Biochemistry and Molecular Biology
2022; 7(4): 70-74
http://www.sciencepublishinggroup.com/j/bmb
doi: 10.11648/j.bmb.20220704.11
ISSN: 2575-5064 (Print); ISSN: 2575-5048 (Online)

Nucleoside Reverse Transcriptase Inhibitors Resistance in Children and Adults with Virological Failure in a Context of Systematic Switching to a Dolutegravir-Based Combination

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To cite this article:

Dechi Jean-Jacques Renaud, Toni Thomas D'Aquin, Cisse-Camara Massara, N'din Jean-Louis Philippe, Ake Aya Jeanne Armande, Siransy Kouabla Liliane, Sekongo Yassongui Mamadou, Gogbe Leto Olivier, Brou Emmanuel, Fieni Flore, Wawa Amedee Junior, Aby Roland, Kouakou Kouadio, Chenal Henri. Nucleoside Reverse Transcriptase Inhibitors Resistance in Children and Adults with Virological Failure in a Context of Systematic Switching to a Dolutegravir-Based Combination. *Biochemistry and Molecular Biology*. Vol. 7, No. 4, 2022, pp. 70-74. doi: 10.11648/j.bmb.20220704.11

Received: September 15, 2022; **Accepted**: October 4, 2022; **Published**: October 17, 2022

Abstract: Switching to dolutegravir (DTG)-based triple therapy is done without taking into account the level of viral replication and resistance developed by the virus against nucleoside reverse transcriptase inhibitors (NRTIs). The objective of the study was to evaluate the resistance to NRTIs in children and adults with virological failure in Côte d'Ivoire in a context of systematic switching to a DTG-based combination. The study population was constituted at the Abidjan Integrated Bioclinical Research Center (CIRBA) from a cohort of children in virological failure from 2012 to 2013 and HIV-1 infected adults with an indication for genotypic resistance testing from 2015 to 2017. Biological analyses were performed using ANRS techniques and algorithm (www.hivfrenchresistance.org). A total of 243 children and adults with virological failure were included in the study. The frequency of resistance to NRTIs was 65% (n = 159/243). It was 92% (n = 146/159) to lamivudine/emtricitabine (3TC/FTC), 52% (n= 82/159) to zidovudine (ZDV), 45% (n = 71/159) to abacavir (ABC) and 18% (n = 29/159) to tenofovir (TDF). Commonly encountered mutations were M184V/I (90%; n = 143/159) for 3TC/FTC, T215I/N/V/Y/F (42%; n = 67/159) for ZDV, L74V/I (22%; n = 35/159) for ABC. K65R (4%; n = 6/159) and K70E (6%; n = 9/159) for TDF. This study highlights the need to guide the switch to triple therapy containing two NRTIs and one dolutegravir molecule by genotypic resistance testing.

Keywords: HIV-1, NRTI Resistance Mutation, Switched to Dolutegravir (DTG) Therapy

1. Introduction

Early antiretroviral therapy (ART) with sustained adherence has been shown to reduce the likelihood of HIV transmission and improve clinical outcomes for people living with HIV (PLHIV) [1, 2].

It is in this sense that the World Health Organization (WHO) has published guidelines. These guidelines are adopted and adapted according to the country. Thus, in Côte d'Ivoire, in the context of achieving the objectives of eliminating the HIV/AIDS epidemic by 2030 and achieving the 90-90-90 objectives of accelerating the national response to AIDS by 2020, the Ministry of Health and Public Hygiene (MSHP) has adopted the "Test and Treat All" approach as a

new strategy for the management of PLHIV [3]. This approach consists of placing any person who tests positive for HIV on ART without any condition of eligibility for treatment [3]. A 2019 WHO guideline update recommends for first-line treatment, dolutegravir (DTG) with two nucleoside reverse transcriptase inhibitors (NRTIs) in all adults, adolescents, infants and children [4]. In addition, it is recommended that PLHIV on ART be routinely switched to this new combination therapy regimen [4].

Routine switching to this new combination therapy regimen should involve the results of virological tests such as viral load and genotypic resistance testing (GRT). However, these virological markers are not commonly used in clinical practice in Côte d'Ivoire, due to the centralization of techniques in reference laboratories, their costs and the lack of trained human resources. Thus, this switching is done without taking into account the level of viral replication and the resistance developed by the virus towards NRTIs which have a lower genetic barrier than second generation integrase inhibitors such as DTG [5-8]. Therefore, treatment efficacy may be compromised from the outset by the presence of NRTI-resistant virus in PLHIV who routinely switch to this new combination therapy regimen.

As a result, PLHIV who switch to this new combination therapy regimen may end up with a single active HIV drug in a context where combination therapy options remain limited and the number of PLHIV on ART is increasing.

It therefore seems appropriate to monitor NRTI-resistant viruses in PLHIV who must systematically switch to this new combination therapy regimen to optimize treatment effectiveness.

The objective of our study was to assess NRTI resistance in children and adults with virological failure in Côte d'Ivoire in the context of routine switching to a DTG-based combination.

2. Material and Methods

2.1. Study Population

Our study population was constituted from two (02) cohorts of HIV-1 infected individuals followed regularly at the Centre intégré de recherche bioclinique d'Abidjan (CIRBA). The first cohort, conducted from 2012 to 2013, consisted of children under 18 years of age with a viral load greater than 1000 Copies/mL. The second cohort conducted from 2015 to 2017 consisted of adults with an indication for genotypic resistance testing (GRT). The study was approved by the National Health and Life Sciences Ethics Committee (CNESVS) (reference numbers: 138-18/MSHP/CNESVS-km).

2.2. Determination of NRTI Resistance Mutations in the Reverse Transcriptase Gene

The determination of resistance mutations in the reverse transcriptase gene was performed using the reference techniques of the AC43 Resistance group of the ANRS-MIE (National Agency for Research on AIDS - Emerging Infectious Diseases) (http://www.hivfrenchresistance.org). For this purpose, the reverse transcriptase gene was amplified after viral RNA extraction with the QIAAMP VIRAL RNA MINI KIT (Qiagen, Germany). The TITAN ONE TUBE RT-PCR SYSTEM kit (Roche Diagnostics, Manheim, Germany) was used for the first PCR. The enzyme concentration was 20 µM. The primer pair was MJ3/MJ4 (5'-AGT AGG ACC TAC ACC TGT CA-3'/5'-CTG TTA GTG CTT TGG TTC CTC T-3') and amplified 941 base pairs (bp) of reverse transcriptase (RT). The EXPAND HIGH FIDELITY PCR SYSTEM kit (Roche Diagnostics, Manheim, Germany) was used for the second PCR. The enzyme concentration was 30 µM. The primer pair consisted of A35/NE135 (5'-TTG GTT GCA CTT TAA ATT TTC CCA TTA GTC CTA TT-3'/ 5'-CCT ACT AAC TTC TGT ATG TCA TTG ACA GTC CAG CT-3') and amplified 731 bp of the reverse transcriptase. The amplicons obtained were purified with the QIAQUICK PCR PURIFICATION KIT (Qiagen, Germany). The sequence reaction was performed with the BIGDYE TERMINATORS V3.1 SEQUENCING KIT (Applied Biosystem, Courtaboeuf, France) and the primer pair was the same as for the second PCR (A35/NE135). The products of the sequence reaction were precipitated and purified by an ethanolic purification process according to the manufacturer's recommendations (Applied Biosystem, Courtaboeuf, France). Electrophoretic migration of the purified products was performed with the Genetic Analyser 3130 sequencer (Applied Biosystem, Courtaboeuf, France) for sequence determination. The reverse transcriptase region sequenced was from codon 20 to codon 240. The obtained sequences were aligned to the HIV-1 reference (HIV-1 HXB2, GenBank accession number: K03455) using SeqScape software (Applied Biosystem, Courtaboeuf, France). The list of defined mutations is that of the International Aids Society (IAS, http://www.iasusa.org). Interpretation was done using the ANRS algorithm (ANRS - AC 43: RESISTANCE GROUP GENOTYPE INTERPRETATION: NUCLEOSIDE AND NUCLEOTIDE REVERSE TRANSCRIPTASE INHIBITORS, October 2021 - Version n°32) (http://www.hivfrenchresistance.org). Table 1 shows the positions of the primers on the HIV-1 reference sequence.

Table 1. *Primer positions on the HIV-1 reference sequence (HIV-1 HXB2, GenBank accession number: K03455).*

Primer name	Sequence	Length	Direction	HXB2 Position	PCR
MJ3	AGTAGGACCTACACCTGTCA	20	Sense	2480 -2499	1st PCR
MJ4	CTGTTAGTGCTTTGGTTCCTCT	22	Anti-sense	3399 -3420	1st PCR
A35	TTGGTTGCACTTTAAATTTTCCCATTAGTCCTATT	35	Sense	2530 -2558	2nd PCR and Sequencing reaction
Ne135	CCTACTAACTTCTGTATGTCATTGACAGTCCAGCT	35	Anti-sense	3300 -3334	2nd PCR and Sequencing reaction

2.3. Data Processing and Statistical Analyses

Microsoft Excel 2013 was used for data processing and SPSS Statistics 17.0.1 was used for statistical analyses.

3. Results

3.1. Characteristic of the Patients in the Study

The number of people with virological failure included in our study was 243. The number of children was 61 out of 260 (13%) and the number of adults was 182 out of 182 (100%). The median age was 39 years (3-75). Females were represented by 54% (n = 131/243) of the total number of patients with a sex ratio (Male/Female) of 0.85.

3.2. NRTI Resistance in Children and Adults with Virological Failure

The frequency of resistance to NRTIs recommended in Côte d'Ivoire for the therapeutic management of people living with HIV-1 was 65% (n = 159/243) in children and adults with virological failure in our study. It was 92% (n = 146/159) for 3TC/FTC, 52% (n = 83/159) for ZDV, 45% (n = 72/159) for ABC and 18% (n = 29/159) for TDF (Figure 1).

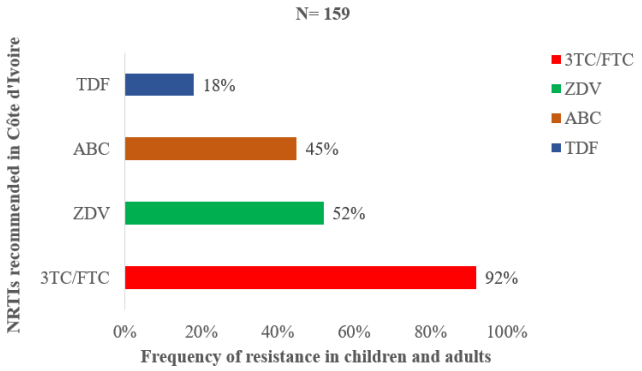

Figure 1. *Frequency of HIV-1 resistance to NRTIs in children and adults with virological failure in Côte d'Ivoire.*

3.3. Resistance to the Combination of 2 NRTIs in Children and Adults with Virological Failure

The frequency of resistance to the combination of 2 NRTIs recommended in Côte d'Ivoire as part of the therapeutic management of people living with HIV-1 was 48% (n = 76/159) for ZDV+3TC, 42% (n = 67/159) for ABC+3TC, and 16% (n = 25/159) for TDF+3TC in children and adults in virological failure in our study (Figure 2).

3.4. NRTI Resistance Mutations and Impacted ARVs in Children and Adults with Virological Failure

Analysis of the reverse transcriptase sequences of children and adults with virological failure made it possible to identify the mutations responsible for resistance to the NRTIs recommended in Côte d'Ivoire for the therapeutic management of people living with HIV-1. The M184V/I (90%; n = 143/159), K65R (4%; n = 6/159) mutations were incriminated in the resistance to 3TC/FTC. Mutations T215I/N/V/Y/F (42%; n = 67/159) and Q151M (1%; n = 1/159) were responsible for resistance to ZDV. L74V/I (22%; n = 35/159), Y115F (6%; n = 10/159), K65R (4%; n = 6/159), and Q151M (1%; n = 1/159) mutations were incriminated for resistance to ABC. For TDF resistance, the mutations found were K65R (4%; n = 6/159) and K70E (6%; n = 10/159) (Figure 3).

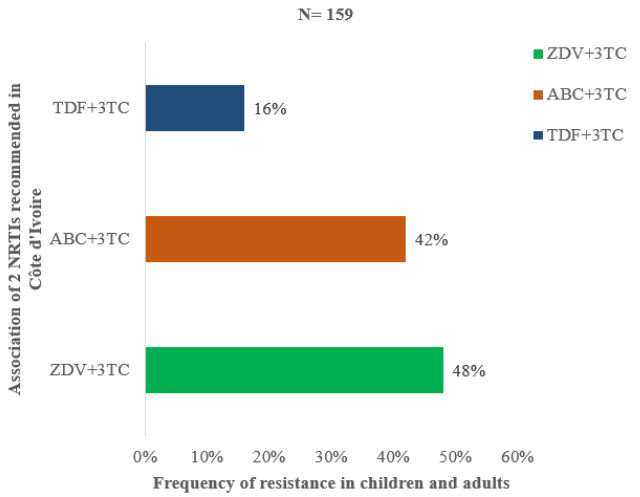

Figure 2. *Frequency of HIV-1 resistance to the combination of 2 NRTIs in children and adults with virological failure in Côte d'Ivoire.*

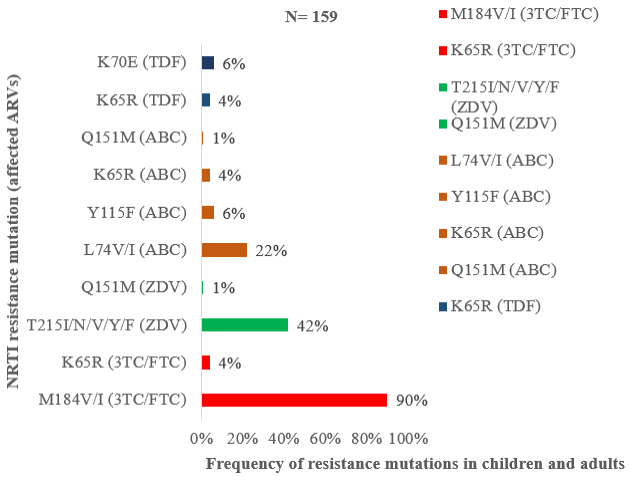

Figure 3. *Frequency of HIV-1 resistance mutations to NRTIs and impacted ARVs in children and adults with virological failure in Côte d'Ivoire.*

3.5. Resistance to the Combination of 2 NRTIs in Children with Virological Failure

The frequency of resistance to the combination of 2 NRTIs recommended in Côte d'Ivoire as part of the therapeutic management of people living with HIV-1 was 48% (n = 76/159) for ZDV+3TC, 42% (n = 67/159) for ABC+3TC, and 16% (n = 25/159) for TDF+3TC among children in virological failure in our study (Figure 4).

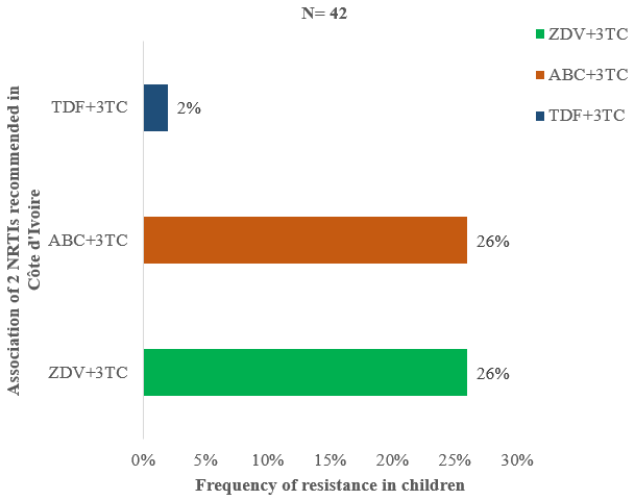

Figure 4. *Frequency of HIV-1 resistance to the combination of 2 NRTIs in children with virological failure in Côte d'Ivoire.*

3.6. Resistance to the Combination of 2 NRTIs in Adults with Virological Failure

The frequency of resistance to the combination of two NRTIs recommended in Côte d'Ivoire for the treatment of people living with HIV-1 was 56% (n = 66/117) for ZDV+3TC, 49% (n = 57/117) for ABC+3TC, and 21% (n = 25/117) for TDF+3TC among adults with virological failure in our study (Figure 5).

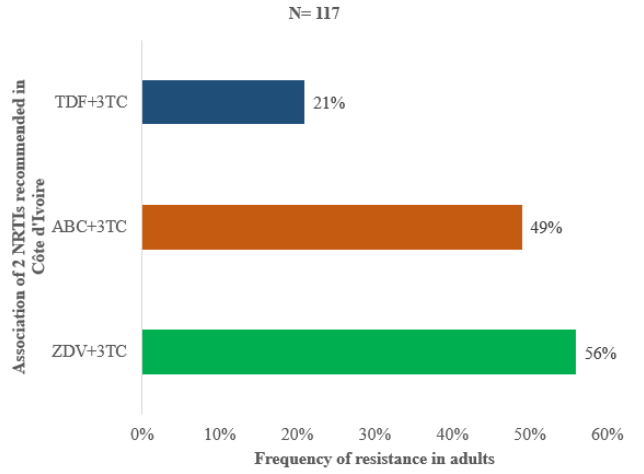

Figure 5. *Frequency of HIV-1 resistance to the combination of 2 NRTIs in adults with virological failure in Côte d'Ivoire.*

4. Discussion

Our study assessed NRTI resistance in children and adults with virological failure in Côte d'Ivoire in a context of systematic switching to a DTG-based combination.

We observed that 65% of children and adults in virological failure had viruses resistant to NRTIs recommended in Côte d'Ivoire as part of their therapeutic management. It was 92% for 3TC/FTC, 52% for ZDV, 45% for ABC and 18% for TDF. It should be noted that the frequency of resistance to 3TC/FTC was very high. In 90% of cases, the M184V/I mutation was responsible for this resistance. This suggests that the majority of children and adults would be on dual therapy if they switched to a TDF-based regimen. Similarly, a proportion would be on monotherapy given the proportions of resistance to ZDV, ABC and TDF.

Studies have shown that the presence of the M184V/I mutation does not affect the efficacy of DTG-based therapy. For example, a review by Ndashimye and Arts in 2021 presents a table listing twelve studies that show that first-line ART-failed or suppressed patients with an M184V/I mutation can achieve or maintain virological suppression when switching to regimens consisting of DTG + NRTI [9]. Similarly, Blanco and collaborator in 2018, concluded in a 24-week study based on two simplified DTG-based strategies that dual therapy (DTG+3TC) showed no warning signs, while the DTG monotherapy arm showed an unacceptable risk of viral failure with development of integrase inhibitor cross-resistance mutations [10]. Hocqueloux and collaborator in 2019, addressed the same issue by stating that although DTG monotherapy was found to be non-inferior to combination ART at week 24, virological failure occurred thereafter and led to the emergence of DTG resistance [11]. Therefore, despite its high resistance barrier, DTG monotherapy is not recommended due to an excess risk of treatment failure and resistance [12, 13].

The most frequently observed resistance mutations in our study for ZDV, ABC and TDF were T215I/N/V/Y/F (42%), L74V/I (22%) and K70E (6%), K65R (4%) respectively. These mutations could be the likely cause of DTG-based treatment failure, including: TDF+3TC+DTG, ABC+3TC+DTG and ZDV+3TC +DTG. However, less resistance to the combination of TDF+3TC as NRTI was observed in children and adults in contrast to the combination of ABC+3TC and ZDV+3TC. Our study is therefore in line with WHO guidelines which recommend switching to the TDF+3TC+DTG combination regimen as first-line therapy [4].

5. Conclusion

The study showed a high frequency of NRTI resistance in children and adults with virological failure in Côte d'Ivoire. Resistance was observed for all four NRTIs used in the country. Mutations to watch for are M184V/I, T215I/N/V/Y/F, L74V/I K65R and K70E. Less resistance to TDF was observed compared to 3TC/FTC. This study highlights the need to guide the switch to triple therapy containing two NRTIs and one DTG molecule by genotypic resistance testing for better therapeutic management of PLHIV in Côte d'Ivoire.

6. Recommendations

The popularization of virological tests (viral load and resistance genotyping) and access to new therapeutic classes such as entry inhibitors should be the focus of HIV control programs in resource-limited countries like Ivory Coast.

Acknowledgements

Our thanks go to the patients, the Strategic Support Program for Scientific Research (PASRES) in Côte d'Ivoire and the Integrated Center for Bioclinical Research of Abidjan (CIRBA).

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