ABSTRACT
The transmission and occurrence of resistance associated mutations (RAMs) are among the challenges for a successful HIV Treatment. Therefore, continuous HIV drug resistance surveillance of prevalence and incidence is recommended. The objective this study was to analyze the prevalence and impact of transmitted drug resistance (TDR) on the first-line ART effectiveness. This is a retrospective observational study that evaluated HIV-1-infected treatment-naïve individuals with pre-antiretroviral therapy (ART) genotype resistance test results, between 2014 and 2015, in Minas Gerais state’s, Brazil. The ART effectiveness was compared in individual with and without TDR, considering viral load (VL) <50 copies/mL at the 12 and 24-month endpoint. Reverse transcriptase and protease sequences were genotyped by Sanger sequencing. A total of 170 individuals were selected. HIV-1 subtype B was highly prevalent (63.5%) and prevalence of TDR was 14.1%. That included 62.5% RAMs associated with non-nucleoside reverse-transcriptase inhibitors (NNRTIs), 29.2% with nucleos(t)ide transcriptase inhibitors (NRTIs) and 25% with protease inhibitors (PIs). The overall ART effectiveness measured at 12 and 24 months was, respectively: 91.0% and 93.4% between individuals without TDR; 88.2% and 93.3% in individuals with TDR.
Among the individuals starting first-line ART with less than three active drugs (16/24), seven individuals achieved VL <50 copies/mL in the presence of K103N mutation and receiving efavirenz-containing ART. Moderate prevalence of TDR was observed in this study, although observed that some NNRTI TDR, did not impact on the effectiveness of efavirenz-based regimens, without major differences on the effectiveness of first-line ART between the groups with or without TDR.

Keywords: HIV-1, effectiveness, antiretroviral therapy, transmitted drug resistance.

INTRODUCTION

The Brazilian government's policy guarantees free access to antiretroviral therapy (ART) for people living with HIV (PLWH). Approximately 660,000 PLWH are undergoing ART treatment in Brazil\(^1\). Minas Gerais is the fourth state with the highest number of new HIV infections in country\(^2\). Therefore, continuous human immunodeficiency virus type-1 (HIV-1) surveillance for monitoring the prevalence and incidence of resistance associated mutations (RAMs) is important for the maintenance of Brazilian HIV/Aids program success.
and end of the AIDS epidemic\textsuperscript{3,4}. In addition, a health service accessible among PLWH to ensure clinical / laboratory monitoring and adequate treatment\textsuperscript{5–7}.

The genetic changes in the structure of HIV-1 may impact on the effectiveness of first-line ART. This RAMs can occur in different ways according to the World Health Organization (WHO)\textsuperscript{3}. Amongst them, the Transmitted Drug Resistance (TDR), that occur in ART naïve individuals with no history of ART exposure, limiting initial therapeutic\textsuperscript{8,9}. The TDR can lead to treatment unsuccessful, decreasing the genetic barrier ART, increasing the risk of virological failure and providing the development of new mutations. The detection of these RAMs are routinely performed by conventional HIV-1 genotyping methods with HIV-1 variants representing more than 20% of the virus population\textsuperscript{10–12}.

Studies report that the frequent use of with non-nucleoside reverse-transcriptase inhibitors (NNRTIs)-based regimens, with a low genetic barrier, may have favored the emergence of RAMs of this class, specifically due to high prevalence of K103N mutation, which could compromise the effectiveness of first-line treatment with efavirenz (EFV)-containing ART\textsuperscript{13–15}. A further increase in the number of countries reporting pre-treatment resistance (≥ 10%) to the NNRTI drug class, which includes PLWH with treatment-naïve or reinitiating first-line ART or prior antiretroviral drug exposure\textsuperscript{16}. This fact revealed the need to adopt new guidelines in the first-line ART, with dolutegravir (DTG)-based regimens\textsuperscript{17}. In addition, undesirable effects are most commonly reported with EFV – class of NNRTIs compared to DTG - class of integrase strand transfer inhibitors (INSTIs)\textsuperscript{18,19}. In 2017, Brazil modified the preferential first-line ART regimen for DTG-based regimen, according to the WHO; although it was not among the countries with a high level of pre-treatment resistance to the NNRTI drug class\textsuperscript{3}. EFV-based regimens remained as alternatives, mainly in tuberculosis/HIV co-infection and HIV-positive childbearing age women\textsuperscript{4,20}. The main objective of this study was to analyze the TDR prevalence prior to the introduction of DTG-based regimen and to evaluate its impact on the effectiveness of ART at 12 and 24 months, in HIV-1-infected treatment- naïve individuals, Minas Gerais state, Brazil.

2 METHODS

This is a retrospective observational study, whose data are part of the research routine of the Laboratory of Immunology and Molecular Biology, Infectious and Parasitic Diseases Service, at the School of Medicine, Federal University of Minas Gerais (UFMG). We
reviewed reports of pre-ART genotype resistance test results of HIV-1-infected treatment-naïve adults (age ≥ 18 years) performed between January 2014 and December 2015. ART and laboratory tests were evaluated respectively by the Brazilian National System of Drugs Logistic Control (SICLOM) and the Control System Laboratory Tests of the National Lymphocyte Count CD4+ (CD4⁺ T-cell)/CD8+ and Viral Load (SISCEL), both are in the public domain developed by the Unified Health System.

The first-line ART effectiveness was considered viral load (VL) < 50 copies/mL at 12 (360 ± 90 days) and 24 months (720 ± 90 days) from first-line ART initiation, considering the VL closest to the target time point. In individuals receiving partly active first-line ART (less than three active drugs) was evaluated the achievement of viral suppression (VL < 50 copies/mL) at the 6 (90 – 270 days), 12 (271 – 450 days), 18 (451 – 630 days) and 24-month (631 - 810 days) endpoint after ART initiation. ART switches were assessed within 24 months after starting treatment.

The CD4⁺ T-cell count was performed by measuring Flow Cytometry (FACScalibur - Multitest) and HIV-1 RNA quantification by the reverse transcription polymerase chain reaction (RT-PCR) Abbott RealTime HIV-1 (Abbott Molecular). A pre-ART genotype resistance test was performed using Sanger-based sequencing of the viral protease (PT) and the reverse transcriptase (RT). The RAMs were defined according to Calibrated Population Resistance based on the WHO surveillance of RAMs list21. Viral subtypes were defined using REGA HIV-1 subtyping tool, version 3.0. The susceptibility to the first-line ART was evaluated according to the Stanford HIVdb-algorithm, version 8.8. Data were analyzed using R version 4.0.2.

3 RESULTS

We analyzed 170 HIV-1-infected treatment-naïve adults with amplify nucleic acid sequences for pre-ART genotype resistance test results. The majority of the study participants were male (78.8%) and 55.4% living in the state’s capital or metropolitan region. The median age was 29.5 years old, baseline CD4⁺ T-cell count median was 415 cells/mm³ and baseline VL median was 4.6 log10 copies/mL.

Overall, 14.1% (24/170) of HIV-1-infected treatment-naïve showed evidence of TDR in the RT and /or PT region. Of these, 87.5% were resistant to only one drug class, 8.3% to dual-class - nucleoside/tide reverse transcriptase inhibitors (NRTIs) and protease
inhibitors (PIs) and 4.2% to triple-class. Most of these RAMs associated with NNRTIs drug class (62.5%) followed by NRTIs (29.2%) and PIs (25%).

The HIV-1 subtype B was the most prevalent (63.5%), followed by subtype F (20%). We also found subtype C (7.6%), 1.8% of circulating recombinant forms (CRF) - CRF29_BF, CRF42_BF and CRF02_AG – and 7.1% of unique recombinant forms (URFs). The prevalence of RAMs is shown in Figure 1.

The initial antiretroviral regimen most used was NNRTI-based regimen (92.4%): 2.9% with nevirapine (NVP); 89.5% EFV-containing ART, being 28% fixed-dose-combined scheme - EFV + lamivudine (3TC) + tenofovir (TDF). The PI-based regimen was 7.6%: 5.9% with lopinavir/ritonavir (LPV/R) and 1.7% with atazanavir/ritonavir (ATV/R).

The prevalence of first-line ART effectiveness in individuals without and with TDR were respectively: 91.0% (101/111) and 88.2% (15/17) for the 12-month endpoint; 93.4% (85/91) and 93.3% (14/15) for the 24-month endpoint. The absence of laboratory measurements in both endpoints was 15.1% among individuals without TDR and 25.0% among those with TDR. The switch to second-line ART occurred in 17.1% (n=24) of the individuals without TDR and 20.8% (n=5) with TDR.

Among individuals with TDR (n=24), 33.3% (n=8) had for at least three fully active antiretroviral drugs and 66.7% (n=16) started receiving partly active first-line ART (less than

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**Figure 1** – Prevalence of HIV-1 subtypes and resistance associated mutations in treatment-naïve adults, Minas Gerais state, Brazil

- URF: unique recombinant forms; CRF: circulating recombinant forms; PI: protease inhibitor; NRTI: nucleoside/tide reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse-transcriptase inhibitor.
three active drugs). Of those individuals who started with partially active regimens, two needed ART switch: one case of AZT adverse reaction – switch after 14 months of ART initiation and individual already in viral suppression; one case of EFV virological failure – switch after 5 months, maintaining VL detectable after two switches for PIs-based regimens and delay ART-dispensing. Four cases of laboratorial loss to follow-up (LTFU), these three cases of treatment abandonment. Others ten cases had maintaining laboratory and therapeutic follow-up with first-line ART, achieving viral suppression - seven individuals with high-level of resistance to EFV, three cases with high-level of resistance to 3TC or intermediate-level to TDF or NVP (table 1).

4 DISCUSSIONS

The Southeast region was the Brazilian region with the highest number of cases of HIV infection, from 2013 to 2015\textsuperscript{22}. Although AIDS detection rate in this region shows a downward trend, between 2015 and 2018\textsuperscript{23}. Minas Gerais is among the Brazilian states with the highest number of new HIV infections, considering the last two decades\textsuperscript{2}. The prevalence of TDR observed in this study was 14.1%, considered moderate according to the WHO\textsuperscript{24}. Previous studies carried out in the same region found similar prevalence: 12.8% (6/47) among HIV seropositive blood donors, between 2007 and 2011\textsuperscript{25} and 14.1% (9/64) among a cohort of men who have sex with men, between 1996 and 2012\textsuperscript{26}. The moderate prevalence had already been observed in the southeast region (11.2%), during the period from 2013 to 2015\textsuperscript{27}. This is in contrast to previous researches in others Southeastern capitals of Brazil that observed high TDR prevalence: 16.3% (7/43) in Rio de Janeiro state’s\textsuperscript{28} and 31.2% (25/80) in São Paulo state’s\textsuperscript{29}. Some factors can influence these proportions, given the methodological differences and population heterogeneity among these studies.
Table 1 – Evaluation of TDR in individuals starting the first-line ART with partly active antiretroviral regimens, Minas Gerais state’s, Brazil

<table>
<thead>
<tr>
<th>ID*</th>
<th>Mutations</th>
<th>Resistance profile</th>
<th>First-line ART</th>
<th>Switch</th>
<th>Baselines VL / CD4⁺ T-cell count</th>
<th>VL after ART initiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PI</td>
<td>NRTI</td>
<td>Low</td>
<td>Intermediate</td>
<td>High</td>
<td>6 months</td>
</tr>
<tr>
<td>140273</td>
<td>-</td>
<td>M41L, D67N, T69D, L210W, T215D</td>
<td>-</td>
<td>ABC, TDF</td>
<td>AZT</td>
<td>Yes, AZT adverse reaction</td>
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<tr>
<td>140327</td>
<td>150L, F53L, V82A</td>
<td>M41L, M184V, L210W, T215Y</td>
<td>K103N</td>
<td>LPV/r</td>
<td>TDF</td>
<td>EFV + 3TC + TDF</td>
</tr>
<tr>
<td>140330</td>
<td>-</td>
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<td>K103N</td>
<td>-</td>
<td>EFV, NVP</td>
<td>EFV + 3TC + TDF</td>
</tr>
<tr>
<td>140334</td>
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<td>K103N</td>
<td>-</td>
<td>EFV, NVP</td>
<td>EFV + 3TC + TDF</td>
</tr>
<tr>
<td>140387</td>
<td>-</td>
<td>-</td>
<td>K103N</td>
<td>-</td>
<td>EFV, NVP</td>
<td>EFV + 3TC + TDF</td>
</tr>
<tr>
<td>140429</td>
<td>-</td>
<td>-</td>
<td>K103N</td>
<td>-</td>
<td>EFV, NVP</td>
<td>EFV + 3TC + TDF</td>
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<tr>
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<td>-</td>
<td>EFV, NVP</td>
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<tr>
<td>140487</td>
<td>-</td>
<td>L210W, M41L, T215D</td>
<td>-</td>
<td>ABC, TDF</td>
<td>AZT</td>
<td>EFV + 3TC + TDF</td>
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</tbody>
</table>
Continuation of Table 1 – Evaluation of TDR in individuals starting the first-line ART with partly active antiretroviral regimens, Minas Gerais state’s, Brazil

The prevalence of HIV-1 subtype B, followed by subtype F is very similar with others.

<table>
<thead>
<tr>
<th>ID*</th>
<th>Mutations</th>
<th>Resistance profile</th>
<th>First-line ART</th>
<th>Switch</th>
<th>Baselines VL / CD4⁺ T-cell count</th>
<th>VL after ART initiation</th>
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<td>12 months</td>
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<td>Inter-</td>
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<td></td>
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<td></td>
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<td></td>
<td>24 months</td>
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<td>–</td>
<td>K103N</td>
<td>–</td>
<td>–</td>
<td>EFV, NVP</td>
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<td>–</td>
<td>–</td>
<td>EFV, NVP</td>
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<tr>
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<td>–</td>
<td>M184V</td>
<td>ABC</td>
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<td>EFV, NVP</td>
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<tr>
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<td>–</td>
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<td>ETR</td>
<td>–</td>
<td>–</td>
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<td>EFV, NVP</td>
</tr>
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</tbody>
</table>

*ID - GenBank accession numbers; ART - antiretroviral therapy; CD4⁺ T-cell - Lymphocyte Count CD4⁺ (cells/mm³); LTFU - loss to follow-up; PI - protease inhibitor; NRTI - nucleoside/tide reverse transcriptase inhibitor; NNRTI - non-nucleoside reverse-transcriptase inhibitor; VL - viral load (copies/mL); **3TC - Lamivudine; ABC – Abacavir; ATV/r - Atazanavir/ritonavir; AZT - Zidovudine; DRV/r - Darunavir/ritonavir; EFV - Efavirenz; ETR - Etravirine; LPV/r - Lopinavir/ritonavir; NVP - Nevirapine; TDF - Tenofovir; Bold antiretrovirals indicate individuals starting first-line ART with partly active antiretroviral regimens.
Brazilian data\(^{13,25,30,31}\). In addition, 36.5\% of individuals were classified as non-B variants. A trend towards an increase in non-B subtype had already been documented in Minas Gerais state’s (9.1\% in 2002 to 33.3\% in 2006)\(^{30}\). Regarding CRF strains, the CRF29\_BF also had already been described in the Southeast, South and Central-West regions of Brazil. The CRF02\_AG is more frequent in the northeast region\(^{27}\), but this CRF strain has also been described in others Southeastern capitals of Brazil – Rio de Janeiro and São Paulo capitals\(^{32,33}\) and it was also observed in this study, demonstrating the circulation of strains between regions in Brazil. In addition, the CRF42\_B /F strain originates from Brazil and is observed in others countries that have high migration rates\(^{34,35}\). The importance of understanding the circulation of these HIV-1 subtypes is due to the fact that these different subtypes could influence the development of specific mutations. For example, the K103N mutation that has been observed to be less prevalent in subtype C when compared to other subtypes - B, F and CRF02\_AG\(^{36}\).

We observed that RAMs associated with NNRTIs drug class was more than double those observed in NRTIs and PIs classes, consistent with other studies\(^{14,15,37,38}\). In Minas Gerais state’s, between 2002 and 2012, a tendency of increasing number of RAMs to NNRTIs had already been observed in individuals exposed to ART\(^{13}\). A meta-analysis shows that the prevalence of pretreatment drug resistance to NNRTIs in Brazil increased almost four times more between the periods from 2000 to 2005 and 2006 to 2015\(^{39}\). On the other hand, the pre-ART genotype resistance test is not performed in all PLWH, only in pregnant woman, co-infected with tuberculosis or partners of individuals who already using ART, limiting the sample size in all these studies.

The eradication of (NNRTI)-based first-line regimens has been progressively taking place in several countries\(^{16}\). Few studies reported about first-line ART effectiveness when in the presence of most prevalent NNRTI drug mutation, K103N. Paredes 2010, did not observed an increased the risk of virologic failure when in the presence of minority populations of HIV-1 expressing the K103N mutation and NNRTI-based regimen. But this risk was more than 3-fold in interference of the Y181C mutation\(^{40}\). Others studies demonstrated that the use of the first-line ART with NNRTI-based regimens a greater risk of virological failure in the presence of K103N, with some exceptions\(^{9,14,41}\), of which are not clearly discussed. However, the low sample number of TDR presence, it is difficult to interpret these results.
Approximately 67% of the subjects with partly active first-line ART that maintained the initial regimen for 24 months, achieved viral suppression, even in the presence of the K103N mutation - except one subject who had virological failure. A study in south of Africa observed virological response similar between individuals who had only NNRTI pretreatment drug resistance compared those without resistance. They found longer time to VL <50 copies/mL when in the presence dual-class NRTI/NNRTI pretreatment drug resistance detected at the 5% threshold\(^{42}\). Beck et al. (2020), also observed rates of virologic failure with EFV-based regimens in the presence only the K103N mutation similar to those with wild-type genotypes and higher failure rates for NVP-based regimens\(^{43}\). This finding can corroborate with our data regarding the lower impact observed of NNRTI TDR on the effectiveness of NNRTI-based regimens. None of the individuals of our study presented dual-class TDR (NRTI/NNRTI) and only one subject had resistance to triple-class but had LTFU. Greater LTFU was seen in individuals with low CD4\(^+\) T-cell count, probably at the greatest risk of death when in advanced stages of immunosuppression. In addition, poor progress in early diagnosis has already been observed in the Minas Gerais state’s\(^7\).

Superior effectiveness of NNRTIs-based regimens compared to PIs was observed previously by Cardoso and collaborators (2014). The outcome was observed in approximately three-quarters of the population with VL exam in the first 12 months after the ART initiation\(^{44}\). Other studies in Brazil observed first-line ART effectiveness of approximately 84% when using the fixed-dose-combined (EFV, 3TC and TDF) in 12-month endpoint\(^{45,46}\). In our study, the overall effectiveness was greater than 90% at 12 and 24 months after ART initiation. We did not observe any major differences on the effectiveness of first-line ART between the groups with or without TDR, especially 24-month endpoint, considering the results of available VL tests. A recent large cohort study from the real-world setting observed a similar results of treatment success between INSTI and NNRTI drugs classes in treatment-naïve adults or experimenting (no previous experience of modified antiretroviral), but the better immunologic responses was observed with INSTI-based regimens\(^{47}\). Primary INSTI resistance is still rare and pre-ART genotype resistance test to this class is not currently recommended. Some studies have reported resistance to dolutegravir in treatment-naïve individuals\(^{48-50}\). Therefore, the monitoring of transmitted INSTI resistance is important, as its wide commercialization may increase the prevalence of dolutegravir resistance\(^51\).
The limitations of this study are inherent to the nature of the secondary data. The small sample size is due to the restrictions for pre-ART genotype resistance test. The absence information related to VL Testing after the ART starting, possibly due to LTFU (treatment abandonment, death, transfer or testing in private laboratories) or because the VL test was performed in a period not corresponding to our inclusion criteria. The samples were part of the laboratory's research routine, and it is not possible to analyze a specific group with indication for pre-ART genotype resistance test. The comparison of RAMs with other studies may provide divergent information by the fact of existing several algorithms for interpreting mutations. Further studies are required to assess the presence of TDR and its impact on the effectiveness of first-line ART given the updates HIV-1 treatment guidelines. In addition, strategies to prioritize actions for the active search of individuals with RAMs are extremely important to minimize the risk of TDR.

5 CONCLUSIONS

In this study, we observed that some RAMs associated with NNRTIs, did not impact on the effectiveness of efavirenz-based regimens, without major differences on the effectiveness of first-line ART between the groups with or without TDR. However, given the changes in the HIV-1 treatment guidelines, the diversity of clades and moderate prevalence of TDR observed in this study, we suggest that further studies are needed for a better evaluation the impact of RAMs on the effectiveness of first-line ART in long-term.
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