

RESEARCH

Open Access



Increased HIV-1 pretreatment drug resistance with consistent clade homogeneity among ART-naive HIV-1 infected individuals in Ethiopia

Mulugeta Kiros^{1,2*}, Dawit Hailu Alemayehu², Eleni Geberekidan³, Adane Mihret², Melanie Maier⁴, Woldaregay Erku Abegaz⁵ and Andargachew Mulu²

Abstract

Background: The development of pretreatment drug resistance (PDR) is becoming an obstacle to the success of antiretroviral therapy (ART). Besides, data from developing settings including Ethiopia is still limited. Therefore, this study was aimed to assess HIV-1 genetic diversity and PDR mutations among ART-naive recently diagnosed HIV-1 infected individuals in Addis Ababa, Ethiopia.

Methods: Institutional based cross-sectional study was conducted from June to December 2018 in Addis Ababa among ART-naive recently diagnosed individuals. Partial HIV-1 pol region covering the entire protease (PR) and partial reverse transcriptase (RT) regions of 51 samples were amplified and sequenced using an in-house assay. Drug resistance mutations were examined using calibrated population resistance (CPR) tool version 6.0 from the Stanford HIV drug resistance database and the International Antiviral Society-USA (IAS-USA) 2019 mutation list.

Results: According to both algorithms used, 9.8% (5/51) of analyzed samples had at least one PDR Mutation. PDR mutations to Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) were the most frequently detected (7.8% and 9.8%, according to the CPR tool and IAS-USA algorithm, respectively). The most frequently observed NNRTI-associated mutations common to both algorithms were K103N (2%), Y188L (2%), K101E (2%), and V106A (2%), while E138A (2%) was observed according to IAS-USA only. Y115F and M184V (mutations that confer resistance to NRTIs) dual mutations were detected according to both criteria in a single study participant (2%). PDR mutation to protease inhibitors was found to be low (only G73S; 2% according to the CPR tool). Phylogenetic analysis showed that 98% (50/51) of the study participants were infected with HIV-1C virus while one individual (2%) was infected with HIV-1A1 virus.

Conclusions: This study showed an increased level of PDR and persistence HIV-1C clade homogeneity after 15 years of the rollout of ART and 3 decades of HIV-1C circulation in Ethiopia, respectively. Therefore, we recommend routine baseline genotypic drug resistance testing for all newly diagnosed HIV infected patients before initiating treatment. This will aid the selection of appropriate therapy in achieving the 90% of patients having an undetectable viral load in consonance with the UN target.

Keywords: HIV-1, HIV-1 genetic diversity, Pretreatment drug resistance, HIV-1 subtype, ART-naive, Ethiopia

*Correspondence: mulugetakiros@gmail.com

² Armauer Hansen Research Institute, Addis Ababa, Ethiopia

Full list of author information is available at the end of the article



© The Author(s) 2020. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Background

Human Immunodeficiency Virus (HIV) is a major global public health issue with millions of people infected worldwide [1]. Africa is the most affected region among other continents in the globe with the highest rise of the illness concentrating in the eastern and southern parts [2]. In Eastern and Southern Africa, which is the home of 54% of the world's people living with HIV [3], there were an estimated 800,000 new HIV infections which account for around 47% of the total global new infection and 310,000 people dying from Acquired Immuno Deficiency Syndrome (AIDS)-related illness in the same year [3].

In Ethiopia, HIV/AIDS is among the top ten leading causes of age-standardized premature mortality and death [4]. In 2018, there were an estimated 23,000 new infections and 11,000 AIDS-related deaths [3]. The rate of new infections showed an increment from the previous year which was 16,000 new infections in 2017 [5]. There were an estimated 690,000 people living with HIV in the country in 2018 [3]. According to data from the Federal HIV/AIDS Prevention and Control Office, the HIV prevalence in the country in 2018 was 0.9% [6].

Although ART is contributing a lot in prolonging the life of HIV infected individuals [7], scaling up of this treatment option along with the absence of drug resistance testing in resource-limited settings is paralleled by an increased prevalence of pretreatment drug resistance (PDR) [8, 9]. This is becoming a significant obstacle in maintaining suppression of HIV replication leading to a higher probability of early virological failure [10] that further hinders the widespread use of ART [11, 12]. Likewise, it is becoming a potential threat to the long-term success of ART and is emerging as a threat to the elimination of AIDS as a public health problem [13].

According to the Ethiopian Ministry of Health updated guideline for HIV prevention, care and treatment, the current preferred first-line regimen combination for adults and adolescents is TDF + 3TC + DTG/EFV. The alternative drug combinations include AZT + 3TC + EFV or TDF + 3TC + NVP. AZT + 3TC + ATV/r or LPV/r are drug of choices in the second-line ART. In the third line treatment strategy, the combination is as follows; DRV/r + ABC + 3TC + EFV or NVP [14].

Although it has been 15 years since the rollout of ART in Ethiopia, only a few studies are done with regard to PDR with the majority of them being from the Northern part of Ethiopia [15–18] and data from the capital city is limited [19, 20]. Thus, this study was aimed at generating updated information about genetic diversity and magnitude of PDR in Addis Ababa that may be utilized in alleviating the problem of treatment failure and consequently reducing the burden of the disease.

Methods

Study subjects and study design

Institutional based cross-sectional study was conducted among 51 ART-naive HIV infected asymptomatic adult individuals in Addis Ababa. The study was conducted in four government-affiliated voluntary counseling test centers in Addis Ababa from June to December 2018. The sites were selected based on the availability of a high flow of individuals in their testing center. Study subjects who are asymptomatic, ≥ 18 years old, and willing to participate in this study were sequentially included in this study. Pregnant women, individuals with known chronic illnesses, or any previous ART use were excluded from this study. The sample size used in this study is concurrent with the WHO recommendation for the surveillance of HIVDR in resource-limited settings. According to the recommendation, it is advised to analyze sequences from a minimum of 47 individuals [21].

Sample collection

A blood sample (10 ml) was collected by trained medical personnel aseptically. Vacutainer test tubes containing ethylene diamine tetra-acetic acid (EDTA) were used to collect the blood samples. The sample was labeled with appropriate patient information. Plasma was then separated from blood cells within 2 h of collection in the laboratory by centrifugation at the speed of 1200g (3000 rpm) for 10 to 15 min in accordance with the recommendation of WHO. The plasma was aliquotted into Nunc tubes of 1.5-ml capacity, and transported in an icebox on the same day to the AHRI laboratory, where it was stored at -80°C until required for molecular analysis.

Laboratory investigations

Viral load and RNA extraction

HIV-1 RNA extraction and viral quantification were done using Abbott Real-time HIV-1 M2000rt extraction machine (Abbott Laboratories, Abbott Park, USA).

Reverse transcription polymerase chain reaction (RT-PCR)

cDNA for the entire PR [99 codons] and partial RT [the first 308 codons] regions was synthesized in a 20 μl reaction mixture using superscript IV Reverse Transcriptase enzyme and HIVrt primer (5'-TGTTTACATCATTA GTGTG-3', HXB2 location: 3630–3649). The thermal cycling for cDNA synthesis was; 50°C for 1 h [17]. After cDNA was synthesized, we used Platinum Taq High Fidelity (Invitrogen, Carlsbad, CA, USA) polymerase enzyme and two in-house outer primers (HIVpcrFor1: 5'-TGATGACAGCATGTCAGGGAGTGG-3', HXB2 location 1826–1849 and HIVpcrRev1: 5'-GGCTCT TGATAAATTTGATATGTCCATTG-3', HXB2 location 3555–3583) for the first-round PCR. The reaction was

performed in a 50 µl reaction mixture and the cycling conditions used were as follows: initial denaturation at 94 °C for 2 min, 35 cycles of 30 s at 94 °C, 1 min at 54 °C, 1 min at 72 °C followed by a final extension for 5 min at 72 °C [17]. This yields 1757 bp amplicon (covering the entire protease [99 codons] and partial reverse transcriptase [the first 308 codons] regions).

Nested PCR and DNA purification

Second-round PCR was performed to re-amplify an amplicon from first-round PCR. This was carried out using High-Fidelity Taq polymerase (Life Technologies, USA) and two inner in-house primers (HIVpcrFor2: 5'-AGCCAACAGCCCCACCAG-3', HXB2 location 2150–2167 and HIVpcrRev2: 5'-CTGTATTTCTGCTATTAAGTCTTTTG-3', HXB2 location 3514–3539). The reaction was performed in a 50 µl reaction mixture similar to the first-round PCR and the thermal cycling consisted of initial denaturation at 94 °C for 2 min, 35 cycles of 30 s at 94 °C, 1 min at 54 °C, 1 min at 72 °C followed by a final extension for 5 min at 72 °C [17]. Agarose gel electrophoresis (using 1.5% agarose gel) was then performed to confirm the final amplified PCR product (1389 bp; covering the entire protease region [99 codons] and partial reverse transcriptase [the first 308 codons of the RT region]) [17]. This was followed by DNA purification using GeneJET Gel Extraction and DNA Cleanup Micro Kit (Thermo Fisher Scientific, Inc., United States), following the manufacturer's instruction. The Quality and concentration of purified DNA were checked using both NanoDrop and agarose gel electrophoresis.

DNA sequencing

Cycle sequencing reaction (by Sanger sequencing method) for each purified DNA sample was performed using Big Dye Terminator Cycle Sequencing Ready Reaction mix v.3.1 (Applied Biosystems, USA) and four in-house inner primers (HIVpcrFor2: 5'-AGCCAACAGCCCCACCAG-3', HXB2 location 2150–2167, HIVSeq 1: 5'-GTTAAACAATGGCCATTGACAGA-3', HXB2 location 2610–2632, HIVSeq 4: 5'-CCATCCCTGTGGAAGCACATT-3', HXB2 location 2988–3008, HIVpcrRev2: 5'-CTGTATTTCTGCTATTAAGTCTTTTG-3', HXB2 location, 3514–3539). The thermal cycling consisted of initial denaturation at 96 °C for 2 min, 39 cycles of 30 s at 96 °C, 15 s at 56 °C, 5 min at 60 °C and a final extension for 5 min at 60 °C [17]. Following cycle sequencing, the excess dye terminators were removed using DyeEx 2.0 Spin Kit (Qiagen, Germany) following the manufacturer's instruction. The analyte was further dried using vacuum centrifuge, and stored at – 20 °C at AHRI laboratory until sequenced. These dried DNA samples were then treated with 20 µl of formamide and subsequently

processed with an automated ABI 3500 xL Genetic Analyzer (Applied Biosystems).

Data processing and analysis

Data management and quality assurance

A structured questionnaire was used to collect socio-demographic data like age, sex, occupation, etc. of the study participants. The questionnaires were then checked for any errors and completeness of the response given by participants before data entry to ensure the quality of the result. In addition, visual inspection of the reagent bottles and expiration date checks were performed before each laboratory work. The laboratory procedures were conducted in separately designated laboratory spaces for quality control purposes. Both positive and negative controls were used for each laboratory work. Overall, standard operating procedures were strictly followed to ensure the quality and accuracy of the test result. Quality of sequence data was checked using an online data management and quality assurance tool found in the Los Alamos HIV sequence database (<http://hiv-web.lanl.gov>).

Statistical analyses

Demographic and clinical data recorded from questionnaire responses of each participant (age, gender, etc.) were checked for completeness and entered into Epi data v3.1 software and exported to SPSS version 25.0 (SPSS Inc. the United States) for analysis. Logistic regression was used to assess the associations between PDR and demographic or virological characteristics. The PDR prevalence was determined with a confidence interval (CI) of 95%. A p-value < 0.05 was considered significant. Drug resistance mutations for PI, NRTI, and NNRTIs were characterized by their frequency and percentages.

Sequence analysis

Sequence editing, alignment, subtype determination and phylogenetic analysis

SeqA5.4 software, which was contained in the ABI PRISM® 3500 xL Genetic Analyzer (Applied Biosystems) collects, processes, and stores the data automatically after each run. Then sequences were exported to other computer and were first edited using chromas software v.2.6.6 (<http://technelysium.com.au/wp/chromaspro/>) and Geneious prime® v.2019.2.1 (<https://www.geneious.com/academic/>). Then the fragment sequences were aligned using the latter software. Then HIV-1 subtype determination was done using the REGA HIV subtyping tool (Leuven University, Leuven, Belgium; <https://www.genomedetective.com/app/typingtool/hiv>) and were further confirmed by phylogenetic analysis using reference sequences from Los Alamos National Laboratory HIV Sequence Database

(<http://hiv-web.lanl.gov>) (Fig. 2). The Geneious prime® v.2019.2.1 software was used to draw neighbor-joining trees under the Tamura Nei genetic distance model. The statistical robustness of the neighbour-joining tree and reliability of the branching patterns were confirmed by bootstrap analysis (1000 replicates).

Pretreatment drug resistance determination

PDR determination was performed using the Stanford Genotypic Resistance calibrated population resistance (CPR) tool version 6.0 contained in the Stanford HIVdb (<http://StanfordHIVdb.stanford.edu>) algorithm and the IAS-USA 2019 mutation list. Classification of PDR level (low: < 5%, moderate: 5–15%, or high: > 15%) was made based on the WHO threshold survey protocol [21].

Results

Sociodemographic characteristics of study participants

Partial HIV-1 pol sequence covering the complete PR [99 codons] and partial RT [the first 308 codons] regions of 51 study participants were successfully sequenced, analyzed, and submitted to Genbank (accession numbers MT416661–MT416711). Of those 51 studied subjects, 54.9% were female. The average age of study participants was 37 years. Most (80.4%) of the study participants reported having a secondary and/or less than secondary school attendance as shown in the table (Table 1). With regard to viral load, 41.2% of study participants had 100,001–500,000 copies/ml (Table 1). No significant associations were observed between participants' demographic characteristics and Pretreatment Drug Resistance Mutations (PDRMs) (Table 1).

Table 1 Sociodemographic and virological characteristics of included study participants

Characteristics	Frequency (N)	Percentage (%)	Individuals with PDRM (N)	
			Based on CPR tool	Based on IAS-USA
Sex				
Male	23	45.1	2	2
Female	28	54.9	3	3
Age category				
18–28	23	45.1	1	2
29–38	17	33.3	2	1
39–48	8	15.7	2	2
> 49	3	5.9	0	0
Baseline viral load (copies/ml)				
2000–10,000	2	3.9	0	0
10,001–100,000	19	37.3	2	3
100,001–500,000	21	41.2	3	2
> 500,000	9	17.6	0	0
Occupation				
Unemployed	31	60.8	3	4
Employed	20	31.2	2	1
Marital status				
Married	19	37.3	1	1
Single	14	27.5	0	1
Divorced	14	27.5	4	3
Widowed/widower	4	7.8	0	0
Educational status				
No schooling	8	15.7	0	
Primary	15	25.4	1	2
Secondary	18	35.3	2	2
College (diploma)	3	5.9	1	1
University degree	7	13.7	1	0

The magnitude of pretreatment drug resistance

According to both the CPR tool and IAS-USA algorithms, 9.8% (5/51, CI (3.3–21.4)) of analyzed samples had at least one PDR Mutation. PDR mutations to NNRTIs were the most frequently detected mutations (4/51; 7.8%, CI (2.2–18.9) and 5/51; 9.8%, CI (3.3–21.4)) according to the CPR tool and IAS-USA mutation list, respectively followed by NRTIs (1/51, 1.96%, CI (0.05–10.5) by both criteria) and PIs (1/51, 1.96%, CI (0.05–10.5) according to the CPR tool only). Simultaneous resistance to NNRTI and NRTI was observed in 1/51 (1.96%, CI (0.05–10.5)) patients.

Both CPR tool and IAS-USA algorithms were concordant in identifying five NNRTIs resistance-associated mutations: K103N in one patient (1.96%), Y188L, and H221Y in another patient (1.96%), K101E in one patient (1.96%), and V106A in another patient (1.96%). However, L234I; which is detected in one individual (1.96%), was recognized by the CPR tool only (Table 2). On the contrary, E138A mutation, which is associated with reduced RPV and ETR susceptibility, was detected by the IAS-USA mutation list only in two individuals (3.92%) (Table 2).

Y115F and M184V PDR mutations that are recognized by both criteria, which confer limited resistance against NRTI drugs (abacavir, emtricitabine, lamivudine), were observed in one patient (1.96%) (Table 2). G75S PDR mutation that confers low-level resistance to PI drug class atazanavir/ritonavir was observed in another study participant according to the CPR tool only (Table 2).

In addition to the above-mentioned major mutations, several additional minor drug-related mutations and/or polymorphisms recognized by both algorithms were also observed on the RT and PR of all sequenced samples (Fig. 1). Single accessory resistance mutations and/or polymorphisms were present in over 15.6% (n=8) of RT sequences with A98S (17.6%) and V179I (3.9%)

being the most frequently observed mutations, while all PR sequences harbored at least two minor PIs resistance mutations and/or polymorphisms (Fig. 1). The most frequent mutations observed in the PR sequence were H69K (100%) and M36L (98%), followed by L89M (58.8%), I15V (25.5%), K20R (25.5%), T74S (17.6%) and L89I (5.88%) (Fig. 1).

HIV-1 genetic diversity

With regard to HIV-1 genetic diversity, phylogenetic analysis using the REGA HIV-1 subtyping tool (<https://www.genomedetective.com/app/typingtool/hiv>). showed that 98% (50/51) of the study participants were infected with HIV-1C virus while one individual (2%: 1/51) was infected with the HIV-1A1 virus. These results were further confirmed using reference sequences retrieved from the Los Alamos HIV sequence database (<http://hiv-web.lanl.gov>) (Fig. 2). All except one (MT416664; subtype A1, which clustered with subtype AE reference sequence with a bootstrap value of 93%) were clustered with HIV1C reference sequence with a bootstrap value of 89% (Fig. 2). Based on the phylogenetic analysis eight closely related transmission clusters with a bootstrap value of $\geq 99\%$ were observed (Fig. 2).

Discussion

Drug resistance poses a challenge for viral suppression, which in turn jeopardizes prevention plan against HIV infection [22]. In this study, we sequenced the partial pol gene from supposedly ART naive HIV infected individuals to show the current viral diversity and the rate of Antiretroviral Drug (ARV) resistance mutations in Addis Ababa, Ethiopia. The finding of this study indicates that 98% (50/51) of the study participants were infected with HIV-1C virus while one individual (2%) was infected with HIV-1 A1 virus. In agreement with previously published studies [16, 17], this finding

Table 2 Pretreatment drug resistance mutations detected and their resistance pattern to common drugs

Sample ID	Age/Sex	Viral load (copies/ml)	Mutations type					
			NNRTIs	Resistant to	NRTIs	Resistant to	PIs	Resistant to
MT416676	32/F	134,263	None		None		G73S*	ATV/ra
MT416698	37/F	124,246	K103N	EFV ^c , NVP ^c	None		None	
MT416672	45/M	19,652	Y188L, H221Y, L234I*	ETR ^a , DOR ^c , EFV ^c , NVP ^c , RPV ^c	Y115F, M184V	ABC ^c , FTC ^c , 3TC ^c	None	
MT416680	40/M	443,919	K101E	ETR ^a , EFV ^a , NVP ^b , RPV ^b , DOR ^a	None		None	
MT416667	25/F	66,791	V106A, E138A	ETR ^a , DOR ^c , EFV ^b , NVP ^c , RPV ^a	None		None	
MT416710	27/F	14,498	<i>E138A</i>	ETR ^a , RPV ^a	None		None	

F Female, M Male, ATV/r Atazanavir/ritonavir, ABC Abacavir, FTC Emtricitabine, 3TC Lamivudine, DOR Doravirine, EFV Efavirenz, ETR Etravirine, NVP Nevirapine, RPV Rilpivirine, NRTI nucleoside reverse transcriptase inhibitor; NNRTI non-NRTI, PI protease inhibitor

^a Low-level resistance, ^bIntermediate-level drug resistance, ^cHigh-level drug resistance

Mutations in bold, are PDRMs by both IAS-USA and the CPR tool; Mutation in Italics are reported by IAS-USA only. While indicated by * only detected by the CPR tool

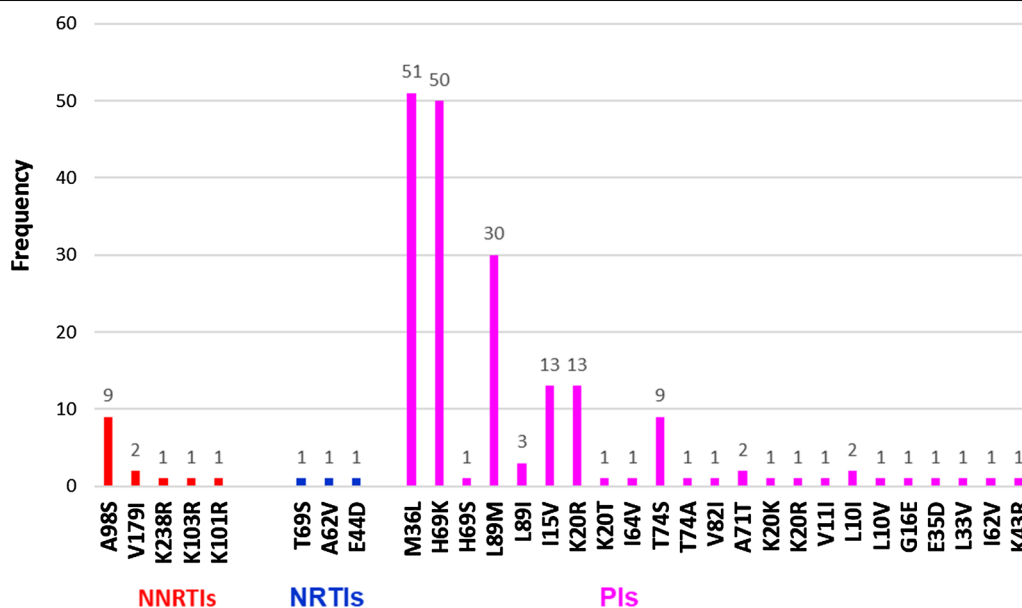


Fig. 1 Minor drug-resistant mutations and/or polymorphisms

indicated that the HIV-1C virus still dominates the HIV-1 epidemic in Ethiopia. Therefore, this is the latest evidence that showed persistence HIV-1C clade homogeneity in the country.

WHO categorizes PDR into three: low (<5%), moderate (5–15%) or high (>15%) categories [21]. Based on this PDR classification, this study revealed that the prevalence of PDR is moderate (9.8%, CI (3.3–21.4)) in the study area but with an increased level of resistance albeit few sample size (wide CI). In contrast to this finding, low levels of PDR among pregnant women [19] and ART-naive adult individuals [23] were reported previously from the same study area. Similarly, a study done in Brazil among ART-naive pregnant women indicated a lower PDR detection rate [24]. In addition to the above previously published data from Ethiopia, this finding is in agreement with studies done in India [25], Brazil [26–28], China [29], and Iran [30], which reported a moderate magnitude of PDR among HIV-1 antiretroviral adults. The absence of PDRM screening before starting ART and poor level of adherence might have contributed to this increment of PDR in the current study. In agreement with the finding of this study, a moderate level (5.6–7.2%) of PDR among HIV-1 ART-naive individuals was reported from Northern Ethiopia [15–17]. This study, combined with all the above previously published reports in Ethiopia, showed a moderate level (but slowly increasing) level of PDR 15 years after the rollout of ART in the country (Fig. 3).

Similar to previously published studies in Ethiopia [16, 23], NNRTI-associated mutations were the most common mutations observed relative to other major mutations (NRTI and PI-associated). However, the frequency of these NNRTI-associated mutations observed in this study (7.8%) was significantly higher compared to reports from previous PDR studies done in Ethiopia (1.5–3.6%) [16, 23, 31], except for the moderate (6%) frequency reported from Gondar [15]. This increased PDR in the current study might be due to the absence of drug resistance testing before ART initiation and consecutively high transmission rate of viruses harboring resistance-conferring mutations.

The lower frequency of NRTI and PI associated mutations observed in this study are in agreement with previously conducted studies among ART-naive individuals in Ethiopia [16, 17, 23, 31, 32]. Likewise, similar NRTI and PI associated PDRMs were reported in other countries like Brazil [26] and India [25].

In agreement with our report, K101E, K103N, and E138A mutations of the RT region had been reported previously in Ethiopia among ART-naive individuals [17, 23, 31]. On the other hand, V106A (Mutation in the RT region that confers resistance to NNRTIs), M114V and Y115F (Mutations in the RTs region that confer resistance to NRTIs) and G73S (Mutation in the PI region that confers resistance to PIs) were all detected for the first time (Fig. 4) among ART-naive individuals in Ethiopia.

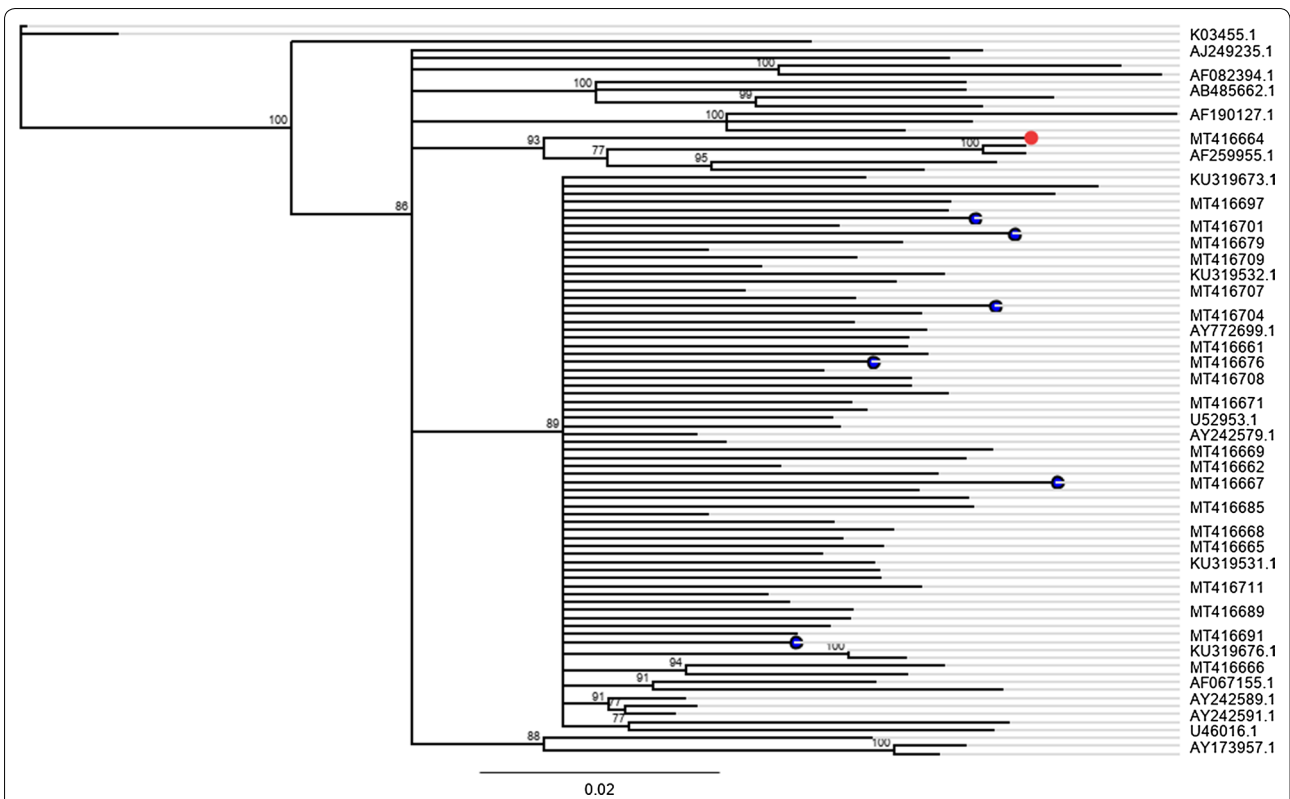


Fig. 2 Neighbor-joining phylogenetic tree of the pol gene (PR and RT nucleotide sequences) of 51 samples along with reference sequences from the Los Alamos HIV database (<http://hiv-web.lanl.gov>). Those with GenBank accession number MT416661–MT416711 are sequences from this study and sequences with PDR mutations are highlighted with a blue node while one (Subtype A1) is highlighted with a red node. The rest are HIV-1 reference sequences retrieved from the Los Alamos HIV database. Reference sequences: U46016, AY242579–AY242597, KU319672–KU319798 (C-Ethiopia), AY772699 (C-S. Africa), AF067155 (C-India), U52953 (C-Brazil), AX149771 (BC-China), and the rest reference sequences are non-C. Only bootstrap values > 70% are indicated at each node

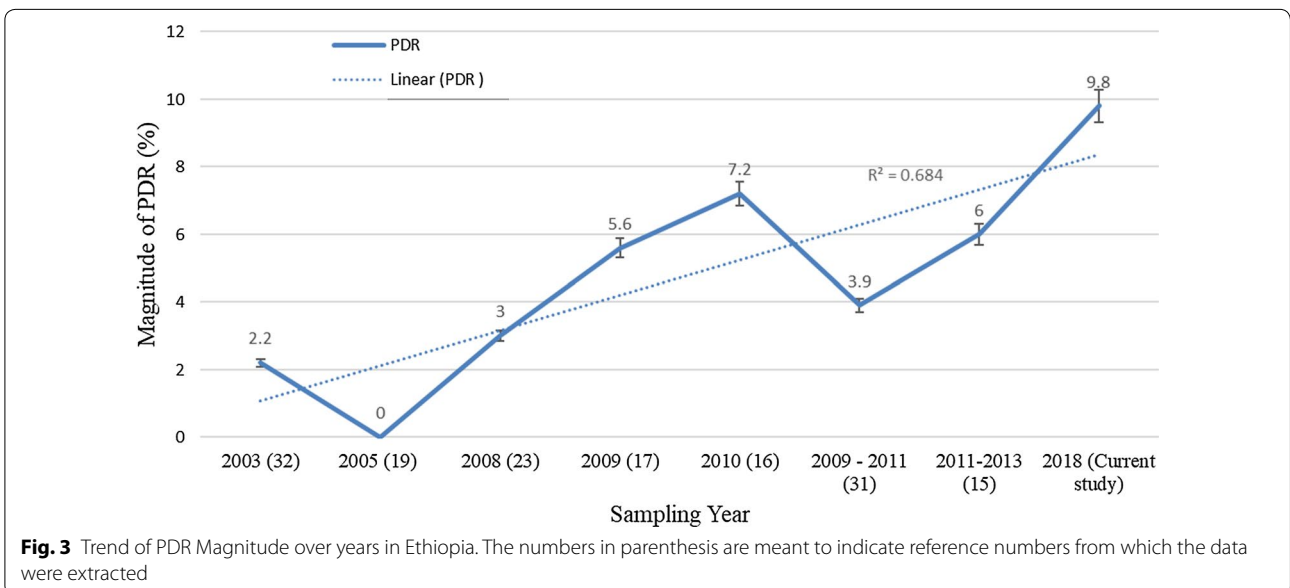
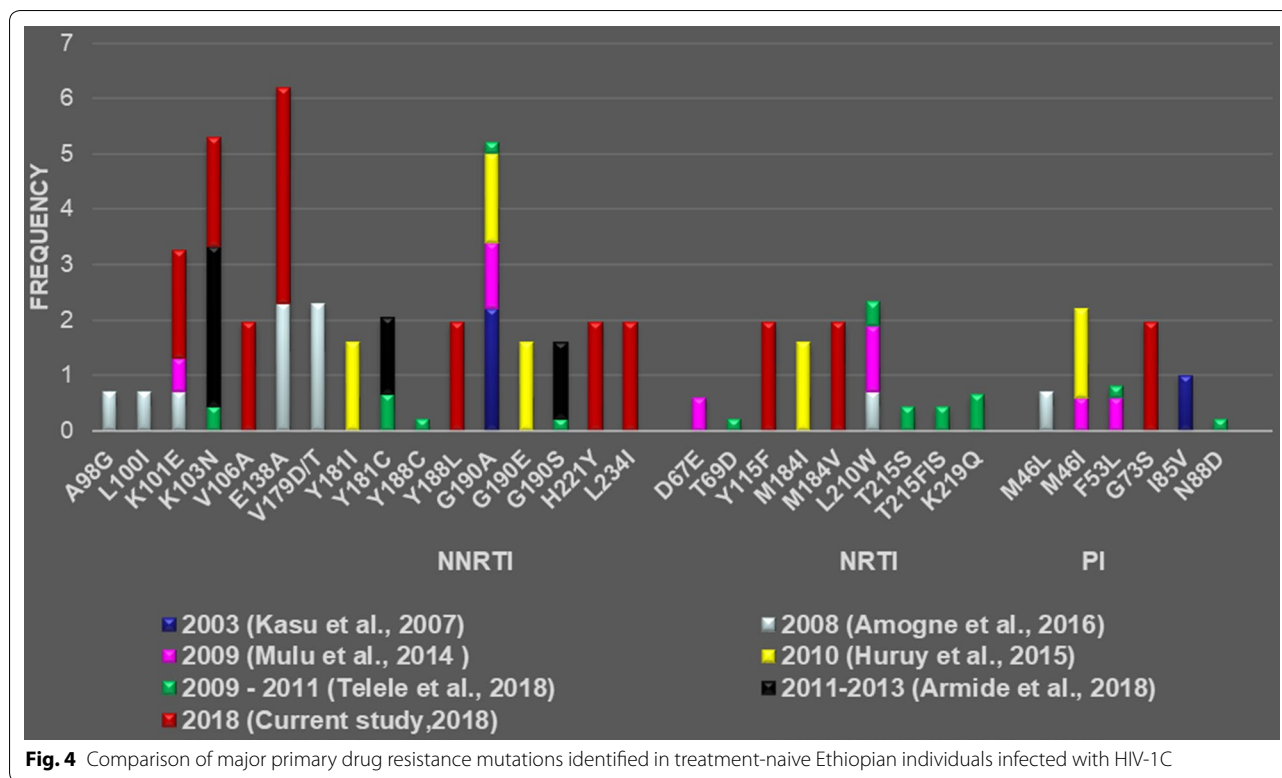


Fig. 3 Trend of PDR Magnitude over years in Ethiopia. The numbers in parenthesis are meant to indicate reference numbers from which the data were extracted



The sources of these mutations could be transmission from individuals on ART already failing treatment since they are likely to be engaged in a risky sexual activity. The other possibility could be due to a natural variation in subtype C, which might have led to the emergence of these resistance conferring mutations. Although they are seen for the first time among ART-naive individuals in the country, these mutations were reported among ART-naive individuals from other countries [33].

E138A mutation, which is associated with a decreased response to ETR and RPV according to the IAS-USA mutation list, was found in 3.9% (2/51) of the specimens showing a similar frequency of detection in previous reports from Ethiopia [17]. In agreement with the previous report from Gonder [16], one individual had PDR mutations associated with two combined drug classes (NRTIs plus NNRTIs).

With regard to minor mutations and/or polymorphisms in this study, a high rate of polymorphic change was observed most frequently in the PR region than in the RT region. This, the so-called accessory mutations or resistance-related mutations, may not reflect PDR but may represent natural HIV-1 genetic variability with possible clinical implications if present with other mutations. The most frequent minor mutations observed in the PR sequence were H69K (100%) and M36L (98%), followed by L89M (58.8%), I15V (25.5%), K20R (25.5%), and T74S

(17.6%). These mutations were reported with relatively the same frequencies from another study in Ethiopia [17] and India [34]. The presence of these mutations indicates a natural variation in HIV-1C virus across the globe.

Conclusions

In conclusion, this study presents additional evidence on the HIV-1C clade homogeneity after three decades of HIV-1 circulation in Ethiopia. The study also showed an increased level of HIV-1 PDR 15 years after the roll-out of ART in Ethiopia. Therefore, high follow-up and counseling strategies are warranted to those who are experiencing virological failure to prevent further dissemination of drug resistant viruses.

In general, this study showed increased levels of HIV-1 PDR, with NNRTIs associated mutations being the most frequently detected in Ethiopia that potentially compromise the effectiveness of ARV drugs especially those based on NNRTI regimens. This highlights the need for routine HIV-1 drug resistance testing before initiation of ART, and a broader public health action to prevent the emergence and transmission of drug-resistant variants. In addition, consistent follow-up and strengthening of adherence patterns, and robust monitoring of viral load to identify early treatment failure is warranted for successful ART programs and overall prevention of HIV

transmission in the country and to support the global efforts in achieving the third 90 of the UNAIDS target.

Abbreviations

AHRI: Armauer Hansen Research Institute; AIDS: Acquired Immuno Deficiency Syndrome; ART: Antiretroviral treatment; ARV: Antiretroviral drugs; EDTA: Ethylene diamine tetra acetic acid; HIV: Human Immunodeficiency Virus; NRTI: Nucleoside reverse-transcriptase inhibitor; NNRTI: Non-nucleoside reverse-transcriptase inhibitor; PCR: Polymerase chain reaction; PDR: Pretreatment drug resistance; PDRM: Pretreatment drug resistance mutation; PI: Protease inhibitor; PR: Protease; RT: Reverse transcriptase.

Acknowledgements

We would like to thank Addis Ababa University and Armauer Hansen Research Institute for financial support. We would like also to thank the study participants and all Armauer Hansen Research Institute staff for their unreserved help.

Authors' contributions

MK drafted the research project, conceived and designed the work, participated in laboratory work, data acquisition, initial and final manuscript writing for publication. DHA, and EG involved in laboratory Work and data acquisition. AMi, MM, WEA, and AMu involved in conceiving and designing the work, data analysis, initial, and final manuscript writing. All authors read and approved the final manuscript.

Funding

This research project was funded by Addis Ababa University and the Armauer Hansen Research Institute.

Availability of data and materials

Sequences from this study can be found from GenBank. (Have been given Accession Number: MT416661–MT416711).

Ethics approval and consent to participate

Ethical approval was obtained from Addis Ababa University, College of health sciences, department of Medical Microbiology, Immunology and Parasitology research and ethical review committee (DRERC) (Reference Number: DERC//17/18/02-H), and Armauer Hansen Research Institute (AHRI)/All Africa Leprosy Rehabilitation and Training Center ethical review committee (Protocol Number: PO16/18). Written informed consent was obtained from the study participants before including them to the study. Samples were coded to keep the confidentiality of the study participants' personal information.

Consent for publication

All authors have approved the manuscript for publication.

Competing interests

The authors declare that they have no competing interests.

Author details

¹ Department of Medical Laboratory Science, College of Medicine and Health Science, Debre Tabor University, Debre Tabor, Ethiopia. ² Armauer Hansen Research Institute, Addis Ababa, Ethiopia. ³ The Ethiopian Public Health Institute, Addis Ababa, Ethiopia. ⁴ Institute of Virology, Leipzig University, Leipzig, Germany. ⁵ Department of Microbiology, Parasitology, and Immunology, School of Medicine, Addis Ababa University, Addis Ababa, Ethiopia.

Received: 25 June 2020 Accepted: 23 September 2020

Published online: 29 September 2020

References

1. The Joint United Nations Programme on HIV/AIDS (UNAIDS). FACT SHEET—GLOBAL AIDS UPDATE 2019. 2019.
2. UNAIDS (Joint United Nations Program on HIV/AIDS). UNAIDS DATA 2017.
3. The Joint United Nations Programme on HIV/AIDS (UNAIDS). UNAIDS Data. 2019.
4. Misganaw A, Haregu TN, Deribe K, Tessema GA, Deribew A, Melaku YA, et al. National mortality burden due to communicable, non-communicable, and other diseases in Ethiopia, 1990–2015: findings from the Global Burden of Disease Study 2015. *Popul Health Metrics*. 2017;15(1):29.
5. World Health Organization (WHO). Ethiopia HIV Country Profile 2017 2019 <http://cfs.hivci.org/country-factsheet.html#>.
6. Federal HIV/AIDS Prevention and Control Office. HIV Prevention in Ethiopia National Road Map 2018–2020. 2018.
7. De Clercq E. Antiretroviral drugs. *Curr Opin Pharmacol*. 2010;10(5):507–15.
8. Stecher M, Chaillon A, Eis-Hubinger AM, Lehmann C, Fatkenheuer G, Wasmuth JC, et al. Pretreatment human immunodeficiency virus type 1 (HIV-1) drug resistance in transmission clusters of the Cologne-Bonn region, Germany. *Clin Microbiol Infect*. 2019;25(2):253.
9. Zazzi M, Hu H, Prosperi M. The global burden of HIV-1 drug resistance in the past 20 years. *PeerJ*. 2018;6:e4848.
10. Panichsillapakit T, Smith DM, Wertheim JO, Richman DD, Little SJ, Mehta SR. Prevalence of transmitted HIV drug resistance among recently infected persons in San Diego, CA 1996–2013. *J Acquir Immune Defic Syndr*. 2016;71(2):228–36.
11. Yendewa GA, Sahr F, Lakoh S, Ruiz M, Patino L, Tabernilla A, et al. Prevalence of drug resistance mutations among ART-naïve and -experienced HIV-infected patients in Sierra Leone. *The Journal of antimicrobial chemotherapy*. 2019;74(7):2024–9.
12. World Health Organization (WHO). HIV DRUG RESISTANCE REPORT 2019. Geneva, Switzerland: World Health Organization; 2019 (WHO/CDS/HIV/19.21). Report No.: Licence: CC BY-NC-SA 3.0 IGO.
13. Do HT, Nguyen DT, Nguyen LAT, Do DH, Le HX, Trinh XMT, et al. An alarmingly high proportion of HIV-1 isolates carrying mutations corresponding to resistance to antiretroviral drugs among HIV-positive High-Risk Groups in Central Vietnam: a Substudy of the National Sentinel Survey. *Jpn J Infect Dis*. 2017;70(6):621–7.
14. Federal Ministry of Health (FMOH). National guidelines for comprehensive HIV prevention, care and treatment. 2018.
15. Arimide DA, Abebe A, Kebede Y, Adugna F, Tilahun T, Kassa D, et al. HIV-genetic diversity and drug resistance transmission clusters in Gondar, Northern Ethiopia, 2003–2013. *PLoS ONE*. 2018;13(10):e0205446.
16. Huruy K, Maier M, Mulu A, Liebert UG. Limited increase in primary HIV-1C drug resistance mutations in treatment naïve individuals in Ethiopia. *J Med Virol*. 2015;87(6):978–84.
17. Mulu A, Lange T, Liebert UG, Maier M. Clade homogeneity and Pol gene polymorphisms in chronically HIV-1 infected antiretroviral treatment naïve patients after the roll out of ART in Ethiopia. *BMC Infect Dis*. 2014;14(1):158.
18. Ethiopian Public Health Institute. HIV Drug Resistance Early Warning Indicators Survey in Ethiopia. 2013/14.
19. Abegaz WE, Grossman Z, Wolday D, Ram D, Kaplan J, Sibide K, et al. Threshold survey evaluating transmitted HIV drug resistance among public antenatal clinic clients in Addis Ababa, Ethiopia. *Antiviral Ther*. 2008;13:89.
20. Ekici H, Amogne W, Aderaye G, Lindquist L, Sonnerborg A, Abdurahman S. Minority drug-resistant HIV-1 variants in treatment naïve East-African and Caucasian patients detected by allele-specific real-time PCR. *PLoS ONE*. 2014;9(10):e111042.
21. Bennett DE, Myatt M, Bertagnolio S, Sutherland D, Gilks CF. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. *Antiviral Ther*. 2008;13:25.
22. Chauhan CK, Lakshmi PVM, Sagar V, Sharma A, Arora SK, Kumar R. Primary HIV drug resistance among recently infected cases of HIV in North-West India. *AIDS Res Treat*. 2019;2019:1525646.
23. Amogne W, Bontell I, Grossmann S, Aderaye G, Lindquist L, Sonnerborg A, et al. Phylogenetic analysis of Ethiopian HIV-1 subtype C near full-length genomes reveals high intrasubtype diversity and a strong geographical cluster. *AIDS Res Hum Retroviruses*. 2016;32(5):471–4.
24. Pimenta ATM, Correa IA, Melli P, Abduch R, Duarte G, Couto-Fernandez JC, et al. HIV-1 genetic diversity and resistance to antiretroviral drugs among pregnant women in Ribeirão Preto (SP), Brazil Cross-sectional study. *Sao Paulo Med J*. 2018;136(2):129–35.
25. Nesakumar M, Haribabu H, Cheedarla N, Karunaianantham R, Kailasam N, Sathyamoorthy P, et al. Transmitted HIV-1 drug resistance in a treatment

- naive cohort of recently infected individuals from Chennai, India. *AIDS Res Hum Retroviruses*. 2019;35(8):775–9.
26. Tanaka TSO, Leite TF, Freitas SZ, Cesar GA, de Rezende GR, Lindenberg ASC, et al. HIV-1 molecular epidemiology, transmission clusters and transmitted drug resistance mutations in Central Brazil. *Front Microbiol*. 2019;10:20.
 27. Coelho LPO, Matsuda EM, Nogueira RS, de Moraes MJ, Jamal LF, Madruga JVR, et al. Prevalence of HIV-1 transmitted drug resistance and viral suppression among recently diagnosed adults in Sao Paulo, Brazil. *Arch Virol*. 2019;164(3):699–706.
 28. Bahls LD, Canezin PH, Reiche EMV, Fernandez JCC, Dias JRC, Meneguetti VAF, et al. Moderate prevalence of HIV-1 transmitted drug resistance mutations in southern Brazil. *AIDS Res Ther*. 2019;16(1):4.
 29. Song YX, Xin RL, Li ZC, Yu HW, Lun WH, Ye J, et al. Prevalence of transmitted drug resistance among HIV-1 treatment-naive patients in Beijing. *Epidemiol Infect*. 2018;146(3):339–44.
 30. Vahabpour R, Bokharaei-Salim F, Kalantari S, Garshasbi S, Monavari SH, Esghaei M, et al. HIV-1 genetic diversity and transmitted drug resistance frequency among Iranian treatment-naive, sexually infected individuals. *Adv Virol*. 2017;162(6):1477–85.
 31. Telele NF, Kalu AW, Gebre-Selassie S, Fekade D, Abdurahman S, Marrone G, et al. Pretreatment drug resistance in a large countrywide Ethiopian HIV-1C cohort: a comparison of Sanger and high-throughput sequencing. *Sci Rep*. 2018;8(1):7556.
 32. Kassu A, Fujino M, Matsuda M, Nishizawa M, Ota F, Sugiura W. Molecular epidemiology of HIV type 1 in treatment-naive patients in north Ethiopia. *AIDS Res Hum Retroviruses*. 2007;23(4):564–8.
 33. World Health Organization (WHO). The HIV drug resistance report. Geneva: Switherland World Health Organization; 2017. Report No.: CC BY-NC-SA 3.0 IGO.
 34. Azam M, Malik A, Rizvi M, Rai A. Zero prevalence of primary drug resistance-associated mutations to protease inhibitors in HIV-1 drug-naive patients in and around Aligarh, India. *J Infect Dev Ctries*. 2014;8(01):079–85.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

