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Antiretroviral resistance following immunological monitoring in a resource-limited setting of western India: A cross-sectional study

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Abstract

Background

The free antiretroviral therapy (ART) program in India still relies on the clinico-immunological monitoring for diagnosis of treatment failure. As the nucleoside reverse transcriptase inhibitor (NRTI) backbone is shared in first- and second-line regimens, accumulation of drug resistant mutations (DRMs) can compromise the efficacy of NRTI. This study was undertaken to describe the pattern of HIV DRMs following immunological monitoring and investigate its impact on the cycling of NRTI between first- and second-line ART.

Methods and findings

This cross-sectional study was performed at a state-sponsored ART clinic of Pune city in western India between January and June 2016. Consecutive adults receiving first-line ART with immunological failure (IF) were recruited for plasma viral load (PVL) estimation. Randomly selected 80 participants with PVL >1000 copies/mL underwent HIV drug resistance genotyping. Of these, 75 plasma sample were successfully genotyped. The median CD4 count and duration of ART at the time of failure were 98 (IQR: 61.60–153.50) cells/μL and 4.62 (IQR: 3.17–6.15) years, respectively. The prevalence of NRTI, non-NRTI, and major protease inhibitor resistance mutations were 89.30%, 96%, and 1.33%, respectively. Following first-line failure, sequences from 56.67% of individuals indicated low- to high-level resistance to all available NRTI. The proportion of sequences with ≥2 thymidine analogue mutations (TAMs) and ≥3 TAMs were 62.12% and 39.39%, respectively. An average of 1.98 TAMs per sequence were observed following IF as compared to 0.37 TAMs per

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sequence following targeted PVL monitoring at 12 months of ART from a prior study; this difference was significant (p<0.001).

Conclusion

The option of cycling of NRTI analogues between first- and second-line regimens would no longer be effective if individuals are followed-up by immunological monitoring due to accumulation of mutations. Introduction of routine PVL monitoring is a priority for the long-term sustainability of free ART program in India.

Introduction

In India, the free antiretroviral therapy (ART) program has scaled up considerably from eight ART centers in 2004 to over 519 by 2015 [1]. In last decade, successful implementation of the National AIDS Control Program has contributed to a decline in the estimated adult HIV prevalence, from 0.34% (0.25–0.43%) in 2007 to 0.26% (0.22%-0.32%) in 2015 [2]. With the rapid rollout of ART, the consequential emergence and propagation of drug resistance is inevitable. Prior studies from India have reported variable HIV drug resistance (HIVDR) prevalence (93.80%-100%) following clinico-immunological failure (IF) of first line ART [3–7]. The prevalence of resistance mutations were lower following targeted plasma viral load (PVL) testing at 12 months of ART [8–10]. In order to make midcourse corrections in the decade-old program, periodic monitoring of HIVDR is essential.

As per national guidelines, first-line ART in adults consists of two nucleoside analogue reverse transcriptase inhibitors (NRTI) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) [11]. Following failure of the first-line regimen, the second-line regimen consists of ritonavir-boosted protease inhibitors (rPI) supported by two NRTI, one of which should be new [12]. The PVL monitoring is a sensitive test and is more likely to indicate treatment failure earlier compared to clinical or immunological monitoring [13]. The free ART program in India continues to rely on the WHO criteria for the detection of clinical and immunological failure in the diagnosis of first-line ART failure [14]. As an NRTI backbone is shared in first- and second-line regimens, accumulation of drug resistance mutations (DRMs) can compromise the efficacy of NRTI. The primary objective of this study was to describe the pattern of HIV-DRMs following immunological monitoring in a state-sponsored ART clinic. Secondarily, we also investigated the impact of the accumulation of DRMs on the therapeutic options for NRTIs to be used in second-line ART.

Materials and methods

Study design and sample collection

The study was carried out at the state-sponsored "Centre of Excellence" ART clinic of Pune city in western India between January and June 2016. In this cross-sectional study, we recruited consecutive adults with features of IF who were referred for confirmation of failure by PVL estimation. The eligibility criteria were individuals with age >18 years who were initiated on first-line ART as per national guidelines and had an adherence rate of over 95% in the past three months. IF was defined as per WHO definition of clinical and immunological failure, wherein, fall in CD4 cell count to baseline or below and persistent CD4 cell count of less than 100 cells/μL were considered [14]. Estimation of PVL was performed using Abbott

RealTime HIV-1 *m2000rt* system and virological failure was defined as a single HIV-1 PVL of more than 1000 copies/mL [14]. Five milliliters of whole blood from each individual was collected in EDTA vacutainer tubes and transported to the laboratory for PVL estimation and HIVDR genotyping. For individuals undergoing resistance testing, the details of adherence, socio-demographic profile, prior antiretroviral (ARV) drug exposure during first-line ART, drug substitutions, co-infections, and previous CD4 cell counts were noted from the individual's medical records. Drug adherence was calculated by averaging the medication possession ratio of the recent 3 months. Substitution was defined as replacement of any first-line ARV (usually by an agent of same class) due to toxicity or drug interaction.

HIV drug resistance genotyping

Randomly selected 80 subjects with PVL > 1000 copies/ml underwent HIV drug resistance (DR) genotyping at WHO accreditated HIV drug resistance genotyping laboratory of the National AIDS Research Institute (ICMR), Pune, India. Genotyping of complete protease (PR; codon 1 to 99) and partial reverse transcriptase (RT; codon 1 to 256) region of the *pol* gene was performed by a previously validated method [15]. Following sequencing, resistance pattern was determined by Stanford University genotype resistance interpretation algorithm, HIVdb version 8.3. The thymidine analogue mutations (TAMs) considered for analysis were, TAM-1, which includes M41L, L210W and T215Y; and TAM-2, which includes D67N, K70R, T215F and K219Q/E. Other clinically relevant NRTI mutations included were M184V/I, K65R, K70E, L74V/I and Y115F. Any accessory mutation present in excess of 10% were also mentioned. The NNRTI mutations considered were L100I, K101E/P, K103N/S, V106A/M, E138A/G/K/Q, Y181C/I/V, Y188L/C/H, G190A/S/E and M230L. The predicted susceptibility of an ARV was decided by adding penalty scores associated with each DRMs in a given sequence (https://hivdb.stanford.edu/DR/asi/releaseNotes/#hivdb_ mutationpenaltyHeader). The degree of resistance was categorized into 5 levels based on total penalty score; susceptible (0–9), potential low-level resistance (10–14), low-level resistance (15–29), intermediate resistance (30–59) and high-level resistance (>60). For analysis purpose, two level category was created with a total score of 0–14 was considered as susceptible and a score of >15 was considered resistant. Phylogenetic tree of partial *pol* gene study sequences along with reference sequences was constructed by the maximum likelihood method based on general time reversible model, with MEGA 6.0 software. The reliability of the branching orders was tested by bootstrap analysis of 1000 replicates.

Statistical analysis

Characteristics of the study participants were summarized by median and interquartile range (IQR) for continuous variables and by proportion for categorical variables. The proportion of sequences with thymidine analogue mutations (TAMs; M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E) from the individuals with immunological failure in present study was compared with the sequences from a virological failure (VF) group of prior study by Karade et al. [9]. In this study, virological suppression was assessed in 847 participants at 12 ± 2 months of ART initiation and HIV drug resistance genotyping was performed in 80 individuals with virological failure (VF) [9]. Differences between these two groups were compared using the Student's t-test for continuous variables and by Pearson's Chi-square test for categorical variables.

Ethical consideration

The study was approved by Ethics committee of National AIDS Research Institute and the participants were enrolled after obtaining written informed consent.

Results

A total of 387 individuals on first-line ART with evidence of immunological failure (IF) reported for PVL testing. Eighty plasma samples, with PVL > 1000 copies/mL were randomly chosen for drug resistance genotyping. Of these, 75 samples, including those from 50 males, were successfully genotyped. The demographic characteristics of the participants at the time of first-line ART failure are summarized in **Table 1.** A total of 56% of individuals with IF underwent treatment switches in the past due to the reasons like adverse drug reactions, drug stock out, and change in the national policy. Importantly, 21 out of 33 patients on TDF based failing regimen had prior exposure to AZT or d4T in past. The proportion of individuals with failure of zidovudine (AZT)- and tenofovir (TDF)-based regimens were 56% and 44%, respectively. Phylogenetic analysis of the partial *pol* gene sequences showed that 74 sequences clustered with the reference sequence of the Indian HIV-1 subtype C virus; one isolate clustered with subtype A1 **(Fig 1)**. The comparison of demographic features of IF group and VF group is shown in **S1 Table**.

The prevalence of NRTI, NNRTI, and major protease inhibitor (PI) resistance mutations following immunological monitoring were 89.30%, 96%, and 1.33%, respectively. M184V (88%) and K103N (46.67%) were the predominant NRTI and NNRTI resistance mutations, respectively. M41L (49.33%) and T215Y (41.30%) were the most common TAMs. The clinically relevant NRTI and NNRTI mutations are shown in **Fig 2**. A total of 72% of the sequences indicated the presence of TAMs, with an average of 1.98 TAMs per sequence. The differences in the proportion of sequences with TAM-1 (57.33%) and TAM-2 (49.33%) mutations were not significant **(S2 Table)**. The number of sequences with ≥2 and ≥3 TAMs were 61.3% and 38.67%, respectively. The average TAMs in patients failing AZT based regimen (2.14 TAMs/sequence) was higher as compared to those on TDF based regimen (1.78 TAMs/sequence), however this difference was not significant ($p = 0.34$). One sequence showed the presence a insertion at codon 69 (T69 insertion) of reverse transcriptase (RT) while another sequence carried the Q151M complex mutations; each of these mutations are known to confer pan-NRTI drug resistance. In addition, another sequence harbored an amino acid deletion at codon 67 of RT. Major PI resistance mutations (M46I and N88S) was observed in a single sequence, whereas four sequences showed accessory mutations Q58E, G73C, T74P, and L10F.

Following first-line ART failure, the susceptibility to various NRTI was ascertained for cycling in second-line ART. Based on the Stanford database HIVDR scoring system, low- to high-levels of resistance to zidovudine (AZT) and tenofovir (TDF) was seen in 65.33% and 49.33% of sequences, respectively. Predicted susceptibility to NRTI in sequences from the present study were compared with our prior study, wherein first-line ART failure was diagnosed by virological monitoring at 12 months of ART [9]. The proportion of sequences with low- to high-level resistance to TDF and AZT were significantly higher following immunological monitoring ($p < 0.005$) **(Fig 3).**

Discussion

In this study, we report the drug resistance outcomes in individuals with failure of first-line ART, diagnosed as per the current national guidelines. The decade-old National AIDS Control Program still relies on immunological monitoring, wherein six-monthly CD4 cell count measurements are performed, while PVL estimation is only reserved for confirmation of first-line ART failure [11]. In a prior study conducted in India, evaluation of the immunological criteria for detecting virological failure in individuals receiving first-line ART indicated a sensitivity and specificity of 22.80% and 94.60%, respectively [16]. Delayed diagnosis of failure not only causes accumulation of DRMs but also increases cross-resistance to NRTI analogues. In a

Table 1. Demographic details of the 75 study participants with failure of first-line ART.

Characteristics of participants	Total, n = 75
Gender, Male, n (%)	50 (66.67)
Patient Age, Median (IQR), in years	39 (34–43)
18–30, n(%)	12 (16)
31–40	32 (46.67)
41 and above	31 (41.33)
Marital status, n(%)	
Married or Living-in partner	52 (69.33)
Divorced/separated/Widow	17 (22.67)
Unmarried	6 (8)
Educational status, n(%)	
Illiterate	16 (21.33)
Primary School	16 (21.33)
Secondary school	33 (44)
College and above	10 (13.33)
Predominant mode of transmission	
Heterosexual, n(%)	70 (93.33)
Past history of tuberculosis	25 (33.33)
CD4 (cells/μL) at failure, Median (IQR)	98 (61.60–153.50)
less than 100, n(%)	39 (52)
100–200	29 (38.67)
201–300	4 (5.33)
more than 300	3 (4)
Median (IQR) VL at failure (log_{10} copies/ml)	4.87 (4.47–5.24)
3–3.9, n(%)	8 (10.67
4–4.9	34 (45.33)
>5	33 (44)
Failing ART regimen, n(%)	
AZT+3TC+NVP	38 (50.67)
AZT+3TC+EFV	4 (5.33)
TDF+3TC+NVP	2 (2.67)
TDF+3TC+EFV	31 (41.33)
First-line ART switch, n(%)	
ART regimen never switched	33 (44)
1 to 2 switches in regimen	35 (46.67)
3 or more switches in regimen	7 (9.33)
Duration of ART (yrs) Median (IQR)	4.62 (3.17–6.15)
≤ 2 years	10 (13.33)
2.1–6 yrs	42 (55.99)
> 6 yrs	23 (30.67)

(Abbreviations; ART—antiretroviral therapy, VL—plasma viral load, IQR—interquartile range, AZT—zidovudine, 3TC—lamivudine, TDF—tenofovir disoproxil fumarate, NVP—nevirapine and EFV—efavirenz.)

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study among Malawians with failure of first-line ART, Hosseinipour et al. reported compromised activity of NRTI agents in 17% of the patients [17]. A prior study from southern India reported TAMs in 53.40% of the subjects following first-line immunological failure with predominance of the M41L (40%) mutation [18]. In our study, 70% of the sequences indicated

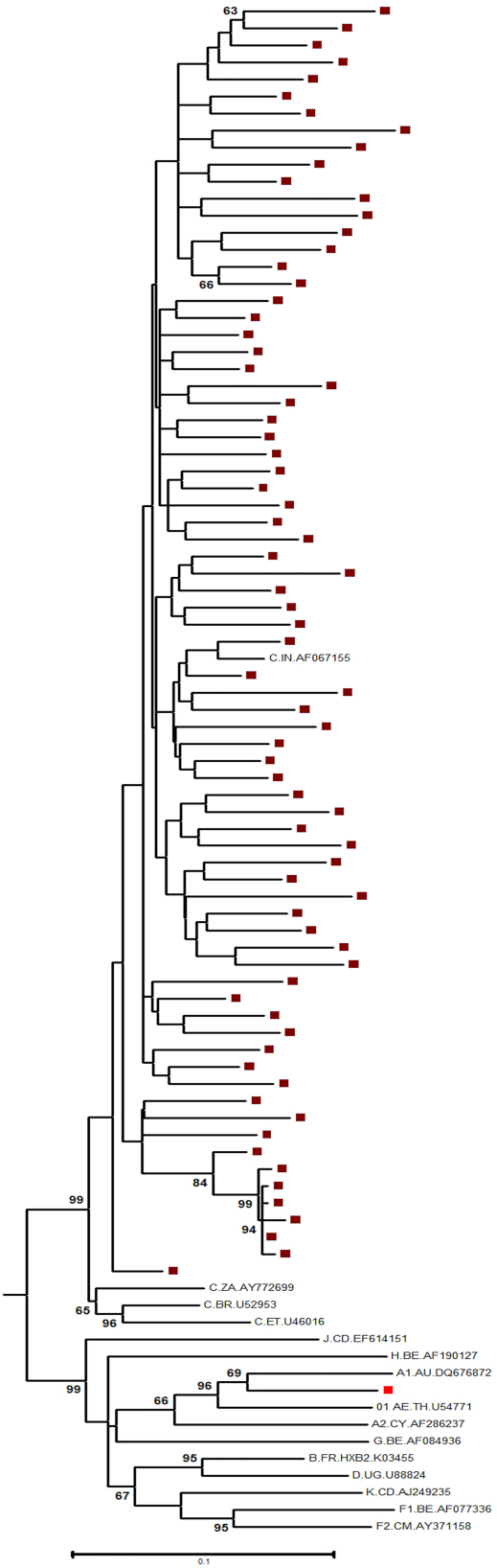

Fig 1. Phylogenetic tree. The phylogenetic tree shows the relationship of 75 partial *pol* gene study sequences with reference sequences. The study sequences are highlighted by solid circles.

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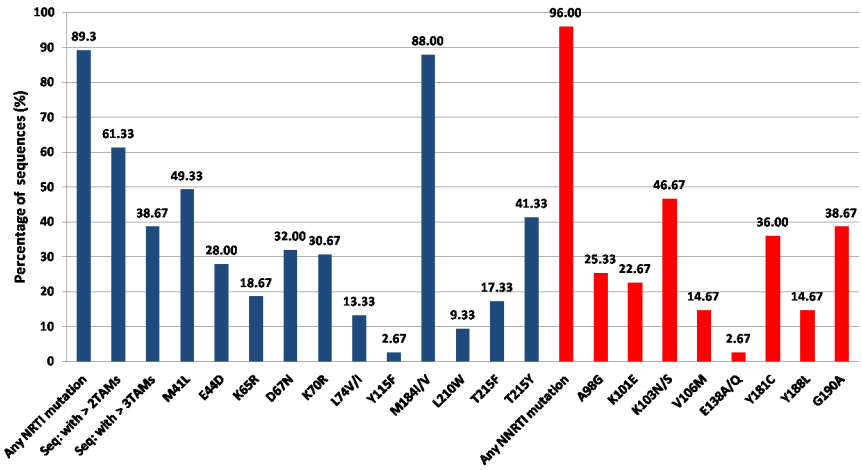

Fig 2. HIV drug resistance pattern. Pattern of NRTI and NNRTI drug resistance mutations following immunological failure. Abbreviations: NRTI—nucleoside/nucleotide analogue reverse transcriptase inhibitor, NNRTI—non-nucleoside reverse transcriptase inhibitor.

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presence of TAMs, with 38.66% showing more than 3 TAMs per sequence (S2 **Table**). Furthermore, 56.67% of the sequences showed intermediate- to high-level resistance to both TDF and AZT, rendering cycling of NRTI option between first- and second-line ART ineffective. Therefore, in absence of PVL and HIVDR testing, over half of the individuals with immunological failure will be exposed to non-productive NRTI backbone in second-line ART.

The evolution of TAMs can be limited by early diagnosis of treatment failure. Reynolds et al. compared the HIVDR pattern between a group monitored by 6-monthly PVL testing (VLM group) and another, monitored by 6-monthly CD4 cell count (IM group) [19]. The study reported significantly higher rates of TAMs in the IM group (49%) than in the VLM

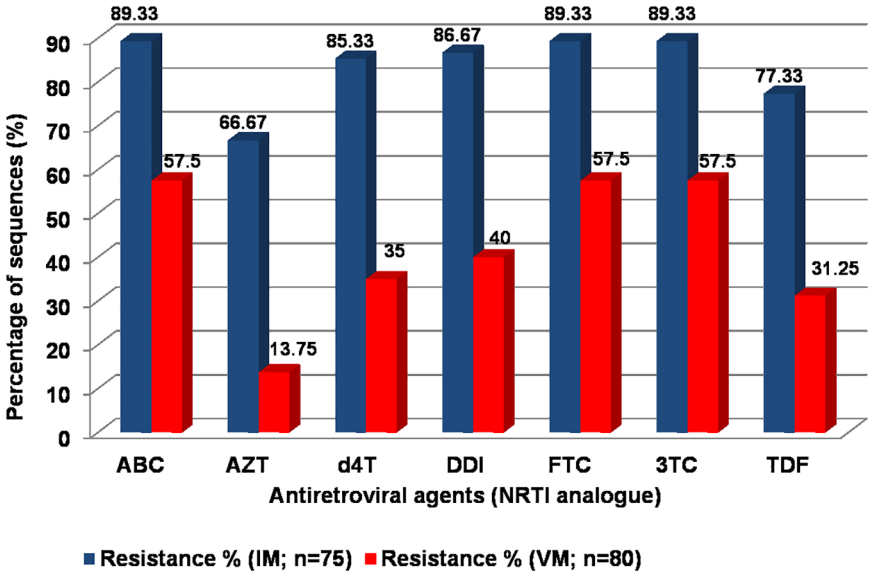

Fig 3. Predicted susceptibility to NRTI. The predicted susceptibility to NRTI following immunological monitoring (IM; n = 75) is compared with those from individuals with virological failure detected by targeted viral load monitoring at 12 months of ART (VM; n = 80).

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group (5%) [19]. Targeted PVL testing at 12 months of ART may be a feasible option for low- and middle-income countries. In a study involving 142 subjects with virological failure from 6 sub-Saharan African countries, the prevalence of TAMs at the end of one year of ART was just 8.50% [20]. Previously, we reported the presence of TAMs in 17.50% sequences following virological failure at 12 months of ART [9]. The predicted susceptibility of NRTI analogues following IM, were significantly lower than that following targeted virological monitoring at 12 months of ART due to accumulation of TAMs.

In the EuroSIDA prospective observational cohort, the rate of accumulation of TAMs in subjects who continued receiving failing regimens was relatively lower (on average, 1 additional TAM accumulated every 4.3 years of exposure to the failing regimen) [21]. Unlike in a research-study setting, where there is dedicated staff and infrastructure, in resource-limited settings, TAM accumulation could be underestimated due to delayed ART initiation, inefficient monitoring, and episodes of ARV stock-out. A primarily subtype-C driven HIV epidemic, lack of pre-treatment genotypic resistance testing facility and suboptimal adherence to ART might further contribute to the adverse resistance outcome in Indian setting [22–24]. A prior study has reported higher frequency of DRMs in HIV-1 subtype C and CRF01_AE, than in subtype B infected people, following first-line ART failure [25].

Evolution of resistance is a continuous process. The prohibitive cost limits inclusion of HIV drug resistance testing into the National AIDS Control Program. The clinico-immunological monitoring identification of treatment-failure results in the development of complex DRMs, thereby compromising the NRTI backbone in second-line ART [17, 26]. Under such circumstances, one can use ritonavir boosted darunavir and/or integrase strand transfer inhibitors (INSTI), such as raltegravir and dolutegravir to support PI. Neither of them may be a feasible option for second-line ART in resource-limited setting. The choice of any of these ARV drugs will also affect options for third-line ART. At the time of failure, the median CD4 count of 98 cells/μL (IQR: 61.60–153.50) and viral load of 4.87 (IQR: 4.47–5.24) $\log_{10}$ copies/mL is indicative of longstanding failure in our study. Lower CD4 cell count and high PVL not only increases the risk of opportunistic infections but also enhances the transmission risk to sexual partners. Therefore, viral load monitoring is essential for early identification of failure. Of note, our study results are limited by the cross-sectional study design and small sample size. In addition, pre-treatment HIVDR reports for the study participants were not available and were presumed to be below 5% [27, 28]. Furthermore, even though we performed PVL and HIVDR testing on same plasma sample of all patients, a delay of 2 weeks was experienced from the time of detection of IF to PVL testing, as many of our patients are referred to us from distant clinics.

To conclude, we report extensive HIV DRMs in patients failing first-line ART. Thus, periodic surveillance of HIVDR at the national level is essential to guide the program. The option of cycling of NRTIs between first- and second-line regimens may no longer be effective if individuals are followed-up by immunological monitoring. Finally, access to periodic PVL monitoring and HIVDR testing is a necessity for long-term preservation of the efficacy of current therapeutic options.

Supporting information

S1 Table. Study group comparison. Comparison of demographic and clinical characteristics of virological monitoring group (VF) and immunological monitoring group (IF).
(DOCX)

S2 Table. Thymidine analogue mutation (TAM) pattern. Comparison of TAMs in sequences from immunological failure group (IM group) with those retrieved from individuals with

virological failure at 12 months of ART (VM group).
(DOCX)

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References

1. National AIDS Control Organization, Department of AIDS Control, Ministry of Health and Family Welfare. Government of India. Annual report 2014–15. Available at http://naco.gov.in/documents/annual-reports
2. National AIDS Control Organization and National Institute of Medical Statistics (ICMR). India HIV Estimations 2015. Technical Report. Available at: http://indiahivinfo.naco.gov.in/naco/resource/india-hiv-estimations-2015-technical-report.
3. Kumarasamy N, Madhavan V, Venkatesh KK, Saravanan S, Kantor R, Balakrishnan P, et al. High frequency of clinically significant mutations after first-line generic highly active antiretroviral therapy failure: implications for second-line options in resource-limited settings. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2009; 49(2):306–9.
4. Sinha S, Shekhar RC, Ahmad H, Kumar N, Samantaray JC, Sreenivas V, et al. Prevalence of HIV drug resistance mutation in the northern Indian population after failure of the first line antiretroviral therapy. Current HIV research. 2012; 10(6):532–8. PMID: 22716105
5. Vidya M, Saravanan S, Uma S, Kumarasamy N, Sunil SS, Kantor R, et al. Genotypic HIV type-1 drug resistance among patients with immunological failure to first-line antiretroviral therapy in south India. Antiviral therapy. 2009; 14(7):1005–9. https://doi.org/10.3851/IMP1411 PMID: 19918105
6. Anquetil D, Deshpande A, Zongo D, Le Bihan L, Pinson PR, Fleury HJ. Susceptibility to etravirine of HIV type 1 subtype C isolates from nevirapine/efavirenz-experienced patients: comparative interpretation of ANRS and STANFORD algorithms. AIDS research and human retroviruses. 2012; 28(12):1793–7. https://doi.org/10.1089/AID.2012.0060 PMID: 22519709

7. Kandathil AJ, Kannangai R, Verghese VP, Pulimood SA, Rupali P, Sridharan G, et al. Drug resistant mutations detected by genotypic drug resistance testing in patients failing therapy in clade C HIV-1 infected individuals from India. Indian journal of medical microbiology. 2009; 27(3):231–6. https://doi.org/10.4103/0255-0857.53205 PMID: 19584504
8. Hingankar NK, Thorat SR, Deshpande A, Rajasekaran S, Chandrasekar C, Kumar S, et al. Initial virologic response and HIV drug resistance among HIV-infected individuals initiating first-line antiretroviral therapy at 2 clinics in Chennai and Mumbai, India. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2012; 54 Suppl 4:S348–54.
9. Karade SK, Ghate MV, Chaturbhuj DN, Kadam DB, Shankar S, Gaikwad N, et al. Cross-sectional study of virological failure and multinucleoside reverse transcriptase inhibitor resistance at 12 months of antiretroviral therapy in Western India. Medicine. 2016; 95(37):e4886. https://doi.org/10.1097/MD.0000000000004886 PMID: 27631260
10. Patil RT, Gupta RM, Sen S, Tripathy SP, Chaturbhuj DN, Hingankar NK, et al. Emergence of drug resistance in human immunodeficiency virus type 1 infected patients from pune, India, at the end of 12 months of first line antiretroviral therapy initiation. Isrn Aids. 2014; 2014:674906. https://doi.org/10.1155/2014/674906 PMID: 25006528
11. Department of AIDS Control. Ministry of health and family welfare. Anti Retroviral Therapy Guidelines for HIV Infected Adults and Adolescents, May 2013. [Internet]. Available at: http://naco.gov.in/care-support-treatment.
12. National AIDS Control Organization, Department of AIDS Control, New Delhi. National Guidelines on Second-line and Alternative First-line ART For Adults and Adolescents May 2013. Available at: http://naco.gov.in/care-support-treatment
13. Rutherford GW, Anglemyer A, Easterbrook PJ, Horvath T, Vitoria M, Penazzato M, et al. Predicting treatment failure in adults and children on antiretroviral therapy: a systematic review of the performance characteristics of the 2010 WHO immunologic and clinical criteria for virologic failure. Aids. 2014; 28 Suppl 2:S161–9.
14. World Health Organization G. Consolidated guidelines on the use of antiretroviral drugs for treating HIV infection. Recommendations for public health approach. Second ed2016. 422 p.
15. Chaturbhuj Devidas N., Nirmalkar Amit P., Paranjape Ramesh S., Tripathy SP. Evaluation of a Cost Effective In-House Method for HIV-1 Drug Resistance Genotyping Using Plasma Samples. PloS one. 2014; 9(2):8.
16. Vallabhaneni S, Chandy S, Heylen E, Ekstrand ML. Evaluation of WHO immunologic criteria for treatment failure: implications for detection of virologic failure, evolution of drug resistance and choice of second-line therapy in India. Journal of the International AIDS Society. 2013; 16:18449. https://doi.org/10.7448/IAS.16.1.18449 PMID: 23735817
17. Hosseinipour MC, van Oosterhout JJ, Weigel R, Phiri S, Kamwendo D, Parkin N, et al. The public health approach to identify antiretroviral therapy failure: high-level nucleoside reverse transcriptase inhibitor resistance among Malawians failing first-line antiretroviral therapy. Aids. 2009; 23(9):1127–34. https://doi.org/10.1097/QAD.0b013e32832ac34e PMID: 19417582
18. Sivamalar S, Dinesha TR, Gomathi S, Pradeep A, Boobalan J, Solomon SS, et al. Accumulation of HIV-1 Drug Resistance Mutations After First-Line Immunological Failure to Evaluate the Options of Recycling NRTI Drugs in Second-Line Treatment: A Study from South India. AIDS research and human retroviruses. 2016.
19. Reynolds SJ, Sendagire H, Newell K, Castelnuovo B, Nankya I, Kamya M, et al. Virologic versus immunologic monitoring and the rate of accumulated genotypic resistance to first-line antiretroviral drugs in Uganda. BMC infectious diseases. 2012; 12:381. https://doi.org/10.1186/1471-2334-12-381 PMID: 23270482
20. Hamers RL, Sigaloff KC, Wensing AM, Wallis CL, Kityo C, Siwale M, et al. Patterns of HIV-1 drug resistance after first-line antiretroviral therapy (ART) failure in 6 sub-Saharan African countries: implications for second-line ART strategies. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2012; 54(11):1660–9.
21. Cozzi-Lepri A, Phillips AN, Martinez-Picado J, Monforte A, Katlama C, Eg Hansen AB, et al. Rate of accumulation of thymidine analogue mutations in patients continuing to receive virologically failing regimens containing zidovudine or stavudine: implications for antiretroviral therapy programs in resource-limited settings. The Journal of infectious diseases. 2009; 200(5):687–97. https://doi.org/10.1086/604731 PMID: 19604043
22. Kantor R, Smeaton L, Vardhanabhuti S, Hudelson SE, Wallis CL, Tripathy S, et al. Pretreatment HIV Drug Resistance and HIV-1 Subtype C Are Independently Associated With Virologic Failure: Results From the Multinational PEARLS (ACTG A5175) Clinical Trial. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2015.

23. Ekstrand ML, Shet A, Chandy S, Singh G, Shamsundar R, Madhavan V, et al. Suboptimal adherence associated with virological failure and resistance mutations to first-line highly active antiretroviral therapy (HAART) in Bangalore, India. International health. 2011; 3(1):27–34. https://doi.org/10.1016/j.inhe.2010.11.003 PMID: 21516199

24. Mehta KG, Baxi R, Patel S, Parmar M. Drug adherence rate and loss to follow-up among people living with HIV/AIDS attending an ART Centre in a Tertiary Government Hospital in Western India. Journal of family medicine and primary care. 2016; 5(2):266–9. https://doi.org/10.4103/2249-4863.192325 PMID: 27843825

25. Huang A, Hogan JW, Luo X, DeLong A, Saravanan S, Wu Y, et al. Global Comparison of Drug Resistance Mutations After First-Line Antiretroviral Therapy Across Human Immunodeficiency Virus-1 Subtypes. Open forum infectious diseases. 2016; 3(2):ofv158. https://doi.org/10.1093/ofid/ofv158 PMID: 27419147

26. Ndembi N, Goodall RL, Dunn DT, McCormick A, Burke A, Lyagoba F, et al. Viral rebound and emergence of drug resistance in the absence of viral load testing: a randomized comparison between zidovudine-lamivudine plus Nevirapine and zidovudine-lamivudine plus Abacavir. The Journal of infectious diseases. 2010; 201(1):106–13. https://doi.org/10.1086/648590 PMID: 19938977

27. Karade S, Patil AA, Ghate M, Kulkarni SS, Kurle SN, Risbud AR, et al. Limited HIV Pre-treatment Drug Resistance (PDR) Among Adults Attending Free Antiretroviral Therapy (ART) Clinic of Pune, India. AIDS research and human retroviruses. 2015.

28. Kannangai R, David S, Sundaresan VC, Sachithanandham J, Mani M, Abraham OC, et al. Frequency of transmitted drug resistance mutations among treatment-naive HIV-1-infected individuals at a tertiary care centre in South India. Molecular diagnosis & therapy. 2015; 19(5):273–5.