Currently, 2.5 million children are living with HIV/AIDS, of whom more than 90% reside in sub-Saharan Africa. Access to antiretroviral (ARV) agents has increased dramatically over the past years. The number of children under 15 years of age receiving antiretroviral therapy increased by 29% between 2008 and 2009. About 356,400 children less than 15 years of age were receiving ARV therapy in low and middle-income countries at the end of 2009, up from 275,300 at the end of 2008. Children represented 6.8% of people receiving ARV therapy and 8.7% of people in need. The ultimate goal of ARV is to achieve virologic suppression and immune reconstitution. Virologic suppression is defined as a reduction in plasma HIV RNA to below the limit of detection or <50 copies/mL (cpm). Immune reconstitution is reflected by an increase in CD4 positive T cell count. HAART has dramatically improved the quality of life and overall survival of in-
individuals infected with HIV-1. Sub-optimal exposure to ARV can rapidly select the development of resistant mutation. These resistant variants can rapidly emerge and ultimately lead to virologic failure (defined as the inability to achieve virologic suppression within 16 to 24 weeks of initiation of ARVs or persistent HIV RNA load >1000 cpm).² In the United States and western Europe, nearly 80% of HIV-1 infected adults who experienced virologic failure harbored virus strains resistant to at least one ARV drug.¹ Few studies on emerging resistant mutations in children were available in selected cohorts of limited size.³–⁵

There are essentially two types of assays for detecting ARV resistance in routine clinical practice. Phenotypic assays measure the susceptibility of the virus to various drugs in a tissue-culture system while genotypic assays detect the presence of resistance mutations. Genotypic assays are most commonly used in clinical practice because they are generally less expensive, laborious and time-consuming than phenotypic assays.¹¹ The objective of this study was to estimate the prevalence and pattern of ARV resistance in HIV-infected children who failed HAART.

**PATIENT AND METHODS**

All perinatally HIV-infected children followed in the King Faisal Specialist Hospital and Research Centre comprehensive HIV care program who received triple ARV therapy or highly active ARV therapy (HAART) according to the recommendation of the working group on ARV therapy and medical management of HIV-infected children¹² were included in the analysis. Genotypic resistance tests were performed in patients with virologic failure (defined as plasma HIV RNA >1000 copies/mL) to optimize the choice of new ARV regimens. The blood samples had genotype tests performed using TRUGENE HIV-1 genotyping kit (DNA-sequencing assay, Bayer Healthcare; healthcare. bayer.com) and interpretable results were based on the manufacturer’s guideline. The analysis was carried out on data obtained between July 2006 and January 2009.

**RESULTS**

Among children receiving HAART, the proportion of children who experienced persistent viral load >1000 copies/mL was 48% (22/46). The characteristics of study population are summarized in Table 1. Overall, a genotype test was performed at least once in the 22 children who experienced virologic failure. The prevalence of resistance to any ARV drugs was 86.4%. Inadequate adherence to ARVs in children with drug resistance was 91%. Twenty-four mutations were detected within the protease coding region and 14 in the RT coding region (Tables 2, 3).

The most common mutation (found in 71% of strains) was piM36I (Table 2). The rtM184V mutation was present in 70% of strains and was associated with cross-resistance to at least two NRTIs—lamivudine and emtricitabine (Table 3). Clinically significant efavirenz resistance was conferred by the rtK103N mutation. ARV resistance was not associated with geographic regions or the CDC pediatric HIV classification. The group of studied children responded satisfactorily to the genotype-guided treatment and intensive family counseling after 52 weeks follow-up.

**DISCUSSION**

This study is the first to estimate the prevalence of genotypic resistance in treated Saudi children who experienced virologic failure defined as HIV RNA >1000 copies/mL. The ultimate goal of ARV therapy is suppression of HIV replication and reducing morbidity and mortality linked to severe immunodeficiency. During the period from July 2006 to January 2009, about 48% of children living in Saudi Arabia and receiving medical care for HIV infection at King Faisal Specialist Hospital and Research Centre had virologic failure. Among the 22 pretreated children with virologic failure, the prevalence of resistance to any drug was 86.4%, which is higher than previously reported.³,⁴,¹³ Randomized trials and observational studies in children have also described a high prevalence of resistance to any drug, increasing with the number of prior ARV drugs received.⁸,¹⁴ In
the United States and western Europe, nearly 80% of HIV-infected adults experiencing virologic failure harbored virus strain resistant to at least one ARV drug. Children seem to be more prone to selection for drug-resistant variants for both biologic and behavioral reasons. Plasma viral loads are much higher following perinatal infection and ARV drugs may not fully suppress viral load during the early years of childhood. Furthermore, drug absorption and pharmacokinetics are highly variable and change with age, resulting in suboptimal levels of ARV drug. This is further compounded by difficulties in adherence resulting from unpalatable liquid formulations and the requirement for frequent dosing.

The assessment of a child with virologic failure should include evaluation of adherence to therapy, medication intolerance, issues related to pharmacokinetics that could result in low drug levels, and evaluation for ARV drug-resistance testing. In our study, 20/22 (91%) viroemic patients provided a clear history of noncompliance with HAART because of patient refusal due to the poor taste of the drug. We have observed a high frequency of possible genotypic resistance to PIs (24 mutations). The most common mutations were piM36I and piL90M, found in 70% and 42% of our patients, respectively. These two mutations confer cross-resistance to various PIs. In the present study, we found the best profile for the PIs to be lopinavir (75.5%) with only four possibly resistant isolates as shown in Table 2. This observation verifies the important fact that lopinavir has the greatest genetic barrier to resistance. The most common mutation in RT regions was rtM184V (70%), which was associated with resistance to 3TC and FTC and possible resistance to AZT.

Table 2. Most common resistance patterns in 24 sequences from patients with resistance mutations to protease inhibitor drug susceptibility.

| Pattern | No | SQV | ATN | IDV | RTV | NPV | LPV | APV |
|---------|----|-----|-----|-----|-----|-----|-----|-----|
| M36I    | 17 | S   | NT  | R   | R   | R   | S   | NT  |
| L90M    | 10 | R   | NT  | R   | R   | R   | S   | S   |
| L10V    | 2  | R   | NT  | PR  | R   | S   | S   | PR  |
| K20M    | 3  | S   | PR  | S   | R   | S   | S   | PR  |
| G16E    | 2  | S   | S   | S   | S   | S   | S   | PR  |
| L63T    | 2  | S   | S   | S   | S   | S   | S   | NT  |
| D30N    | 9  | S   | S   | S   | R   | R   | S   | NT  |
| N88D    | 8  | S   | S   | S   | R   | R   | S   | NT  |

SQv: saquinavir, ATn: atazanavir, idV: indinavir, RTV: ritonavir, NPv: nelfinavir, LPV: lopinavir, APV: amprenavir

Table 3. The resistance pattern in 14 sequences from patients with resistance mutations in the reverse transcriptase coding region.

| Pattern | No | AZT | 3TC | D4T | ABC | DDI | FTC | TDF |
|---------|----|-----|-----|-----|-----|-----|-----|-----|
| M184V   | 10 | PR  | R   | S   | S   | S   | R   | S   |
| D67N    | 3  | S   | R   | PR  | R   | S   | S   | PR  |
| K70R    | 3  | PR  | S   | PR  | S   | S   | S   | S   |
| K219E   | 2  | R   | S   | PR  | S   | S   | S   | S   |
| T215Y   | 3  | R   | R   | R   | PR  | S   | S   | S   |
| M41L    | 2  | R   | S   | R   | S   | S   | S   | S   |

AZT: zidovudine, 3TC: lamivudine, D4T: stavudine, DDI: didanosine, FTC: emtricitabine, TDF: tenofovir, ABC: abacavir

R: resistant, S: susceptible, PR: possible resistance.
high proportion of patients harboring the M184V mutation could be because 80% of our viremic patients were receiving 3TC at the time of testing. The M184V mutation in the RT gene is associated with resistance to 3TC and inverse susceptibility to others, as has been shown in clinical and in vitro studies.\textsuperscript{22,23} RT enzyme possessing the M184V mutation exhibit reduced processability and increased fidelity compared with wild-type enzymes. All patients had genotypic resistance or possible resistance to AZT, D4T and ABC because the pressure of three or more AZT-specific mutations including T214Y is thought to confer resistance to D4T and ABC.\textsuperscript{25,26}

We detected the rtK103N mutation in three viremic children who were receiving efavirenz (NRTI). Our data is similar to those described previously by Bacheler et al.\textsuperscript{27} The K103N mutation was detected in patients receiving efavirenz and who had a plasma viral load rebound. The K103N is the most common RT gene mutation observed following an NNRTI-containing regimen.

Antiretroviral resistance was not associated with geographic region or CDC status. The children had a satisfactory response to genotype-guided treatment. This response was maintained over the follow-up period. Family education concerning adherence was intensive and included training in the administration of the prescribed medication with an emphasis on the importance for adherence to drug regimen.

In conclusion, antiretroviral resistance is common among HIV-infected Saudi children failing HAART. Inadequate adherence is the most common cause of ARV failure in children. The clinician needs to assess the likely contribution of adherence problems to the failure of the drug regimen. M361, M184V and K103N mutations were frequent for the PI, NRTI, and NNRTI classes, respectively. These mutations are extremely important as they confer cross-resistance among drugs within the same antiretroviral class. Genotype resistance testing is important to assess reasons for current virologic failure and to identify future selection of active ARV medications. The provided data will help to improve the clinical management of HIV-infected children in Saudi Arabia.
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