1 subtype C-infected Indian population.

Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral therapy, ARV = antiretrovirals, AZT = zidovudine, d4T = stavudine, DDI = didanosine, DRMs = drug resistance mutations, EFV = efavirenz, ETR = etravirine, FTC = emtricitabine, NACP = National AIDS Control Program, India, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse

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transcriptase inhibitor, NVP = nevirapine, PLHA = people living with HIV/AIDS, RPV = rilpivirine, TAMs = thymidine analog mutations, TDF = tenofovir, VF = virological failure.

Keywords: antiretroviral therapy, HIV drug resistance, K65R, mutations, virological monitoring

1. Introduction

India has an estimated population of 2.11 million (1.71–2.64 million) HIV-infected individuals with adult (15–49 years) prevalence of 0.26% in 2015.[1] Initiated on April 1, 2004, the program of provision of free antiretroviral therapy (ART) to the eligible individuals, has scaled up considerably to register second largest number of people living with HIV/AIDS (PLHA) across the globe.[2] Compared to 2000 and 2007, the Indian National AIDS Control Program (NACP) has, respectively, achieved 66% and 32% decline in new cases of HIV infection in 2015.[1] With maturing of the program it is essential to assess the effectiveness of ART by estimating virological suppression among individuals initiated on first-line regimen. Concern over emergence and spread of HIV drug resistance (HIVDR), also necessitates resistance surveillance studies to monitor treatment outcome.[3]

The national program in India implements public health approach for initiation of first-line ART comprising of 2 nucleoside/nucleotide analog reverse transcriptase inhibitors (NRTI) and a non-nucleoside reverse transcriptase inhibitor (NNRTI).[4] The NRTI options available in the program are zidovudine (AZT), abacavir (ABC), lamivudine (3TC), stavudine (d4T), didanosine (DDI), and emtricitabine (FTC), whereas tenofovir (TDF) is the only nucleotide reverse transcriptase inhibitor. The NNRTI options currently available are efavirenz (EFV) and nevirapine (NVP).[4]

In developed nations, viral load (VL) monitoring is a part of standard treatment protocol, whereas our program still relies on immunological and clinical monitoring, though targeted viral load is offered for confirmation of treatment failure.[5] In the absence of periodic plasma viral load estimation, detection of treatment failure is delayed which may result in sequential accumulation of resistance mutations, especially thymidine analog mutations (TAMs).[6] A large study conducted in Sub-Saharan Africa and Southeast Asia indicated virological failure (VF) rate of 11.1% at 12 months of ART.[7] A recent study from southern India among 599 participants recorded cumulative VF incidence of 13.2% in the first year, however drug resistance outcome data were unavailable.[8] A prior study from 2 ART clinics in southern and western India documented viral load suppression (<1000 copies/mL) in 75% and 64.6% patients, respectively, at the end of 12 months.[9]

In the absence of routine virological monitoring, HIVDR outcome studies from India at 12 months of ART are limited by sample size, different inclusion criteria and variable duration of ART treatment before genotyping.[9–13] In one of the prior large study involving genotyping of 138 patients with failure from South India, M184V and Y181C emerged as most common NRTI and NNRTI mutations, respectively.[14] Sinha et al[15] also reported overall drug resistance mutation (DRM) prevalence of 93.8% among 128 individuals from North India with failure of first-line ART. There is a need to have systematically collected data as recommended by WHO to assess effectiveness of first-line ART regimen at 12 months of its initiation.[16]

Conventionally, mutations like K69 Insertion, Q151M complex and multiple TAMs reduce susceptibility to all currently available NRTI.[17] Additionally K65R mutation selected by TDF, ABC, d4T, and DDI impart resistance to all NRTI except AZT.[18] In subtype C-infected Indian population, K69 Insertion and Q151M are seldom reported and common mutations responsible for multi-NRTI resistance includes, K65R and multiple TAMs with or without M184V.[19] M184V is selected by 3TC/FTC and reduces susceptibility to these drugs by 100-fold. In combination with TAMs, M184V also reduces susceptibility to ABC and DDI.[18,20] As NRTI forms an important backbone of both first and second-line regimen, there is a need to estimate the proportion of individuals developing multi-NRTI resistance mutations at 12 months of ART.

Therefore the primary objective of this study was to ascertain the rate of population level virological suppression and factors associated with failure at 12 months of ART. The secondary objective was to characterize HIVDR mutation pattern with special attention to multi-NRTI resistance mutations.

2. Methods

2.1. Study design and participants

This cross-sectional study was carried out between June and August 2014 at 4 State sponsored ART clinics of Pune city in western India. Consecutive ART naive adults (age >18 years) were recruited on completion of 12 ± 2 months of ART for viral load screening. All ART clinics followed the National guidelines wherein, ART was initiated at CD4 count of ≤ 350 cells/μL or else irrespective of CD4 count for individuals in WHO clinical stage III and IV.[4] Individuals with history of exposure to antiretrovirals (ARV) outside program were excluded, except in cases of females, where ARV were offered for prevention of mother to child transmission. The primary study outcome was VF at the end of 1 year of ART initiation and development of resistance mutations was secondary outcome.

The details of participant's age, gender, marital status, ARV treatment history, and immunological profile were recorded from the data maintained at respective clinics. Married and those in "Living-in" relationship were considered to be living with partner. Unmarried, divorced, and separated couples were considered as living without partner. A counselor elicited history of prior ART exposure and adherence to the medication at the time of sample collection. The drug adherence was assessed at the time of monthly drug refill by calculating the proportion of doses missed since last visit. An average of recent 3 months adherence was taken into account for analysis. Ten milliliters of peripheral blood was collected in EDTA collection vials for CD4 cell count, viral load testing, and drug resistance genotyping. Plasma viral load estimation was performed on m2000RT Abbott Real Time HIV-1 assay (Abbott Molecular, Inc., Des Plaines, IL). Individuals with VF, defined as single plasma HIV-1 viral load of more than 1000 copies/mL were subjected to HIVDR genotyping.

2.2. HIV drug resistance genotyping

Genotyping to characterize DRMs was performed by an In-house population-based sequencing protocol, validated previously.[21]

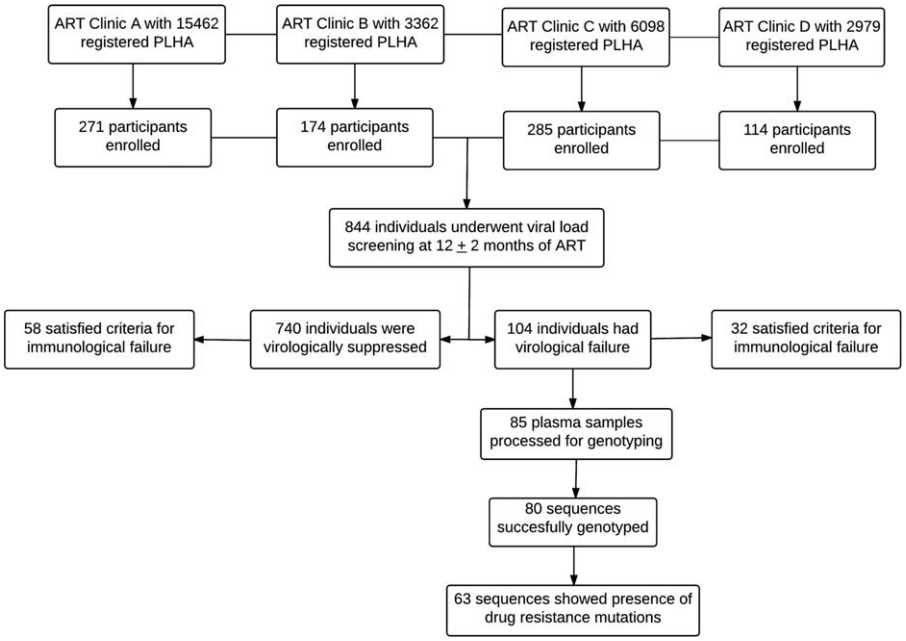

Figure 1. Study flow chart showing participation of 844 people living with HIV/AIDS (PLHA) at 12 ± 2 months of ART.

Bidirectional sequencing of complete protease (PR, 1–99 amino acids) and partial reverse transcriptase (RT, 1–256 amino acids) were performed on ABI 3730XL DNA Sequencer (Applied Biosystems, Inc., Foster City, CA). The quality of PR/RT sequences were screened using Sequence Quality Analysis Tool (SQUAT) and any pair of sequences with genetic distance of less than 0.99 or more than 15 were excluded.[22] Resistance patterns were determined using Stanford University HIVdb Genotypic Interpretation Algorithm version 7.0 dated February 27, 2014.[23] Predicted susceptibility for each NRTI and NNRTI was calculated by adding up penalty score associated with each DRM in a given sequence. A total score of 14 or less was considered susceptible and a score of 15 or more was considered resistant. Phylogenetic trees were constructed by the maximum likelihood method based on general time reversible model, using MEGA 6.0.[24] Multi-NRTI resistance mutations were defined as either presence of K65R or 3 or more TAMs or presence of M184V along with 2 TAMs.

2.3. Statistical analysis

The demographic, clinical, and biological characteristics of study participants were summarized by medians and interquartile range (IQR) for continuous variables and by proportions for categorical variables. Differences between groups were compared using the Mann–Whitney *U* test for continuous data and Pearson Chi-square test for categorical data. Univariable logistic regression analysis was performed to assess factors associated with VF and multi-NRTI resistance mutations. Variables with significant association ($P < 0.05$) with outcome in univariable regression analysis were included in multivariable logistic regression model and were adjusted for age and gender. Adjusted odds ratio (aOR) with 95% confidence intervals (95% CIs) were calculated. All significant tests were 2-sided and "*P*" value of <0.05 was considered statistically significant. Data were entered in a spreadsheet (MS Excel 2010) and analyzed using SPSS version 17.0.

Minimum sample size calculated for our study, assuming proportion of individuals with VF of 15%, precision of 2.5% and alpha error as 5%, was 784. Anticipating nonparticipation by 10%, we targeted recruitment of 863 individuals for our study.

2.4. Ethical consideration

The study was approved by Ethics committee of National AIDS Research Institute (ICMR) and the individuals from each ART clinics were recruited voluntarily after obtaining written informed consent.

3. Results

A total 844 individuals consented to participate at 12 ± 2 months of ART initiation as shown in study flow chart (Fig. 1). Compared to baseline median CD4 count of 213 cells/μL (IQR: 116–309), significant increase in median CD4 count at 12 months of ART (379 cells/μL, IQR: 256–526) was observed (Table 1). At the time of ART initiation 681 (80.7%) and 160 (19.0%) individuals were on AZT- and TDF-based regimen, respectively. Toxicity was the main reason for which 43.7% of individual underwent drug substitution in ART during first year.

On completion of 12 months of ART, successful suppression of plasma viral load to less than 1000 copies/mL was achieved in 87.7% (95% CI: 85.5–89.9%) of individuals. Among 740 virologically suppressed individuals, 58 (7.83%) patients satisfied the criteria for immunological failure.[25] Of these 50 (6.75%) had CD4 cell count of below baseline value suggestive of immuno-virological discordance and 17 (2.28%) had 12 months CD4 count of ≤100 cells/μL. Nine patients in common satisfied both the criteria of 12 months CD4 count of <100 cells/μL as well as below baseline value. Out of 104 individuals with VF, only 32 satisfied the immunological failure criteria. First 85 plasma samples from VF patients were processed for HIVDR genotyping due to budgetary constraints and 80 were sequenced successfully.

Table 1

Demographic and clinical characteristics of study participants.

Characteristics of participants	Total, n = 844	VF, n = 104	VS, n = 740	*P*
Sex, male—n (%)	405 (48.0)	54 (51.9)	351 (47.4)	0.39
Patient age, median (IQR), in years	39 (32–45)	36 (31–43)	39 (33–45)	0.02
18–30, n (%)	154 (18.2)	24 (23.1)	130 (17.6)	
31–40	331 (39.2)	44 (42.3)	287 (38.8)	
41 and above	359 (42.5)	36 (34.6)	323 (43.6)	
Marital status, n (%)				
Married or living-in partner	553 (65.5)	56 (53.8)	497 (67.2)	**0.007**
Unmarried or divorced or separated	291 (34.5)	48 (46.2)	243 (32.8)	
Baseline CD4 cells/μL, median (IQR)	213 (116–309)	161 (90–259)	224 (120–313)	**≤0.001**
Less than 100, n (%)	174 (20.6)	29 (27.9)	145 (19.6)	
100–200	221 (26.2)	35 (33.7)	186 (25.1)	
201–300	216 (25.6)	21 (20.2)	195 (26.4)	
More than 300	233 (27.6)	19 (18.3)	214 (28.9)	
12 mo CD4 cells/μL, median (IQR)	379 (256–526)	240 (140–355)	399 (271–545)	**≤0.001**
Less than 100, n (%)	34 (4.0)	17 (16.3)	17 (2.3)	
100–200	104 (12.3)	29 (27.9)	75 (10.1)	
201–300	152 (18.0)	24 (23.1)	128 (17.3)	
More than 300	554 (65.6)	34 (32.7)	520 (70.3)	
ART regimen at baseline, n (%)				
AZT + 3TC + NVP	579 (68.6)	67 (64.4)	512 (69.2)	0.51
AZT+ 3TC + EFV	102 (12.1)	11 (10.6)	91 (12.3)	
TDF + 3TC + NVP	96 (11.4)	16 (15.4)	80 (10.8)	
TDF + 3TC + EFV	64 (7.6)	10 (9.6)	54 (7.3)	
d4T + 3TC + NVP/EFV	3 (0.3)	—	3 (0.4)	
ART regimen at 12 mo, n (%)				
AZT + 3TC + NVP	436 (51.7)	49 (47.1)	387 (52.3)	0.55
AZT + 3TC + EFV	82 (9.7)	12 (11.5)	70 (9.5)	
TDF + 3TC + NVP	244 (28.9)	32 (30.8)	212 (28.6)	
TDF + 3TC + EFV	72 (8.5)	11 (10.6)	61 (8.2)	
d4T + 3TC + NVP/EFV	10 (1.2)	—	10 (1.3)	
Antiretroviral substitution, n (%)				
Any substitution	369 (43.7)	39 (37.5)	330 (44.6)	0.17*
NRTI substitution	198 (23.45)	21 (20.19)	177 (23.9)	
NNRTI substitution	227 (26.89)	22 (21.15)	205 (27.7)	
Adherence to regimen				
Low adherence, <95%, n (%)	125 (14.8)	40 (38.5)	85 (11.5)	**≤0.001**
Unemployed PLHA, n (%)	300 (35.5)	29 (27.9)	271 (36.6)	0.08
Predominant mode of transmission				
Heterosexual, n (%)	763 (90.4)	94 (90.4)	669 (90.4)	0.26
MSM	2 (0.2)	1 (1.0)	1 (0.1)	
Others	79 (9.4)	9 (8.7)	70 (9.5)	
History of tuberculosis	179 (21.2)	21 (20.2)	158 (21.4)	0.78
Mean VL at 12 mo, $\log_{10}$ copies/mL	—	4.52 (3.7–5.2)	—	
3–3.9, n (%)	—	36 (34.6)	—	
4–4.9	—	37 (35.6)	—	
>5	—	31 (29.8)	—	

3TC = lamivudine, ART = antiretroviral therapy, AZT = zidovudine, d4T = stavudine, EFV = efavirenz, IQR = interquartile range, MSM = men who have sex with men, NVP = nevirapine, PLHA = people living with HIV/AIDS, TDF = tenofovir, VF = virological failure, VL = viral load, VS = virologically suppressed.

* Any substitution versus no substitution is compared.

P value of < 0.05 was considered statistically significant and highlighted in bold.

3.1. Determinants of virological failure at 12 months of initiation of ART

The factors significantly associated ($P < 0.005$) with VF (Table 2) were 12 months CD4 count of <100 cells/μL (aOR −7.11; 95% CI: 3.10–16.31), <95% reported adherence (aOR −4.44; 95% CI: 2.74–7.18) and those living without any partner (OR −1.98; 95% CI: 1.24–3.14).

3.2. HIV-1 diversity

The HIV-1 subtype C was the most predominant subtype, seen in 97.5% of sequences. The phylogenetic tree of 80 partial *pol* gene sequences along with reference sequences retrieved from Los Alamos database is shown in Supplementary Figure, http://links.lww.com/MD/B267. A single sequence (KR816094) clustered with subtype A1 and another sequence (KR816077) was separated away from Indian subtype C cluster suggestive of recombination. Near full-length genomic sequencing of this isolate (KR816077) confirmed unique recombination pattern of CRF01_AE and subtype C.[26]

3.3. HIV drug resistance at 12 months

Of these 80 successfully genotyped sequences, 17 (21.25%) sequences did not show any DRM. The prevalence of DRMs

Table 2

Logistic regression analyses of factors associated with virological failure in (n = 844) individuals at completion of 12 ± 2 months of first-line antiretroviral therapy.

			Univariable logistic regression		Multivariable logistic regression	
Factors	**Total number**	**Virological failure, n (%)**	**Crude odds ratio (95% CI)**	***P***	**Adjusted odds ratio (95% CI)**	***P***
Gender						
Male	405	54 (13.3)	1.20 (0.79–1.81)	0.39	1.43 (0.90–2.29)	0.13
Female	439	50 (11.4)	1		1	
Age, y						
18–30	154	24 (15.6)	1.66 (0.95–2.89)	0.07	1.75 (0.95–3.22)	0.07
31–40	331	44 (13.3)	1.38 (0.86–2.20)	0.18	1.63 (0.98–2.70)	0.05
41 and above	359	36 (10.0)	1		1	
Education						
Illiterate	189	27 (14.3)	1.25 (0.78–2.01)	0.35	—	—
Literate	655	77 (11.8)	1		—	—
Baseline CD4 count, cells/μL						
<100	174	29 (16.7)	1.59 (1.00–2.53)	0.05	0.98 (0.56–1.73)	0.95
>100	670	75 (11.2)	1		1	
12 mo CD4 count, cells/μL						
<100	34	17 (50.0)	8.31 (4.09–16.87)	**≤0.001**	7.11 (3.10–16.31)	**≤0.001**
>100	810	87 (10.7)	1		1	
Marital status						
No partner	291	48 (16.5)	1.75 (1.16–2.66)	**0.008**	1.98 (1.24–3.14)	**0.004**
Living with partner	553	56 (10.1)	1		1	
NRTI in failing regimen						
Tenofovir	316	43 (13.6)	1.18 (0.78–1.79)	0.43	—	—
Zidovudine	518	61 (11.8)	1		—	—
NNRTI in failing regimen						
Nevirapine	688	81 (11.8)	0.77 (0.47–1.27)	0.30	—	—
Efavirenz	156	23 (14.7)	1		—	—
Any substitution						
Yes	369	39 (10.6)	0.75 (0.49–1.14)	0.17	—	—
No	475	65 (13.7)	1		—	—
Reported adherence, %						
<95	125	40 (32.0)	4.82 (3.06–7.59)	**≤0.001**	4.44 (2.74–7.18)	**≤0.001**
>95	719	64 (8.9)	1		1	

95% CI = 95% confidence interval, NNRTIs = nonnucleoside reverse transcriptase inhibitors, NRTIs = nucleoside/nucleotide reverse transcriptase inhibitors.
P value of < 0.05 was considered statistically significant and highlighted in bold.

were 58.75%, 78.75%, and 1.25% for NRTIs, NNRTIs, and protease inhibitors (PIs), respectively (Fig. 2). The most common NRTI and NNRTI mutation were M184V/I (51.25%) and K103N (36.25%), respectively. Only 1 individual had major PI resistance mutation L90M and 5 had minor PI resistance mutations L89M, V77I, L63P, H69K/R/Q, M36I, K20I/M/R/T, G16E, and L10V/I. Out of 80, 17.5% sequences showed at least 1 TAMs and there was no significant difference in the mutations arising from TAM-1 (16.25%) and TAM-2 (21.25%) pathway.

3.4. Stanford resistance score at virological failure

Based on Stanford resistance scoring system (Fig. 3), among NRTI, the predicted susceptibility of AZT was highest (86.25%) followed by TDF (68.75%). Comparison of mutation pattern among individuals failing on TDF- and AZT-based regimen (Supplementary Table, http://links.lww.com/MD/B267) showed statistically significant association of K65R mutation with TDF-based regimen ($P < 0.001$). Among NNRTI, the predicted susceptibility for NVP as well as EFV was only 23.75%. Cross-resistance to second-generation NNRTI, namely etravirine (ETR) and rilpivirine (RPV) was seen in 51.25% and 60% of sequences, respectively.

3.5. Determinants of multi-NRTI resistance mutations at 12 months of ART initiation

Factors significantly associated with presence of multi-NRTI resistance mutations (Table 3) were CD4 count of ≤100 cells/μL at baseline (aOR −13; 95% CI: 2.26–74.77) and TDF-based failing regimen (aOR −20.43, 95% CI: 4.21–99.14). In addition low reported adherence (<95%) was negatively associated with multi-NRTI resistance (aOR −0.046; 95% CI: 0.02–0.66).

4. Discussion

Since 2013, WHO has recommended plasma viral load testing as preferred method to monitor individuals on ART.[25,27] To our knowledge, this is the largest study from India describing virological response and drug resistance outcome after 12 months of initiation of first-line ART. Among 844 participants, who were part of free ART program, we report successful virological suppression in 87.6%, which is well above WHO recommended target of 70%.[28] Prior studies with limited sample size reported VF rate of 19%, 25%, and 35.4%, respectively, at ART clinics of Pune, Chennai, and Mumbai.[9,13] Similar VF rate of 12% was reported in Kigali, Rwanda.[29] McMahon et al[30] carried out the first systematic review to quantify population-level viral

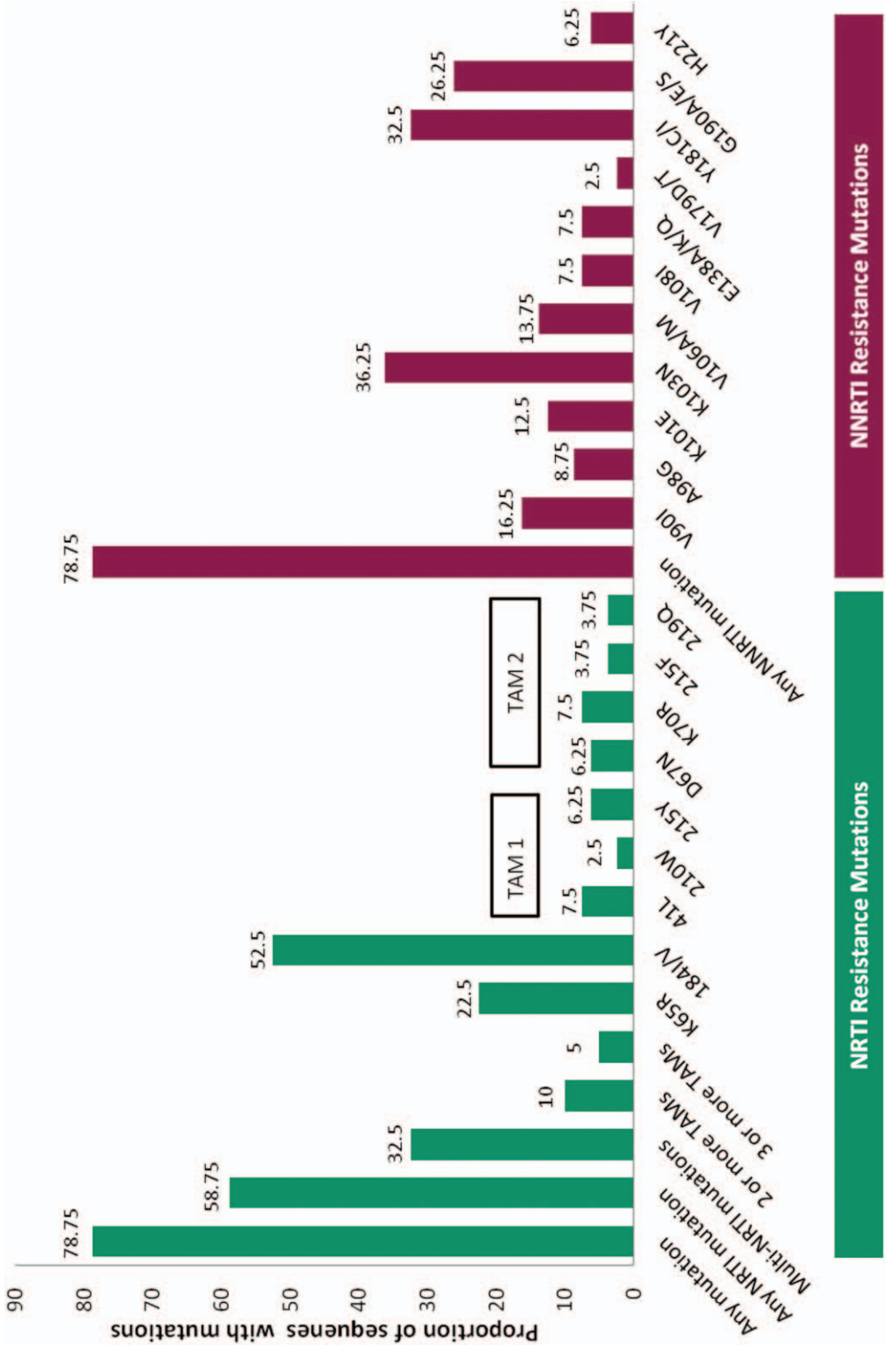

Figure 2. Prevalence of nucleoside reverse transcriptase inhibitors (NRTIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) drug resistance mutations among individuals with virological failures at 12 months of ART initiation.

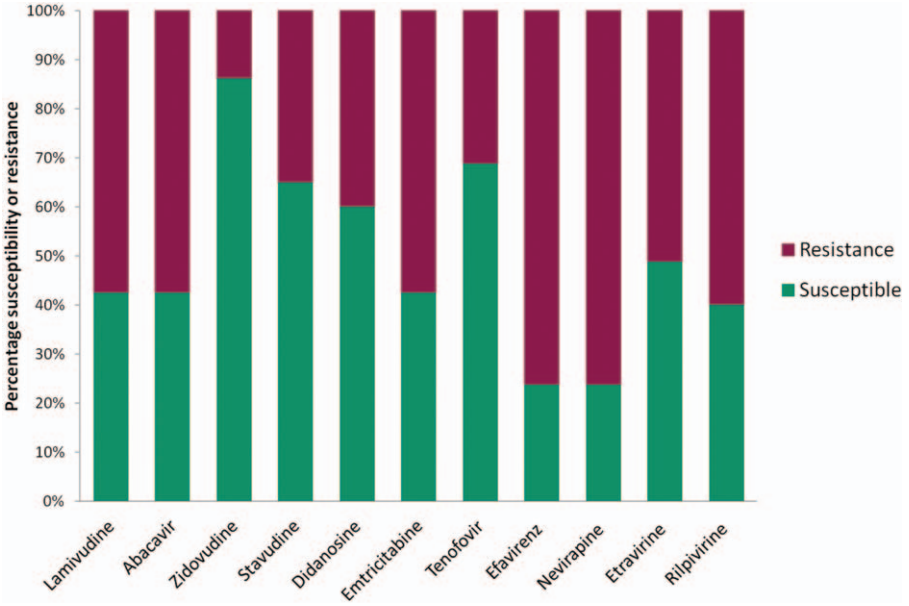

Figure 3. Predicted efficacy of nucleoside reverse transcriptase inhibitors (NRTI) and nonnucleoside reverse transcriptase inhibitors (NNRTI) following virological failure, based on Stanford genotype susceptibility scoring.

suppression 12 months after ART initiation in low and middle income countries (LMIC). In nine cohorts with viral suppression threshold of <1000 copies/mL, the review showed suppression in 83.5% (95% CI: 77.8–88.4; n=3192) of the combined on-treatment populations.[30] Present study reports better virological suppression, that is, in 87.7% of individuals as compared to other LMIC.

In this study, 58 (7.83%) virologically suppressed individuals failed to show adequate immunological recovery at 12 months of ART. Of these, 17 (2.28%) of individuals had 12 months CD4 count of ≤100 cells/μL and 50 (6.75%) had fall in CD4 cell count to baseline or below. Zoufaly et al[31] have indicated increased risk of developing non-AIDS morbidity and mortality among those individuals who failed to achieve CD4 count of above baseline value. In absence of virological monitoring, individuals with immunovirological discordance would be missed and misclassified as treatment failure.[32] Also out of 104 individuals with VF, only 32 meet the WHO immunological failure criteria, indicating the need for plasma viral load testing for early identification of treatment failure.

In the current setting, low adherence to ART (<90%), low CD4 count (<100 cells/μL) and living without a partner emerged as important predictors of VF among PLHA. Several prior studies have indicated suboptimal adherence as important factor for VF in resource-limited settings.[8,33,34] Our study showed that VF was more likely among PLHA who are living alone rather than those living with their partners. This highlights the need for strengthening social support measures in country where HIV/AIDS is still associated with as stigma.

The overall prevalence of DRM in individuals with VF was 78.25%, with NNRTI DRMs leading the list. A prior multi-country WHO HIV drug resistance survey (n=269) reported overall prevalence of 72.1% in patients failing therapy at 12 months with resistance to NNRTI and NRTI as, 69.5% and 62.5%, respectively.[28] The most common NRTI mutations reported were M184V and K65R whereas K103N/S and V106A/M were common NNRTI resistance mutations. The pattern was similar to as observed by other investigators in resource-limited settings.[9,33,35] Thus, the pattern of DRMs is predictable at 12 months of ART in individuals with access to virological monitoring.

In this study, 21.25% sequences from individuals with VL >1000 copies/mL, did not contain any DRM, suggestive of unreported treatment interruption. VF in the absence of DRM has been reported from India and elsewhere.[13,28] Failure without DRM also support the need of virological monitoring complemented with drug resistance genotyping or else 1 in 5 patients with VF may be subjected to costly second-line regimen unnecessarily.

A prior large study involving 6 Sub-Saharan African countries revealed prevalence of resistance to NNRTI and NRTI as 60.6% and 57.8%, respectively.[36] Higher prevalence of NNRTI resistance mutations (79%) in our study can be explained by its low genetic barrier. Earlier, Neogi et al[37] performed temporal analysis of primary drug resistance in India and reported rising trend of NNRTI resistance. Importantly, 51.25% and 60% of sequences respectively also indicated low to high level of resistance to second-generation NNRTI, ETR and rilpivirine (RPV). These NNRTI analogs are not yet introduced in the program and emergence of cross-resistance will reduce their future utility. High cross-resistance to ETR and RPV has been reported previously.[38,39]

TAMs are important in resource-limited settings as they impart resistance to all available NRTIs and the degree of cross-resistance depends on specific mutations and total number of TAMs.[40] In presence of TAMs, M184V also causes additional low-level resistance to DDI and ABC.[17] In this study only 5% of sequences from VF patients had ≥3 TAMs, due to which the susceptibility to AZT was preserved. WHO guidelines also recommends use of AZT in the second line, if TDF is utilized in failing first-line regimen.[41] Accumulation of TAMs in case of immunological monitoring jeopardize NRTI backbone[6] leaving

Table 3

Logistic regression analyses of determinants of multi-NRTI drug resistance mutations (DRM) in individuals with virological failure (n = 80) at 12 months of ART initiation.

			Univariable logistic regression		Multivariable logistic regression	
Variables	**Total (n = 80)**	**Multi-NRTI DRM, n (%)**	**Crude odds ratio (95% CI)**	***P***	**Adjusted odds ratio (95% CI)**	***P***
Gender						
Male	43	14 (32.6)	1.01 (0.39–2.57)	0.99	1.20 (0.21–6.77)	0.84
Female	37	12 (32.4)	1		1	
Age, y						
18–30	22	4 (18.2)	0.36 (0.09–1.46)	0.15	0.66 (0.08–5.57)	0.70
31–40	37	14 (37.8)	0.99 (0.33–2.98)	0.98	1.30 (0.22–7.78)	0.77
41 and above	21	8 (38.1)	1		1	
Marital status						
No partner	37	16 (43.2)	2.51 (0.96–6.57)	0.06	—	—
Living with partner	43	10 (23.3)	1		—	—
Education						
Illiterate	17	17 (11.8)	0.22 (0.05–1.03)	0.05	—	—
Literate	63	63 (38.1)	1		—	—
CD4 at baseline, cells/μL						
<100	22	15 (68.2)	9.16 (3.01–27.83)	**<0.001**	13.00 (2.26–74.77)	**0.004**
>100	58	11 (19.0)	1		1	
CD4 at 12 mo, cells/μL						
<100	15	9 (60.0)	4.24 (1.31–13.67)	**0.01**	1.99 (0.28–14.27)	0.49
>100	65	17 (26.2)	1		1	
Viral load at 12 mo, copies/mL						
$<10^5$	56	20 (35.7)	1.67 (0.57–4.88)	0.35	—	—
$>10^5$	24	6 (25.0)	1		—	—
NRTI in failing regimen						
Tenofovir	35	21 (60.0)	12.00 (3.80–37.88)	**<0.001**	20.43 (4.21–99.14)	**<0.001**
Zidovudine	45	5 (11.1)	1		1	
NNRTI in failing regimen						
Nevirapine	65	23 (35.4)	2.19 (0.56–8.56)	0.26	—	—
Efavirenz	15	3 (20.0)	1		—	—
Any substitution						
Yes	27	9 (33.3)	1.06 (0.40–2.84)	0.91	—	—
No	53	17 (32.1)	1		—	—
Reported adherence, %						
<95	28	5 (17.9)	0.32 (0.11–0.98)	**0.04**	0.11 (0.02–0.66)	**0.01**
>95	52	21 (40.4)	1		1	

95% CI = 95% confidence interval, DRM = drug resistance mutation, NNRTIs = nonnucleoside reverse transcriptase inhibitors, NRTIs = nucleoside/nucleotide reverse transcriptase inhibitors.
P value of < 0.05 was considered statistically significant and highlighted in bold.

PI as only active ingredient in second line. Introduction of newer class of ARV in second-line regimen can be averted if failure is detected early by virological monitoring. Although recent trials have indicated promising results of simplified PI monotherapy, the success of this strategy ultimately depends on viral load monitoring.[42]

In resource-limited settings NRTI analogs are utilized in first as well as second-line regimen. Due to low prevalence of multi drug mutation such as T69Ins or Q151M, we adopted criteria for multi-NRTI DRMs as either presence of K65R or presence of 2 TAMs along with M184V or presence of 3 or more TAMs. Multivariable analysis indicated that low CD4 count at baseline and TDF-based failing regimen were significantly associated with multi-NRTI DRMs. Recently a multicentre retrospective cohort study has reported strong association of low pre-ART CD4 count (<100 cells/μL) with development of TDF resistance.[43] The median CD4 count of study participants at ART initiation was 213 cells/μL (IQR: 116–309) as against the program recommendation of 350 cells/μL for ART initiation.[4] Though early diagnosis and linkage to ART program is challenging in resource-limited settings, initiating ART at higher CD4 count will likely to improve virological suppression and resistance outcome.[27,44,45] A similar study from Asia found multi-NRTI resistant associated mutations (RAMs) in 37% of the patients, wherein, multi-NRTI RAMs were defined as presence of either Q151M; 69Ins; 2 TAMs; or M184V + 1 TAM.[19] In our study, with modified definition, multi-NRTI DRMs were present in 32.5% of sequences.

The emergence of K65R among individuals failing TDF-based regimen is primarily responsible for multi-NRTI resistance. In this study, 52.94% of individuals who were exposed to TDF-developed K65R mutation. Though TDF-based regimen is associated with higher rate of viral suppression as compared to AZT, one must be cautious of the emergence of K65R mutation among individuals failing TDF-based regimen.[46] These results have implications on program as recently we adopted fixed-dose combination of TDF, 3TC, and EFV as primary initiating regimen based on WHO recommendations.[26,27] In a recent study from South Africa, authors have concluded that patients failing on a TDF-containing regimen were almost 5 times more likely to present with a K65R mutation compared to d4T-exposed patients.[47] The selection of K65R is known to be facilitated in subtype C, which is a predominant circulating subtype in India.[48] The lower genetic barrier in

subtype C for K65R may be attributed to enzymatic pausing arising at the end of poly-adenine stretches.[49] In addition, recent reports have suggested antagonism between K65R and TAMs, indicating that both pathways are unlikely to occur simultaneously.[50]

Surprisingly among individuals with VF, higher reported adherence (> 95%) was associated with multi-NRTI DRMs. As we captured adherence data of recent 3 months before failure, it is possible that these individuals had periods of suboptimal adherence initially. In a failing individual high level of adherence provides environment for selective drug pressure.[51] Thus in absence of virological monitoring, continuation of failing regimen despite higher adherence may further lead to development of DRMs.[52] Nevertheless intensive efforts should be made to ensure optimal adherence among individuals initiating ART.

Our study does have few limitations. Firstly, pretreatment drug resistance was not assessed due to financial constraints, which may influence the final outcome.[53] However, recent studies from India indicates transmitted drug resistance to be less than 5%.[54–56] In this study, VF was diagnosed by single plasma viral load estimation at 12 ± 2 months. Thought 2 tests are preferable, most studies resort to single tests as cost-saving measure.[30] Use of population-based genotyping method in this study may underestimate the prevalence of resistance. Finally, it should be recorded that the ART clinics involved in this study were located in large city, catering for urban population and therefore, findings may not be nationally representative.

To conclude, implementation of viral load monitoring into the program is a long-standing priority. Though, this study achieved WHO recommended target for viral load suppression, the program should focus on timely ART initiation and optimal adherence. Psychosocial support of partner is essential for improvement in virological outcome among PLHA. The study highlights the importance of AZT as preferred NRTI option in second-line ART due to selection of K65R by TDF. With recent introduction of fixed-dose combination of TDF, 3TC, and EFV in national program, the emergence of K65R need to be monitored closely among HIV-1 subtype C-infected Indian population.

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