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Antiretroviral resistance in HIV-infected Saudi children failing first-line highly active antiretroviral therapy

Sami Hussain Al Hajjar,[a] Husn Frayha,[a] Sahar Althawadi[b]

From the [a]Pediatric Infectious Diseases and [b]Pathology & Laboratory Medicine, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

Correspondence: Dr. Sami Hussain Al Hajjar · Pediatric Infectious Diseases, King Faisal Specialist Hospital and Research Centre, Riyadh 11211 SaudiArabia · T: +966-464-7272 loc 27761/31983 F: +966-1-442-7784 · hajjar@kfshrc.edu.sa

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BACKGROUND AND OBJECTIVES: The use of a potent combination of antiretroviral (ARV) drugs, so-called highly active ARV therapy (HAART), has dramatically improved the quality of life and overall survival of children with human immunodeficiency virus (HIV) infection. However, these benefits can be compromised by the development of drug resistance. Our objectives were to analyze the prevalence and pattern of HIV-drug resistance among HIV-infected children failing first-line HAART.

DESIGN AND SETTING: Retrospective study based on data obtained from July 2006 through January 2009 of prevalence of genotypic resistance estimated in HAART-treated children who experienced virologic failure (HIV RNA>1000 copies/mL) at a tertiary care center in Riyadh.

PATIENTS AND METHODS: The characteristics of the study population and genotype resistance data were analyzed in ARV-treated children who experience virologic failure.

RESULTS: Among 22 children who underwent resistance testing, the prevalence of resistance to any drug 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 reverse transcriptase (RT) coding region. In 80% of isolates piM36I was detected, while rtM184V was detected in 70% of the isolates and was associated with cross-resistance to at least two nucleoside RT inhibitors (NRTI). Clinically significant non-nucleoside RT inhibitors (NNRTI) resistance was conferred by rtK103N. The best ARV susceptibility was to lopinavir in the PI class. ARV resistance was not associated with geographic regions or the CDC classification status. Study children responded satisfactorily to genotype-guided treatment and intensive family counseling.

CONCLUSION: ARVs resistance is common among HIV-infected Saudi children who experienced virologic failure to HAART. Inadequate adherence is a common cause for resistance to ARVs in children. Mutations M36I and M184V were more frequent for PIs, NRTIs and NNRTIs. Evaluation of genotype tests should be considered in all children with therapeutic failure to guide future selection of ARV regimens. These data will help improve clinical management of HIV-infected children in Saudi Arabia.

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.[1] 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-

dividuals 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.[3] Few studies on emerging resistant mutations in children were available in selected cohorts of limited size.[3-10]

There are essentially two types of assays for detecting ARV resistance in routine clinical practice. Phenotypic assays measures 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 children[12] 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 interpretative results were based on the manufacturer's guideline. The analysis was carried out on data obtained between July 2006 and January 2009.

Table 1. Characteristics of HIV-infected children experiencing therapeutic failure.

Characteristic	
Median age (years)	7
Gender Male Female	 13 (59.1) 9 (40.9)
CDC (Category C)[a]	2 (9.1%)
CDC (AIDS-defining conditions)[a]	2 (9.1%)
Virologic failure[b] and documented resistance	22 (86.4%)
Median CD4 count and range (cells/m^3)	817 (134-1885)
Median HIV-RNA viral load and range (copies/mL)	1352 (1100-20 202)
Inadequate adherence to ARVs	20/22 (91%)

Values are number (%) unless otherwise indicated.

[a]The Centers for Disease Control and Prevention (CDC). Clinical categories for children younger than 13 years (Category C= HIV encephalopathy) years and CDC case definition for AIDS-defining conditions: for adolescent and adult, 13 years of age and older (recurrent bacterial pneumonia=3 episodes in 1 year).

[b]Virologic failure: persistent viremia (HIV-RNA viral load >1000 copies/mL).

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

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	PR	NT
L63T	2	S	S	S	S	S	PR	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

R: resistant, S: susceptible, PR: possible resistance, NT: not tested

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

Pattern	No	Drug Susceptibility						
		AZT	3TC	D4T	ABC	DDI	FTC	TDF
M184V	10	PR	R	S	S	S	R	S
D67N	3	S	R	PR	R	S	PR	S
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: entricitabine, TDF: tenofovir, ABC: abacavir

R: resistant, S: sensitive, PR: possible resistance.

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.[3,4] 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.[2,8,15,16] The assessment of a child with virologic failure should include evaluation to adherence to therapy, medication intolerance, issues related to pharmacokinetics that could result in low drug levels, and evaluation for ARV drug-resistance testing.[17,18] In our study, 20/22 (91%) viremic 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. or possible resistance mutations were detected in the RT regions.[19-21]

The most common mutation in RT regions was rtM184V (70%), which was associated with resistance to 3TC and FTC and possible resistance to AZT. This

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.[22-24] 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.[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.[27] The K103N mutation was detected in patients receiving efivarenz and who had a plasma viral load rebound. The K103N is the most common RT gene mutation observed following an NNRT-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 NNRT 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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