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Diversity and prevalence of antiretroviral genotypic resistance mutations among HIV-1-infected children

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Abstract

Objective: To evaluate genotyping and subtyping in antiretroviral (ARV) naïve and experienced children, as well as drug resistance profiles through genotyping in these children.

Methods: This retrospective study assessed ARV-naïve HIV children and HIV children failing highly active antiretroviral treatment (HAART) followed up at Santa Casa de São Paulo. Genotyping was performed using purified polymerase chain reaction (PCR) products from retrotranscribed RNA using Kit Viroseq HIV-1 Genotyping System 2.0 or nested PCR in-house. Sequencing was performed using automatic equipment (ABI 3100). ARV resistance mutations were analyzed in the Stanford HIV Drug Resistance Database and subtyping was performed at the National Center for Biotechnology Information (NCBI), using SimPlot analysis, together with phylogenetic analysis.

Results: No primary ARV resistance mutation was detected in the 24 ARV-naïve children, although there were mutations that may contribute to resistance to nucleoside analogue reverse transcriptase inhibitors (NRTI) (12.5%) and to protease inhibitors (PI) (95.8%). For the 23 children failing HAART, we found ARV resistance mutations to NRTI in 95.6% and to non-nucleoside analogue reverse transcriptase inhibitors (NNRTI) in 60.8%. For PI, we found ARV resistance mutations in 95.7%, 47.8% of which had only polymorfisms. In the subtyping analyses, 78.3% of the sequences clustered in HIV-1 subtype B, 4.3% in C, 13% in F and 4.4% in recombinant forms.

Conclusion: Our results show low rates of primary resistance in ARV-naïve children and high rates of resistance in children failing ARV treatment, which is compatible with ARV use in these patients.

J Pediatr (Rio J). 2009;85(2):104-109: HIV, resistance, genotype, child, antiretroviral therapy.

Introduction

The introduction of highly active antiretroviral therapy (HAART) has improved morbidity and mortality of HIV-1-infected children. In Brazil, the antiretroviral (ARV) therapy, as well as monitoring tests such as TCD4 counts, viral load and genotyping, are provided free of cost by the government to all HIV-1-infected patients.[1]

The emergence of drug-resistant viruses can limit the efficacy of ARV treatment. Despite the growing number of ARV agents available, options for HIV-1-infected children failing ARV therapy remain limited, especially in cases of resistance to nucleoside analogue reverse transcriptase inhibitors (NRTI), the first class of antiretroviral made available and most commonly used. Although high rates of resistance to ARV in children failing ARV therapy have been demonstrated,[2-5] there is still limited information on drug resistance in the pediatric population, as well as on the impact of resistance tests.

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Table 1 - Sample characteristics

	ARV-naïve children (n = 24)	Treated children (n = 23)
Median age at inclusion (months)	21.5	102
Median age at diagnosis (months)	21.5	61
Median CD4 (cell/mm^3)	888	402
Median VL (log_{10})	5.30	5.04

VL = viral load.

In the past 10 years, the prevalence of primary resistance to HIV has ranged from 0 to 25%, with higher and increasing rates in countries with access to ARV therapy.[6-12] In Brazil, although the ARV therapy has been available free of cost for the past 10 years, low rates of primary resistance are still observed,[13-19] but there is no information about children.

The role of viral diversity in HIV-1 transmission and response to treatment remains unclear. HIV-1 can be classified into three groups: M, O, and N. The M group is responsible for the pandemic; based on genetic distance, it may be classified into 11 clades and 37 circulating recombinant forms (CRF). In Brazil, there is a predominance of infection with HIV-1 clade B, with the cocirculation of HIV-1 F as a minor variant; exceptionally, in the south, HIV-1 clade C prevails. This scenario favors intersubtype recombination, so that different mosaics, including BF and BC CRF, have been described.

This study aims to compare HIV-1 genotyping and subtyping in ARV-naïve and experienced children and to assess drug resistance profiles through genotyping in these children.

Methods

Santa Casa is a referral hospital in the central area of São Paulo (Brazil), with 150 pediatric beds. In the outpatient clinic, around 80 HIV-1-infected children and adolescents are followed up. The study protocol was approved by the Institutional Review Board of Santa Casa Hospital and Adolfo Lutz Institute. All patients signed an informed consent form.

The study was conducted between November 2000 and March 2004. HIV-1-infected, ARV-naïve children and previously treated (experienced) children failing ARV therapy (virological failure) were selected. Patients' charts were reviewed for demographic data, CD4 cell count, viral load, genotyping, and ARV history.

The ARV-naïve group included children aged 0 to 16 years at the diagnosis of HIV infection. The ARV-experienced group included children aged 0 to 16 years using HAART (defined as at least 3 ARV) for at least 3 months, and presenting virological failure (defined as a decrease < 1 log_{10} after 12 weeks of the beginning of HAART or an increase > 0.5 log_{10}).

Laboratory assays

- Quantification of HIV-1 RNA: viral load was measured by using the COBAS Amplicor HIV-1 Monitor Test 1.5, with an assay quantification of 400 copies/mL.
- HIV-1 genotyping and subtyping: genotyping was performed using purified polymerase chain reaction (PCR) products from retrotranscribed RNA using Kit Viroseq HIV-1 Genotyping System 2.0 or nested PCR in-house.[20] Sequencing was performed using automatic equipment (ABI 3 100). ARV resistance mutations were analyzed in the Stanford HIV Drug Resistance Database. Subtyping was performed at the National Center for Biotechnology Information (NCBI), using SimPlot analysis, together with phylogenetic analysis. More detailed molecular analyses of part of the samples are described elsewhere.[21]
- Analysis of drug resistance mutations: reverse transcriptase (RT) mutations associated with resistance were identified according to the International AIDS Society (IAS)[22] and the Stanford HIV Drug Resistance Database.[23]

Results

We included a total of 47 children, of whom 24 were ARV-naïve and 23 had failed ARV therapy. All patients had acquired HIV by vertical transmission. Characteristics of the study population can be found in Table 1.

In the ARV-naïve group, all samples were obtained when the diagnosis was confirmed. The 24 patients were born from mothers who were not treated during pregnancy. Only two received prophylactic AZT in the first 6 weeks of life.

In the group failing ARV therapy, the median duration of ARV treatment was 60 months (3-120), and the median number of ARV treatments was four (1-10). Seven patients received monotherapy, and 10 received double therapy previously. The six remaining patients started treatment with HAART.

At the moment of genotyping, all patients, except two, were receiving HAART. Seventeen patients were already exposed to non-nucleoside analogue reverse transcriptase inhibitors (NNRTI), and 16, to protease inhibitor (PI).

Table 2 - Resistance profile to antiretrovirals according to the Stanford HIV Drug Resistance Database, in the experienced group (n = 23)

Antiretroviral	Resistance (%)	Intermediate resistance (%)	Susceptible (%)
Abacavir	8.6	86.9	4.3
Didanosine	17.4	65.2	17.4
Emtricitabine	56.5	13	30.5
Estavudine	43.5	30.5	26
Lamivudine	52.1	17.4	30.5
Tenofovir	0	74	26
Zidovudine	48	26	26
Delavirdine	56.5	4.3	39.1
Efavirenz	56.5	4.3	39.1
Etravirine	4.3	56.5	39.1
Nevirapine	65.2	0	39.1
Atazanavir	4.3	43.4	52.1
Darunavir	0	30.4	69.5
Fosamprenavir	4.3	39.1	56.5
Indinavir	13	30.4	56.5
Lopinavir	0	43.4	56.5
Nelfinavir	30.4	17.4	52.1
Ritonavir	13	30.3	56.5
Saquinavir	8.6	39.1	52.1
Tipranavir	0	34.8	65.2

All patients had received or were receiving AZT; 91.3%, DDI; 82.6%, 3TC and D4T; 47.8%, Efavirenz; 34.7%, Nevirapin; 54.1%, Ritonavir; 60.8%, Nelfinavir; 8.6%, Amprenavir; and 4.3%, Indinavir, Saquinavir and Lopinavir. The ARV regimens observed at the time of sample collection were: dual-NRTI (AZT + DDI) regimen in 8.6% of the children, 2 NRTI + 1 NNRTI in 30.4%, 1 NRTI + 1 NNRTI + 1 PI in 17.3%, 2 NRTI + 1 PI in 43.4%.

In the ARV-naïve group, no major resistance mutations were observed to any ARV, but secondary mutations that contribute to resistance to NRTI (12.5%) and PI (95.8%) were found. The most frequent resistance mutations were L10I (16.7%), K20R (12.5%), M36I (33%), L63P (37.5%), A71T (8.3%), V77I (25%), V82I (4%), I93L (12.5%), V118I (8.3%), and K219N (4.1%).

In the experienced group, all samples (95.6%), except one, had mutations that confer resistance to NRTI and 14 samples (60.8%) to NNRTI. The most common mutations to NRTI were T215 (69.6%), M184 (56.5%), D67 (47.8%), M41 (43.5%), and K219 (34.8%); and to NNRTI, K103 (39.1%) and Y181 (17.4%). We found nucleotide excision mutation (NEM) in 17 patients (73.9%), with a median of three mutations/patient (0-5). Mutations occurred in 95.7% of the protease; of these, 47.8% had only polymorphisms at codon positions 10, 20, 36, 63, 71, 77, 93, and 47.8% had primary mutations that confer resistance to PIs. The most common mutations were V82, I54, L90 (21.7%), M46 (17.4%). The ARV resistance profile according to the Stanford HIV Drug Resistance Database can be seen in Table 2.

In subtyping analyses, 78.3% of the sequences clustered in HIV-1 clade B, 4.3% in clade C, 13% in clade F, and 4.4% were BF mosaics.

Discussion

In the ARV-naïve group, we did not find any mutations conferring primary resistance to NTRI, NNRTI or PI, but only secondary mutations that contribute to resistance. In the RT gene, two patients had V118I and another had K219N. The latter had received prophylactic AZT in the first 45 days of life, which may explain the presence of this mutation. With regard to protease, one patient, clustered as B, had V82I, a mutation that is more frequent in non-B subtypes (1%), conferring low resistance to PI.[24-28]

Studies evaluating primary resistance to HIV have shown a prevalence ranging from 0 to 25%.[6-12] In Brazil, in spite of the availability of ARV therapy free of cost in the last 10 years,

we still have low rates of primary resistance, except in some cities of the country, such as Santos.[13-19]

Our data did not show high resistance rates among ARV-naïve patients. One aspect that may have contributed to this finding is that all patients were born from non-treated mothers, who were not aware of the diagnosis of HIV infection before pregnancy.

In the group failing ARV therapy, we observed a high rate of genotypic resistance, with a variable pattern of mutations. Other Brazilian studies that also evaluated children failing ARV therapy found similar rates.[2-4,19]

Considering NRTI, the most frequent mutations were T215 (69.6%), M184 (56.5%), D67 (47.8%), M41 (43.5%), and K219 (34.8%). It is common to find four, five and even six NEM in patients failing many ARV treatments. This fact decreases susceptibility to many NRTI, mainly to AZT, D4T and Abacavir, but also DDI and Tenofovir.[22,23]

AZT is the most frequently used drug in the pediatric population. All patients were exposed to AZT, and 74% of them presented mutations associated with resistance. Other studies with children failing ARV therapy show similar rates of resistance to AZT (64-85%).[22,23]

We also observed a high rate of resistance mutations to D4T in exposed (68.4%) and unexposed (74%) patients. We found different results in the literature, with a resistance prevalence ranging from 0 to 20% in exposed children.[4,5,29,30] This discrepancy in results can be explained by different inclusion criteria regarding resistance mutations to D4T. The first study[4] considered only V75T mutations; the second[5] considered T215Y, M184V, K70R, and D67N mutations; the third one[29] considered Q151M and 69I mutations; and the last one[30] did not mention the mutations taken into consideration. Moreover, the definition of resistance varies among studies, and algorithms of interpretation evolve over time. We considered 41, 210, 215, 75T, 151, and 69SS mutations, as published by the IAS[22] and in the Stanford HIV Drug Resistance Database.[23]

3TC is another NRTI frequently used in children. Nine of our patients had been previously exposed to 3TC, and 11 patients were using the drug for a median time of 31 months. We found M184 mutation in 63% of our patients exposed to 3TC and in 25% of the unexposed ones. This low mutation rate in unexposed patients with resistance mutation to 3TC can be explained by the specificity of M184 to this NRTI.

For DDI, when considering 65, 74, and 75T mutations, we found L74V in only one (4.8%) exposed patient who was using the drug at the moment of genotyping. In unexposed patients we did not find any mutation. Different results can be found in the literature, with higher rates of resistance mutations in exposed and unexposed children.[2-5] This difference may be explained by difficulties in defining inclusion criteria regarding resistance mutations, as can be observed with D4T. The M184 mutation was not considered for DDI, based on the IAS publication,[22] which reports that M184 is not associated with resistance to DDI *in vivo*; this fact has been corroborated by other studies.[31-34] The resistance profile to DDI according to the Stanford HIV Drug Resistance Database also revealed a low rate of resistance (17.4%); however, when considering intermediate resistance, the rate of resistance reaches 65.2%.

Many mutations confer cross-resistance to several ARVs within NRTI. Our results corroborate this statement, since even patients without exposure to Abacavir and Tenofovir had mutations associated with resistance to these agents. Similar patterns of resistance have also been shown in other pediatric studies.[5,29]

For NNRTI, the most frequent mutations were K103 (39.1%) and Y181 (17.4%), a class that is also very frequently used in the pediatric population. We had 12 patients failing NNRTI therapy, and all of them had some mutation conferring resistance to NNRTI, which confirms the low genetic barrier of the class. On the other hand, we did not find mutations to NNRTI in patients who had never used it before.

For PI, the most frequent mutations were V82, I54, L90 (21.7%), and M46 (17.4%). The PI most frequently used during the study period in our children were Ritonavir and Nelfinavir. Although other PIs were not used, we observed cross-resistance to some of them, except to Lopinavir/r, Tipranavir and Darunavir, suggesting a potential benefit of these drugs in the rescue therapy. The most frequent polymorphisms were L63 (73.9%), M36, L10, and V77 (34.8%). Polymorphism L63 is really the most frequent in the literature, ranging from 50 to 90%.[22,23]

HIV-1 subtype B predominates in Latin America, including Brazil, but other subtypes, such as F and C, also circulate, favoring CRF. Brazilian studies[2-4] including children show rates from 67 to 78% of subtype B and 6 to 15% of F, results that are similar to ours (78% of subtype B and 13% of F). However, with regard to subtype C, we observed a higher rate (4.3%), a finding that has been observed in the south of Brazil, with rates ranging from 29 to 70%,[13,35,36] showing an epidemiological distinction in that region. CRF were found in 4.4% of our children (B/B-F in 2.2% and F/B in 2.2%), but it would be necessary to sequence the full gene to discard new recombinations in genomic regions.

In conclusion, our results revealed an extensive profile of resistance in children failing ARV regimens, with 95% of the samples showing resistance to at least one class of drugs.

The lack of major mutations in ARV-naïve children is in agreement with previous studies documenting a low prevalence of these mutations in our country, as well as the presence of polymorphisms.

Further and more detailed studies in children are needed to confirm these findings and to evaluate the prevalence and importance of HIV resistance.

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