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ABSTRACT

Although mother-to-child HIV transmission prevention has slowed down pediatric HIV infection in developed countries, large numbers of infants still become infected in developing nations. Data on pediatric HIV infection is however largely scarce. In this study, we have overviewed clinical, laboratory and genotypic data from a large cohort of HIV-infected infants regularly followed at two pediatric HIV outpatient clinics in Rio de Janeiro, Brazil. Children on antiretroviral therapy, as well as drug-naïve, newly diagnosed infants were analyzed. Prevalence of drug resistance mutations, as well as immunological and virological responses to therapy were evaluated. Additionally, HIV-1 subtype frequencies and their distribution over the course of the epidemic were studied. We have found a high prevalence of mutations among ARV-experienced children, whereas mutations were absent in the drug-naïve group. Despite the high levels of resistance among treated infants, an important improvement of their immunological status was observed. HIV-1 subtype distribution followed the trends of the adult population, with the appearance of non-B subtypes and recombinant forms after 1990. To our knowledge, this is the largest pediatric cohort ever analyzed in Brazil, and the data provided is of paramount importance to a better understanding of HIV/AIDS evolution in pediatric settings.

Key words: HIV-1, pediatric, drug resistance, clinical, genotyping, subtype.

INTRODUCTION

The use of potent antiretroviral (ARV) combination therapy as the standard of care for the treatment of HIV-1 has produced a remarkable change in the natural history of HIV disease. Immune restoration has led to a decrease in disease progression, AIDS-related opportunistic infections (OI) and malignancies (Grulich et al. 2001, Ives et al. 2001, van Sighem et al. 2003, Louie et al. 2002). Successful therapy has allowed the discontinuation of primary and secondary prophylaxis for OI and in some cases complete remission of Kaposi’s Sar-
coma without chemotherapy (Kaplan et al. 2002, Lasso et al. 2003). Reduced mortality and hospitalization has been reported worldwide (Gadelha et al. 2002, Nuesch et al. 2002, Selik and Lindegren 2003). Although clinical improvement can be observed even in those with partial viral suppression, maximal suppression of viral replication continues to be the primary goal of therapy in order to prevent the emergence of resistant strains. Unfortunately, sustained virus suppression is not achieved in 20%-50% of the patients initiating highly active ARV therapy (HAART) and can be higher in subsequent treatments (Palella et al. 2002, Phillips et al. 2002, Mocroft et al. 2003). Although many factors are associated with detectable viremia, including poor adherence, drug pharmacokinetics, and suboptimal regimens, the emergence of drug-resistance strains is a leading cause of treatment failure in HIV-infected individuals.

Development of resistance to ARV drugs has a major impact on treatment of HIV-infected individuals due to the fact that it can lead to cross-resistance to other drugs of the same ARV class, limiting therapeutic options (Paolucci et al. 2000, Dionisio et al. 2001, Rousseau et al. 2001). In addition, there is an increasing prevalence of new infections with HIV strains resistant to one or more classes of ARV drugs (Wensing and Boucher 2003). Since 1996, the Brazilian Ministry of Health has provided ARV therapy to all HIV-infected patients, and a few studies that addressed the surveillance of drug-resistance virus in untreated persons in the country were limited to adult populations (Dumans et al. 2002, Brindeiro et al. 2003, Soares et al. 2003a, b, Pires et al. 2004). Rates of HIV drug resistance in children are largely scarce and unknown in Brazil and also worldwide.

The HIV-1 subtype distribution in Brazil is very complex and dynamic. Subtype B still prevails in the country, but other subtypes such as F1, C and D have been described (Cornelissen et al. 1996, Couto-Fernandez et al. 1999, Bongertz et al. 2000). More recent studies, however, have pointed out to a large proportion of subtype C in the southern states of Brazil, as well as increasing rates of that subtype in Rio de Janeiro and São Paulo (Brindeiro et al. 2003, Soares et al. 2003a, b). Moreover, a few studies from our group and others have shown that different HIV-1 subtypes may have distinct responses to ARV therapy or drug resistance patterns based on their genetic polymorphisms (Descamps et al. 1997, Gonzalez et al. 2003, Dumans et al. 2004). The dynamic nature of Brazilian HIV-1 subtypes therefore highlights the need for a constant surveillance system to enable future epidemiological, vaccine design and clinical trial studies in the country. Similarly as above, subtype data available in Brazil refers only to adult subjects, and systematic studies on HIV-infected children have not yet been conducted.

In view of those needs, we describe in the present study the genotypic impact of dual and triple therapy in a cohort of HIV-infected children in the state of Rio de Janeiro. The prevalence of HIV-1 subtypes in this population and a surveillance of primary drug resistance mutations in a subset of drug-naïve children has also been conducted.

**MATERIALS AND METHODS**

**Study population:** A cross-sectional study was conducted on consecutive HIV-1 infected children attending the outpatient clinic for HIV-infected infants of the Instituto de Puericultura e Pediatria Margarão Gesteira (IPPMG), Federal University of Rio de Janeiro, and at Hospital Jesus, a municipal hospital. Children between 1 month and 14 years of age were eligible to participate in the study if they had been using ARV therapy for at least 3 months. Exclusion criteria included severe anemia (Hgb < 9 g/dl). At the same time, children were eligible to participate in a longitudinal study if HIV-1 infection was confirmed by 2 ELISA and 1 Western blot at ≥ 18 months of age or by 2 RNA PCR assays if < 18 months of age. The research protocol was approved by the Ethical Committee of the Federal University of Rio de Janeiro, the Brazilian National Council in Ethics in Research, and the Institutional
Laboratory evaluation: CD4+ T-lymphocyte percentages and absolute counts were measured by flow cytometry using standardized techniques (FACScan, Becton Dickinson, San Jose, CA, USA). HIV-1 RNA was measured by the nucleic acid sequence-based amplification (NASBA) assay according to manufacturer’s instructions (Organon Teknika, Durham, NC). The detection limit was 80 HIV-1 RNA copies/ml. For the cross-sectional study, the CD4+ T-cell counts and HIV-1 RNA levels at the nearest time point to the genotypic study were used in the analysis of response to therapy.

HIV genotyping: Viral HIV-1 RNA was extracted from plasma, reverse transcribed and sequenced. Two regions were targeted in a 2-round nested PCR: the entire protease (PR) coding region (codons 1-99) and the first 235 codons of the reverse transcriptase (RT) coding region. Resistance mutations were classified as primary or secondary based on recommendations of The International AIDS Society-USA (D’Aquila et al. 2003). Phylogenetic analysis was conducted in ClustalW (Thompson et al. 1994) and MEGA v.2.0 (Kumar et al. 2001) software with 2,000 bootstrap sampling replications. Representative sequences of HIV-1 group M subtypes obtained from the Los Alamos HIV-1 database (http://hiv-web.lanl.gov) were used to construct phylogenetic trees and to evaluate bootstrap robustness values. A SIVcpz sequence was used as an outgroup. To assess HIV-1 drug resistance by genotyping, patient’s sequences were submitted to the Stanford HIV resistance interpretation algorithm (http://hivdb.stanford.edu). Sequences described in this work have been assigned the GenBank accession numbers AY313299-312, 335-362, 373-400, 411-425, AY390036, 038-042, 044-050, 052-060, 062-066, 068-071, 073-075, 082, 085-086, 089-097, 100-105, 107-109, 111-123, 126, 128-134, 136-174, 186-187, 215-217, AY393067, AY502094-095, 098, 101-102, AY530160-161, and AY569830-970.

Statistical analyses. Exploratory analyses included the examination of frequency distributions using parametric and non-parametric methods. Frequencies were expressed as percentages. Means and standard deviations of quantitative results were computed. Chi-square or Fisher’s exact test, whenever indicated, were used to compare the presence or absence of resistance mutations in association with virological or CD4+ T-cell count responses and with viral subtypes. A p-value of <0.05 was considered statistically significant.

RESULTS

1. Patients on Dual Therapy

Resistance mutations

Genotypic data was available from 53 children out of 60 on dual therapy. Two patients without genotypic data had an undetectable viral load and additional 3 patients had a VL < 5000 copies/ml. Thirty-nine patients were using zidovudine (ZDV) + didanosine (ddI) (6 have been subjected to monotherapy with ZDV before introduction of ddI). Fourteen have been treated with ZDV + lamivudine (3TC) (10 patients) or ZDV + stavudine (d4T) (4 patients), 10 of whom were previously treated with ZDV + ddI before the second-line regimen. Treatment exposure times at sample collection for genotyping was not different between both groups and had an average of 2.89 yr ± 1.4.

The prevalence of RT mutations on both groups is shown in Figures 1A and B. M184V was the most prevalent mutation in those taking 3TC and was rare in the AZT+ddI group. Mutation patterns related to multi-nucleoside RT inhibitor (NRTI) resistance – multiple NRTI-associated mutations (NAMs), the Q151M complex or the 69 insertion complex (Hirsch et al. 2003) – were found in 38.5% of the patients in the AZT+ ddI group. Twelve patients in this group had at least 4 NAMs (M41L, D67N, K70R, L210W, T215Y/F and/or K219Q/E), 2 patients showed the Q151M complex and one patient presented the 69 insertion complex. In the group taking 3TC or d4T, 4 out of 14 presented 4 NAMs.
(28.5%).

Mutations known to cause resistance to ddI were completely absent in both groups. Despite the use of ddI for a long period of time, few samples showed total or intermediate resistance to this drug which was more prevalent in the arm using 3TC, probably by the impact of M184V mutation together with NAMs on the decreased susceptibility to ddI. We have also observed in both groups a high prevalence of intermediate cross-resistance to abacavir (ABC) and tenofovir (TDF), although all patients were naïve to these drugs (Figures 2A and B).

Virological and immunological responses
Mean viral load (VL) at the time of genotypic analysis varied from 110 to 2,200,000 copies/ml (mean 121,000 ± 364, 250 copies/ml) in the ZDV + ddI group and from 390 to 210,000 copies/ml (mean 21,263 ± 54,885 copies/ml) in the ZDV + 3TC/d4T group, with no statistically significant differences between them (not shown). None of the patients had an undetectable VL. As CD4 T-cell counts before therapy were not available to all patients, we compared their CDC immunological stage with the values of %CD4 at the time of sample collection. Even though these patients were failing their ARV therapy and had a high number of resistance mutations, an immunological improvement was still present in 15 patients (50%) of those initially classified in CDC immunological class 2 (15% = CD4 % = 24%) or 3 (CD4% < 15%). Their mean level of CD4 T-cell percentage at the time of the study was around 28% (±6.4).
HIV-1-INFECTED CHILDREN IN RIO DE JANEIRO, BRAZIL

A

0
10
20
30
40
50
60
70
80
90
AZT D4T DDI ABC TDF 3TC

B

0
10
20
30
40
50
60
70
80
90
AZT D4T DDI ABC TDF 3TC

Fig. 2 – Impact of NRTI resistance mutations on susceptibility of individual drugs in the ZDV + ddI (A) and in ZDV + 3TC/d4T (B) groups. Light bars depict full resistance, while dark bars represent intermediate resistance.

2. PATIENTS ON TRIPLE THERAPY

Resistance mutations

We analyzed 76 infant patients on triple therapy. Sixteen patients were not included in the analysis because genotyping was not successful. Ten patients out of those 16 had an undetectable viral load and 4 had a VL < 5,000 copies/ml. Only 11 patients (14.5%) were previously naïve to all classes of ARV drugs. Forty-two patients (55.3%) were NRTI-experienced (18 had experienced 4 NRTI) but naïve to protease inhibitors (PI). Twenty-three patients (30.2%) had previously used one or more PI before their current ARV regimen (19 patients) or all three classes of ARV (4 patients) and 22 patients had used 4 NRTI.

Current treatment for the previously drug-naïve group included nelfinavir (NFV) (9 patients) or ritonavir (RTV) (2 patients). Of the PI-naïve patients, 23 were using NFV (one patient with nevirapine (NVP) included), 16 were using RTV and 3 were using NVP. Among the multi-experienced group, 7 patients were using only one PI (NFV – 6 patients and APV – 1 patient), 7 were using NNRTI (efavirenz – 5 patients; NVP – 2 patients) and 9 were being treated with all 3 classes of ARV. Time of treatment of the current ARV regimen at the time of genotypic study was similar in both previously ARV-naïve and PI-naïve groups (2.05 yr and 2.09 yr, respectively) and larger than in the multi-experienced group (1.18 years; p < 0.0009 when compared with the former 2 groups).

In order to assess whether treatment history would impact on the development of NRTI- and PI-associated resistance mutations, we have compared the percentage of each mutation to these 2 classes of ARV in the 3 different groups. Patients that were PI-naïve and were using only non-nucleoside RT inhibitors (NNRTI) were not included in this analysis. Table I shows the resistance mutations found in the 3 groups and Table II depicts the impact of resistance and cross-resistance to NRTI and PI. In the group of patients using NNRTI as part of their ARV regimen (20 patients), 13 (65%) were resistant to all drugs of this class. Comparison of prevalence of NRTI- and primary PI-associated mutations among groups showed only minor differences between them. In the RT region, a lower frequency of M41L mutation occurred in the ARV-naïve group when compared to the other 2 groups (p=0.02). Analysis of primary mutations in protease region (codons 30, 46, 54, 82, 84 and 90), also did not show significant differences except for a higher prevalence of M46I/L mutation in the multi-experienced group (p=0.04). Differences in prevalence of resistance to current regimen and cross-resistance was only seen when the ARV-naïve group was compared to the other 2 groups (Table II).
**TABLE I**

Drug resistance mutations in 73 patients on triple therapy with 1 protease inhibitor segregated by ARV history.

<table>
<thead>
<tr>
<th>Mutations</th>
<th>ARV-naïve patients (n=11)</th>
<th>PI-naïve patients (n=39)</th>
<th>Multi-experienced patients (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RT mutations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M41L</td>
<td>1 (9.1%)</td>
<td>21 (53.8%)</td>
<td>10 (43.5%)</td>
</tr>
<tr>
<td>E44D</td>
<td>2 (18.2%)</td>
<td>7 (17.9%)</td>
<td>4 (17.4%)</td>
</tr>
<tr>
<td>A62V</td>
<td>0</td>
<td>1 (2.6%)</td>
<td>2 (8.7%)</td>
</tr>
<tr>
<td>K65R</td>
<td>0</td>
<td>1 (2.6%)</td>
<td>1 (4.3%)</td>
</tr>
<tr>
<td>D67N</td>
<td>7 (63.6%)</td>
<td>19 (48.7%)</td>
<td>10 (43.5%)</td>
</tr>
<tr>
<td>T69D</td>
<td>0</td>
<td>4 (10.3%)</td>
<td>1 (4.3%)</td>
</tr>
<tr>
<td>K70R</td>
<td>6 (54.5%)</td>
<td>9 (23.1%)</td>
<td>5 (21.7%)</td>
</tr>
<tr>
<td>L74V</td>
<td>0</td>
<td>0</td>
<td>2 (8.7%)</td>
</tr>
<tr>
<td>V75A/I/M</td>
<td>0</td>
<td>9 (23.1%)</td>
<td>4 (17.3%)</td>
</tr>
<tr>
<td>F77L</td>
<td>0</td>
<td>2 (5.1%)</td>
<td>1 (4.3%)</td>
</tr>
<tr>
<td>A98G</td>
<td>0</td>
<td>3 (7.7%)</td>
<td>1 (4.3%)</td>
</tr>
<tr>
<td>Y115F</td>
<td>0</td>
<td>1 (2.6%)</td>
<td>0</td>
</tr>
<tr>
<td>F116Y</td>
<td>0</td>
<td>1 (2.6%)</td>
<td>1 (4.3%)</td>
</tr>
<tr>
<td>V118I</td>
<td>1 (9.1%)</td>
<td>14 (35.9%)</td>
<td>7 (30.4%)</td>
</tr>
<tr>
<td>Q151M</td>
<td>0</td>
<td>1 (2.6%)</td>
<td>1 (4.3%)</td>
</tr>
<tr>
<td>M184V</td>
<td>0</td>
<td>32 (82.1%)</td>
<td>14 (60.9%)</td>
</tr>
<tr>
<td>L210W</td>
<td>1 (9.1%)</td>
<td>17 (43.6%)</td>
<td>8 (34.8%)</td>
</tr>
<tr>
<td>T215F</td>
<td>2 (18.2%)</td>
<td>8 (20.5%)</td>
<td>4 (17.4%)</td>
</tr>
<tr>
<td>T215Y</td>
<td>4 (36.4%)</td>
<td>21 (53.8%)</td>
<td>8 (34.8%)</td>
</tr>
<tr>
<td>K219Q/E</td>
<td>3 (36.4%)</td>
<td>10 (25.6%)</td>
<td>5 (21.7%)</td>
</tr>
<tr>
<td><strong>Protease mutations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L10F/I/V</td>
<td>4 (36.4%)</td>
<td>20 (51.3%)</td>
<td>12 (52.2%)</td>
</tr>
<tr>
<td>K20M/R</td>
<td>2 (18.2%)</td>
<td>8 (20.5%)</td>
<td>5 (21.7%)</td>
</tr>
<tr>
<td>L24I</td>
<td>1 (9.1%)</td>
<td>3 (7.7%)</td>
<td>1 (4.3%)</td>
</tr>
<tr>
<td>D30N*</td>
<td>1 (9.1%)</td>
<td>10 (25.6%)</td>
<td>2 (8.7%)</td>
</tr>
<tr>
<td>V32I</td>
<td>0</td>
<td>2 (5.1%)</td>
<td>1 (4.3%)</td>
</tr>
<tr>
<td>L33F</td>
<td>0</td>
<td>3 (7.7%)</td>
<td>0</td>
</tr>
<tr>
<td>M36I</td>
<td>4 (36.4%)</td>
<td>15 (38.5%)</td>
<td>9 (39.1%)</td>
</tr>
<tr>
<td>M46I/L</td>
<td>3 (27.3%)</td>
<td>17 (43.6%)</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>F53L</td>
<td>0</td>
<td>0</td>
<td>2 (8.7%)</td>
</tr>
<tr>
<td>I54L/V</td>
<td>1 (9.1%)</td>
<td>13 (33.3%)</td>
<td>6 (26%)</td>
</tr>
<tr>
<td>L63P</td>
<td>7 (63.6%)</td>
<td>25 (64.1%)</td>
<td>19 (82.6%)</td>
</tr>
<tr>
<td>A71V/T</td>
<td>1 (9.1%)</td>
<td>14 (35.9%)</td>
<td>8 (34.8%)</td>
</tr>
<tr>
<td>G73S</td>
<td>0</td>
<td>0</td>
<td>1 (4.3%)</td>
</tr>
<tr>
<td>V77I</td>
<td>1 (9.1%)</td>
<td>6 (15.4%)</td>
<td>7 (30.4%)</td>
</tr>
<tr>
<td>V82A/T/S</td>
<td>1 (9.1%)</td>
<td>13 (33.3%)</td>
<td>5 (21.7%)</td>
</tr>
<tr>
<td>I84V</td>
<td>0</td>
<td>2 (5.1%)</td>
<td>4 (17.4%)</td>
</tr>
<tr>
<td>N88D</td>
<td>0</td>
<td>6 (15.4%)</td>
<td>2 (8.7%)</td>
</tr>
<tr>
<td>N88S#</td>
<td>0</td>
<td>1 (2.6%)</td>
<td>1 (4.3%)</td>
</tr>
<tr>
<td>L90M</td>
<td>6 (54.5%)</td>
<td>9 (23.1%)</td>
<td>9 (39.1%)</td>
</tr>
</tbody>
</table>

*Protease mutations in bold denote major (primary) mutations. # N88S causes hypersusceptibility to amprenavir.
TABLE II
Impact of drug resistance mutations on the susceptibility to used ARV and on cross-resistance to other ARV in patients on HAART therapy.

<table>
<thead>
<tr>
<th>Type of resistance</th>
<th>ARV-naïve (n=11)</th>
<th>PI-naïve (n=42)</th>
<th>Multi-experienced (n=23)</th>
<th>Total (n=76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No NRTI-resistance</td>
<td>2 (18.1%)</td>
<td>2 (4.8%)</td>
<td>5 (21.7%)</td>
<td>9 (11.8%)</td>
</tr>
<tr>
<td>No PI-resistance</td>
<td>4 (36.3%)</td>
<td>9 (21.4%)</td>
<td>8 (34.8%)</td>
<td>21 (27.6%)</td>
</tr>
<tr>
<td>High level resistance to current ARV (any class)</td>
<td>5 (45.4%)*</td>
<td>37 (88%)</td>
<td>20 (87%)</td>
<td>62 (81.5%)</td>
</tr>
<tr>
<td>High level resistance to other ARV not in use (cross-resistance)</td>
<td>1 (9%)#</td>
<td>25 (59.5%)</td>
<td>19 (82.6%)</td>
<td>45 (59.2%)</td>
</tr>
</tbody>
</table>

*p=0.003.  # p=0.0003.

Virological and immunological responses
Overall improvement in CDC immunological status was seen in 38 children (50%) and was of approximately 45% in each group. Mean viral load was 97,700 copies/ml for the previously ARV-naïve group and 133,850 copies/ml and 766,447 copies/ml for the PI-naïve and the multi-experienced group, respectively, with no statistical differences among them.

HIV-1 subtypes and response to therapy
Most of the samples were classified as subtype B by phylogenetic analysis. Among the 133 patients studied we have identified 27 (20.3%) non-B subtype viral isolates. Nine patients (6.8%) were classified as subtype F in both regions studied and the remaining as recombinants of subtype B in one region and subtypes F, C, or D in the other region. We have previously reported (Machado et al. 2004) a lower increase in CD4 percentages in non-B subtypes when compared with subtype B counterparts. As we did not have pre-treatment CD4 percentages of most of the children included in this study we could not confirm our previous results here.

3. NEWLY-DIAGNOSED UNTREATED HIV-INFECTED CHILDREN
Between November 1999 and October 2003 we have enrolled 80 newly diagnosed HIV-infected children in our casuistic. Of all but one, mothers were not tested during pregnancy and the children were the index case because of symptomatic disease or because they were diagnosed due to a symptomatic sibling. The age distribution on these children varied from 0.34 yr to 13.9 yr of age (mean = 5.4 ± 3.6 yr) and 60% were 6 yr or younger. Table III shows clinical and immunological data of these children at the time of diagnosis and as expected older children were more immunosuppressed. Two patients in the older group (> 9 yr) were still in CDC immunological class 1, and they were likely to represent long-term non-progressors. Mean VL was inversely correlated with age, being higher in children less than 3 years old.

Primary resistance mutations and subtype distribution
Analysis of RT and protease regions of untreated children was started in 2000 and 68 samples of a total of 80 children were analyzed. Primary resistant mutations were not observed, but polymorphisms were frequent in both genomic regions. Differences in frequency of different polymorphisms were not seen when the sequences were stratified by the age of the children (not shown). In the protease region the most frequent mutations found were L63P (56%), M36I (30%), V77l (29%), L10l/V (11%) and K20M/R (4.5%). The number of polymorphisms in
TABLE III
Clinical and immunological parameters of drug-naïve HIV-infected children.

<table>
<thead>
<tr>
<th></th>
<th>0-3 yr (n=23)</th>
<th>3-6 yr (n=25)</th>
<th>6-9 yr (n=17)</th>
<th>&gt; 9 yr (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>1.5</td>
<td>4.2</td>
<td>7.3</td>
<td>11.1</td>
</tr>
<tr>
<td>CDC clinical stage C</td>
<td>9 (39.1%)</td>
<td>7 (28%)</td>
<td>9 (53%)</td>
<td>7 (46.7%)</td>
</tr>
<tr>
<td>CDC immunological stage 3</td>
<td>7 (30.4%)</td>
<td>6 (24%)</td>
<td>9 (53%)</td>
<td>9 (60%)</td>
</tr>
<tr>
<td>CD4 %</td>
<td>18.1</td>
<td>20.7</td>
<td>13.4</td>
<td>12.9</td>
</tr>
<tr>
<td>CD8 %</td>
<td>46.5</td>
<td>46.7</td>
<td>49.7</td>
<td>57.1</td>
</tr>
<tr>
<td>Mean VL (log)</td>
<td>6.4</td>
<td>5.5</td>
<td>5.3</td>
<td>5.2</td>
</tr>
</tbody>
</table>

those children varied from 0 to 4 per patient.

Five children were classified as subtype F in both regions studied. Additional 6 comprised subtypes F, D or C in one or both regions, with a prevalence of non-B subtypes of 16.7%.

4. DYNAMICS OF HIV-1 SUBTYPE PREVALENCE IN INFECTED CHILDREN

We analyzed the HIV-1 subtype prevalence over time in infected infants in Rio de Janeiro. Samples were stratified by year of infection and by infecting subtype and were then grouped in 4 times periods: 1985-1989, 1990-1994, 1995-1999 and 2000-2001 (Figure 3). By 1990, the first subtype F viruses appeared in our dataset as well as subtype F-harboring recombinants (F/B and D/F). The prevalence of subtype F over the epidemic course seemed to stabilize from 1990 to 1999. However, a decrease of subtype B (± 82% to 75%), and a concomitant increase of many different recombinant forms (± 9% to 15.6%) between 1995 and 1999 were also observed.

DISCUSSION

Overcoming the emergence of resistant strains during ARV therapy to obtain a durable suppression of virus replication is one of the main goals of combination therapy. Achieving this goal during clinical management is difficult and it is even more challenging when dealing with pediatric population. Adherence in this population is a difficult task taking into account the bad taste of the medications, high pill burden and the young age of some children. Pharmacokinetics studies of NFV in children have shown that plasma concentration can vary with weight or age, suggesting that doses should be individualized, instead of a uniform standard dose (Bergshoeff et al. 2003, Floren et al. 2003). Due to all those factors, resistance in children is expected to be high. This cross-sectional study shows the impact of long-term (over 2 years) dual or triple therapy on genotypic outcomes in HIV-infected children. As NRTI are an important component of combination ARV therapy, detecting cross-resistance after failure to a first-line regimen is an important issue in the management of subsequent treatments. Also, recycling of NRTI is more difficult when compared with PI. Some of these difficulties reside in the fact that increased levels of one drug do not occur when 2 NRTI are combined together as it occurs with the combination of RTV-boosted PI combinations. Finally, the lack of an agent that produces levels much higher than the inhibitory concentration of 50% (IC50), increasing the genetic barrier to resistance mutations as is the case of the PI lopinavir (Kempf et al. 2001), is not available in the NRTI class.

In the group on dual therapy we have seen that almost one-third of the patients presented patterns related to multiple NRTI resistance, a high prevalence of M184V mutation in those taking 3TC and an absence of specific resistance mutations to ddI and d4T. However, the most prevalent resistance profile in this study was the presence of multiple mutations
related to the thymidine analogues ZDV and d4T. Such mutations were first related to the use of ZDV, but can be also developed by the use of d4T, although less frequently (Maxeiner et al. 2002). Decreased sensitivity to d4T has been show to be related to the number of NAMs, being higher in samples with more than 3 NAMs (Mouroux et al. 2001, Maxeiner et al. 2002) and may explain the high prevalence of cross-resistance to d4T seen in the ZDV+ddI group. Specific resistance mutations to d4T (I50T or V75T) or ddI virtually never occur in clinical specimens, as it has been show by some studies (Salomon et al. 1998, Vidal et al. 2002), and may explain the lack of specific mutations to these drugs in our casuistic and also the lack of resistance to ddI.

Prevalence of M184V was very high in the group taking 3TC. Although it leads to complete resistance to 3TC, its appearance has been shown to partially reverse the resistance of ZDV, d4T and TDF. There is an increasing trend to maintain this profile of resistance in order to increase the sensitivity to NRTI (as it is the case of the association 3TC + TDF) in clinical settings. Phenotypic studies in samples showing NAMs have shown different phenotypic impact on NRTI resistance when accompanied by M184V mutation (Whitcomb et al. 2003). Concomitantly, there was an overall intermediate resistance to ABC and TDF, drugs that were not used in this cohort, in 70% and 50% of the samples studied, respectively. Comparison of the Stanford algorithm used in our study with a virtual phenotype has shown a good correlation between both methods to predict cross-resistance in the setting of NNRTI and PI mutations but discrepancies are common when comparing samples with NRTI mutations (Puchhammer-Stöckl et al. 2002).

In some cases, a predicted resistance to NRTI shows a sensitive virtual phenotype. Also, introduction of TDF in heavily NRTI-experienced patients has been able to produce a reduction in VL of at least 0.5 log for up to 96 weeks (Margot et al. 2003). One of the limitations of this study is the lack of phenotypic estimation concomitant with the genotypic study and there was a likely overestimation of resistance, at least to TDF.

In the triple therapy group, there were very few patients with an undetectable VL (only 10 patients among 92 children on HAART). The low level of sustained viral suppression in our study contrasts with other reports (Nadal et al. 2000, Resino et al. 2003) and emphasizes that improving adherence in this population is urgently required. We detected a high rate of primary drug resistance mutations to NRTI (M184V was the most common) and PI (> 80%) in both PI-naïve and multi-experienced groups, and even in the ARV-naïve group it was...
much higher than previous reports (Eshleman et al. 2001, Mullen et al. 2002). A possible explanation for this discrepancy is that our genotypic study was conducted after the treatment failure had already become established and resistance mutations have accumulated due to long exposure to the same drug regimen. The presence of primary mutations to NRTI has been associated with a better virologic outcome in children treated subsequently with NVP (Eshleman et al. 2001), confirming the finding of a hypersusceptibility to NNRTI in NRTI-experienced patients described in adults (Haubrich et al. 2002). When groups were segregated by length of treatment, these differences are emphasized showing that salvage therapy is increasingly less efficient as expected. It has been shown that virologic response to a second-line regimen is lower and it took longer to be achieved (Resino et al. 2003).

Immunological recovery was still present in those heavily treated children and was seen in 50% of the children, independent of the ARV regimen used (dual or triple), and is in accordance with other reports of a continuing immunological benefit despite the presence of resistant mutants and/or virological failure (Jankelevich et al. 2001, Chiappini et al. 2003). This observation could be explained by a potential decrease in virus’ replication capacity (RC) with the acquisition of drug resistance mutations selected by heavy treatment. Such impairments in RC have been reported to correlate with persistence of immune response in patients with viro-immunological discordances (Sarmati et al. 2004).

Our genotypic study in untreated children born between 1985 and 2001 has shown a complete absence of primary resistance mutations to any ARV drug. As most of the children were the index case in the family, it suggests that most of the viruses transmitted were from individuals not aware of their HIV status. Surveillance of ARV resistant strains has not been done in untreated children in Brazil. Few studies in ARV-naive adults in Rio de Janeiro and in Rio Grande do Sul, mostly conducted among blood donors and Army personnel, have shown a prevalence of primary mutations of 0-2% (Dumans et al. 2002, Brindeiro et al. 2003, Soares et al. 2003a, b, Pires et al. 2004). These studies represent only a subset of newly infected persons in the country, and surveillance for drug-resistant viruses should continue in a larger scale as circulation of these strains varies from state to state in Brazil. Concerns about transmission of resistance strains reside in the possibility that it can compromise the efficacy of ARV treatment. Although previous reports of immunological and virological outcomes in patients with ZDV mutant virus are the same when compared with patients with wild-type virus (Imrie et al. 1996), more recent studies have shown that some drug-resistance isolates can have equal or even higher rates of infectivity than drug-susceptible isolates (Simon et al. 2003), a long permanence in the host (Torti et al. 2003) and can also lead to rapid decline in CD4 T-cell counts (Chan et al. 2003).

Clinical characteristics of this cohort showed a higher level of VL in children of age 3 or less and a decay of 1 log in older counterparts. Previous reports have shown that mean HIV-1 RNA levels in perinatally-infected children rises within the first 2 months of life to fall slowly until the age of 24 months, contrasting with the more rapid control of replication seen in adults who reach stable HIV-1 RNA levels around 6 months after infection (Shearer et al. 1997). During the first year of age, 20-25% of the children will progress to AIDS or death (Tovo et al. 1992). Although our cohort is not appropriate to draw conclusions about survival in children in general as children who survived are overrepresented, almost 40% was composed of children of 6 years of age or older. Only larger prospective studies with children followed since birth can confirm if this represents the natural outcome of perinatal infection in our country.

HIV-1 subtype distribution in children was similar to what is seen in adult population in the state of Rio de Janeiro (Pires et al. 2004, Brindeiro et al. 2003, Dumans et al. 2002), with a predominance of subtype B followed by subtype F. We have also
observed an increase of non-B subtype strains as the epidemic progressed, in particular after 1990. The more recent introduction of non-B subtypes and their expansion have also been suggested in the adult population in Brazil (Soares et al. 2003a,b), and that pattern seems to be followed in the pediatric setting.

It is still unclear whether there are differences in the evolution of disease or in response to ARV therapy among distinct HIV-1 subtypes. Although some studies addressing different clades have failed to show differences in virological response after treatment or in the prevalence of resistance mutation in patients failing therapy (Pillay et al. 2002, Kantor and Katzenstein 2004), subtle differences are starting to emerge. Recent reports have shown an increased prevalence of mutations associated with NVP and a faster progression to disease in patients with subtype D (Eshleman et al 2004, Kaleebu et al. 2002). Also, the mutation L90M occurs more frequently in non-B subtypes in adults but these differences were not noted in HIV-infected children (Pillay et al. 2002).

In summary, we describe here our genotypic and clinical findings in drug-naïve and ARV-treated infants, as well as their HIV-1 subtype distribution. The results of DRM suggest that more efforts have to be done to increase the adherence in this population. More studies addressing heavily-treated children with a high rate of DRM are necessary to determine the optimal regimen to be used in those cases.

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RESUMO


Palavras-chave: HIV-1, pediatria, resistência a drogas, clínica, genotipagem, subtipo.

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