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PEDIATRIC INFECTIOUS DISEASES (I BROOK, SECTION EDITOR)

Antiretroviral Treatment and Resistance Patterns in HIV-Infected Children

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Abstract Paediatric HIV-infected patients have higher risk of developing resistance to antiretroviral drugs, and from public health perspective, drug resistance remains a limiting factor for effective management of HIV infection in children. We reviewed the current evidences available on the antiretroviral treatment and resistance patterns in HIV-infected children. Prevalence of HIV drug resistance varied among the three main classes of antiretroviral drugs, namely nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors in both treatment naïve and treatment-experienced children in different countries. Most of the patients with extensive triple-class drug-resistant mutations were found to be considerably exposed to the three main classes of antiretroviral agents. Identification of genetic factors linked with susceptibility to perinatal transmission of HIV may be key in understanding the development of resistance due to waning antiviral effectiveness. Children who were less likely to achieve viral re-suppression were more likely to have resistance mutations. Newer drugs such as etravirine can be used as alternatives in case of resistance to efavirenz while newly developed diagnostic method such as next-generation sequencing is a platform for improving quality of detections especially minor variant drug resistance mutations.

Keywords Human Immunodeficiency Virus · Antiretroviral resistance · Antiretroviral treatment · Drug resistance mutations · Children

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Introduction

Introduction of highly active antiretroviral therapy (HAART) for the management of HIV-exposed and HIV-infected children during pregnancy, delivery and after breastfeeding periods has gone a long way in preventing mother-to-child transmission of HIV. It has significantly reduced the morbidity resulting from HIV, enhanced the immunological systems and improved clinical outcomes and quality of life [1]. The World Health Organization (WHO) recommended that antiretroviral (ARV) treatment regimens for children infected with HIV should consist of a three-drug combination comprising of two nucleoside reverse transcriptase inhibitors (NRTIs) with either or non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI) [2]. As shown in Table 1, there are preferred regimens and alternative regimes across age range in children.

The major limitation to the successful suppression of HIV viral load (VL) is the development of resistance against antiretroviral (ARV) compounds which results from biochemical changes such as substitution of various amino acid sequences in the HIV reverse transcriptase and protease enzymes. These enzymes are the main targets of the ARV agents [3]. In spite of the fact that the introduction of effective ARV combination

Table 1 Summary of WHO-recommended first- and second-line ARV regimens for children (including adolescents) [2]

	Children (including adolescents)	First-line ARV regimen	Second-line ARV regimen
LPV/r-based first-line regimen	Younger than 3 years	ABC+3TC+LPV/r AZT+3TC+LPV/r	No change[a]
	3 years and older	ABC+3TC+LPV/r	AZT+3TC+EFV
		AZT+3TC+LPV/r	ABC (or TDF)[b]+3TC+EFV
NNRTI-based first-line regimen	All ages	ABC+3TC+EFV (or NVP) TDF[b]+3TC (or FTC)+EFV (or NVP)	AZT+3TC+LPV/r[c]
		AZT+3TC+EFV (or NVP)	ABC or TDF+3TC[c] (or FTC)+LPV/r[c]

ABC abacavir, *AZT* zidovudine, *3TC* lamivudine, *EFV* efavirenz, *NVP* nevirapine, *TDF* tenofovir, *LPV/r* lopinavir/ritonavir, *FTC* emtricitabine, *NNRTI* non-nucleoside reverse transcriptase inhibitor, *WHO* World Health Organization

[a] No change is recommended unless in the presence of advanced clinical disease progression or lack of adherence specifically because of poor palatability of LPV/r. In this case, switching to a second-line NVP-based regimen should be considered. Based on the recent approval of the use of EFV in children less than 3 years, an EFV-based regimen could be considered as an alternative

[b] TDF may only be given to children >2 years

[c] ATV/r can be used as an alternative to LPV/r in children older than 6 years

regimens has increased the thresholds for drug resistance mutation (DRM) [4], there are more than 100 mutations leading to various levels of resistance [5]. Development of ARV drug resistance to a large extent depends on antiviral efficacy of the ARV regimen in use and the continuous replication of the virus while treatment is ongoing [6]. The similarities in the molecular structure of ARVs of the same class make the agents to be vulnerable to cross-resistance whereby once a member of a class is resistant to the virus, there is a high chance of other members of the class developing same on the long run [6].

Emergence of drug resistance (DR) HIV variants could be traced to the use of suboptimal antiretroviral drug exposure, thereby leading to viral replication under a selective pressure [6]. Sub-optimal drug exposure can be as a result of non-adherence with ARV, changes in drug metabolism and tissue and cellular sanctuaries for HIV [3]. Adherence in children and adolescence is a big challenge due to the issues associated with palatability of some of the paediatric formulations, reliance on caregivers who may be an older sibling almost in the same age group as the patients or other members of the community and the numerous challenges associated with growing adolescents. Paediatric HIV-infected patients have higher risk of developing DR due to high VL [7], less effective immunological responses [8] and problems with sub-optimal drug concentrations because of frequent varying dosages [9]. Resistance of HIV to ARVs hampers the expected clinical benefits of ART in HIV-infected children due to the loss of antiviral treatment efficacy of the drugs. This is a major public health concern because of the effects of resistance on long-term treatment options, taking into cognisance the lengthy duration of ART that is expected in HIV-infected children from infancy to adulthood.

Antiretroviral Drug Resistance Mechanism

Nucleoside Reverse Transcriptase Inhibitors Resistance Mechanism

The role of NRTIs is very critical in the composition of HAART in the treatment of HIV-infected children as it makes up two of the three drug regimes. Drug resistance to NRTIs can occur via two mechanisms: the first being through mutations at the end or near the drug-binding site of the reverse transcriptase gene [10]. The second mechanism involves mutations which tend to undo the action of the NRTIs even if they bind in the right way within the reverse transcriptase gene with the occurrence of pyrophosphorolysis also known as thymidine analogue mutations (TAMS), especially in zidovudine and stavudine [10]. Table 2 adapted from the Stanford HIV Drug Resistance Database [11] shows the discriminatory, thymidine and multidrug resistance mutations for various NRTIs.

Non-nucleoside Reverse Transcriptase Inhibitors Resistance Mechanism

Drug resistance mechanism of the NNRTI-resistant HIV strains is due to the mutations mainly in and around the NNRTI binding pocket [10]. The NNRTI binding ability could be directly affected by changing the shape, size and polarity in diverse areas of the pocket or in some cases by indirectly affecting the access to the pocket. The mutations are mainly seen in domains of amino acids 98–108, 178–190 or 225–238 of the p66 subunit [12]. Table 3 shows different major NNRTI resistance mutations.

Table 2 Major nucleoside reverse transferase inhibitor resistance mutations

	Discriminatory mutations					Thymidine analog mutations (TAMs)						Multidrug resistance mutations	
Consensus	M	K	K	L	Y	M	D	K	T	T	K	T	Q
3TC	*VI*	*R*										*Ins*	*M*
FTC	*VI*	*R*										*Ins*	*M*
ABC	VI	*R*	*E*	*VI*	*F*	L			W	FY		*Ins*	*M*
DDI	VI	*R*	*E*	*VI*		L			W	FY		*Ins*	*M*
TDF		*R*	*E*		F	L		R	W	FY		*Ins*	*M*
D4T		*R*	*E*			*L*	*N*	*R*	*W*	*FY*	QE	*Ins*	*M*
AZT						*L*	*N*	*R*	*W*	*FY*	QE	*Ins*	*M*

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

Protease Inhibitors Resistance Mechanism

Most mutations in the viral protease gene that lead to resistance to the protease inhibitors are due to amino acids that are not close to the binding sites for the antiretroviral class [10]. The major PI resistance mutations are shown in Table 4.

We hereby review current evidences available on the antiretroviral treatment and resistance patterns in the HIV-infected children vis-à-vis prevalence and types of ARV drug resistance mutations, use of ARV for prevention of mother-to-child transmission (PMTCT) of HIV monitoring, diagnosis and alternative treatment regimens.

Prevalence and Different Types of Drug Resistance Mutations

The knowledge of the prevalence and different types of resistant viruses in HIV-infected children is essential in determining the best ART combination regimens needed among a particular cohort of patients. This also helps in deciding whether to carry out resistance test before ART initiation [13]. There are evidences that most cases of HIV DRMs developed in HIV-infected infants during the postpartum period primarily because of exposure to maternal ARV drugs via breast milk

Table 3 Major non-nucleoside reverse transferase inhibitor resistance mutations

Consensus	100 L	101 K	103 K	106 V	138 E	181 Y	188 Y	190 G	230 M
NVP	*I*	*EP*	*NS*	*AM*		*CIV*	*LCH*	*ASE*	*L*
EFV	*I*	*EP*	*NS*	*AM*		CIV	*LC*H	*ASE*	*L*
ETR	*I*	E*P*			AGKQ	*CIV*	L	AS*E*	*L*
RPV	*I*	*EP*			*AGKQ*	*CIV*	*L*	AS*E*	*L*

EFV efavirenz, *ETR* etravirine, *NVP* nevirapine, *RPV* rilpivirine

[14]. An investigation was carried out to determine transmitted HIV-1 DRMs among vertically infected children aged between 6 weeks and 5 years in Kenya. The results indicated that the prevalence of HIV-1 DRMs of various types among these group of Kenyan children was 22.6 %. None had any major PI-related mutations while minor mutation in L10I/V was 41.5 % (22/53). Analysis of the reverse transcriptase region showed that major NRTI and NNRTI resistance mutations were present in the children [15]. Many of the treatment-experienced perinatally HIV-infected adolescents had been heavily exposed to various ART regimens over the years. They developed different strains of drug-resistant virus and in need of treatment for comorbidities [16]. The need for non-antiretroviral medications for comorbidities may also predispose them to clinically significant drug–drug interactions [16]. In order to have a better understanding of all the aforementioned factors, an Argentine study evaluated the prevalence of concomitant medications and clinically significant drug–drug interactions in treatment-experienced perinatally HIV-infected adolescents. The findings revealed a high prevalence of comorbidities, co-medications and clinically significant drug–drug interactions in the adolescents. Significant percentage of these adolescents had drug–drug interactions which could result in sub-therapeutic ARV concentrations. This is a concern in patients who have drug-resistance viruses [17].

Assessment of the prevalence and the characteristics of HIV-1 DR in paediatric patients with virological failure following an ART from 2005 to 2012 in Yunnan Province, China, was carried out by Zhong et al. [18]. Findings revealed that about 73 (10 %) of 729 children had virological failure while 53 developed resistance for ARVs. The dominant DRMs mutations were M184V/I, K103N, T215F/Y, G190A, Y181C and K101E. The percentage of drug resistance to individual ARVs was as follows nevirapine (NVP) (61.6 %), lamivudine (3TC) (54.8 %), efavirenz (EFV) (47.9 %), stavudine (D4T) (13.7 %), zidovudine (AZT) (12.3 %) and (abacavir) ABC (5.5 %). One patient had intermediate resistance to lopinavir/

Table 4 Major protease inhibitor resistance mutations

Consensus	30 D	32 V	33 L	46 M	47 I	48 G	50 I	54 I	76 L	82 V	84 I	88 N	90 L
ATV/r		I	F	IL	V	**VM**	*L*	VTALM		ATFS	*V*	*S*	*M*
DRV/r		**I**	F		**VA**		*V*	**LM**	**V**	F	V		
FPV/r		*I*	**F**	**IL**	**V***A*		*V*	VTA*LM*	*V*	ATS*F*	*V*		**M**
IDV/r		**I**		**IL**	V			*VTA*LM	*V*	*AFTS*	*V*	S	**M**
LPV/r		**I**	F	IL	V*A*	VM	**V**	**VTALM**	*V*	*AFTS*	**V**		M
NFV	*N*		F	**IL**	V	*VM*		*VTALM*		*AFTS*	*V*	*DS*	*M*
SQV/r						*VM*		**VTA**LM		AT	*V*	S	*M*
TPV/r		I	F	IL	**VA**			**VAM**		*TL*	**V**		

ATV/r atazanavir/ritonavir, *DRV/r* darunavir/ritonavir, *FPV/r* fosamprenavir/ritonavir, *IDV/r* indinavir/ritonavir, *LPV/r* lopinavir/ritonavir, *NFV* nelfinavir, *SQV/r* saquinavir/ritonavir, *TPV/r* tipranavir/ritonavir

The resistance mechanism tables list the most common clinically significant resistance mutations. Mutations in italics are associated with the highest levels of reduced susceptibility or virological response to the relevant antiretroviral drugs. Mutations in bold reduce ARV susceptibility or virological response. Mutations in plain texts contribute to reduced susceptibility in combination with other drug-resistant mutations

The description of HIV drug resistance mutations and their interpretation can be found at http://hivdb.stanford.edu [11]

ritonavir (LPV/r). The main cause of virological failure in this paediatric cohort was drug resistance to ARVs [18].

A cross-sectional survey was conducted to assess the mutations in the reverse transcriptase and protease genes of HIV-1 in both antiretroviral experienced and naïve HIV-1 infected Indian children. Major resistance associated mutations in reverse transcriptase gene were observed in about one in three of the ART naïve and experienced children respectively while minor mutations were observed in protease gene in only five out of the total 140 children involved in the study [19]. Sagna et al. [20] identified resistant strains to ARV drugs and determined the subtypes and circulating recombinant forms (CRF) of HIV-1 among mother–infant pairs in a study carried out in Burkina Faso. The study revealed genetic mutations that induce resistance such as M184V, Y115F, K103N, Y181C, V179E and G190A. The main strains of infection in this study group were CRF06_CPX and CRF02_AG [20].

A study that was conducted in Tanzania to evaluate durability of ART and predictors of virologic failure among children at 4-year follow-up found that subtypes A and D were most prevalent among those who failed on first-line therapy [21]. The key NRTI mutations were M184V in 89 % of the individuals and one or more TAMs present in 49 %. NNRTI mutations were found in 89 % of the individuals, including K103N (40 %) and Y181C (23 %). However, there was no K65R mutation. Intermediate resistance to NRTI was predicted in 29 % of the participants and only of 40 % of this subgroup achieved full suppression while on a second-line regimen and the remaining were failing second-line in spite of reported good adherence in some of them. Two of the patients with high predictive resistance (6 TAMs and 3 TAMs) did very well on second-line with good adherence and supportive physician care. Many switched to second-line therapy and achieved virologic suppression notwithstanding multiple resistance mutations [21].

An assessment of the outcome of first-line ART was carried out among HIV-1 vertically infected Vietnamese children. The total study population was 86, with 68 children having VL <1000 copies/ml and 64 having full viral suppression with VL <400 copies/ml after 24 months of ART. There was no significant difference between successfully treated patients and failure groups in terms of clinical parameters and demographics at the baseline [22]. Among the children with treatment failure, one developed reverse-transcriptase inhibitor resistance mutations by 24 months of treatment. None of the successfully treated patient developed DRMs. The most dominant NNTRIs and NRTIs resistance mutations were Y181C and M184V/I, respectively [22].

Resource-limited countries often times limit recommended DR genotyping investigations for children who are on failing second-line therapy regimens due to cost and other logistical issues [23]. On the account of the limited data, a review explored the knowledge and summary of HIVDR, transmitted HIVDR (TDR) and DRM rates of the three main classes of ARVs in both treatment naïve and pre-treated children in different settings. The review included 41 studies with HIVDR data from 2538 patients (558 naive and 1980 pre-treated) from 30 countries. Findings showed that 9 studies reported on both TDR and DRM prevalence, 6 for TDR only and 26 reported on DRM only. Prevalence of HIVDR varied across different countries and HIV-1 non-B variants were prevalent in 18 out of the 24 countries that reported the subtypes. Five countries, namely Brazil, Spain, Thailand, Uganda and UK, had resistance data that were equal or more than 200 patients [24].

Lange et al. [25] characterised multi-class drug resistance using single genome sequencing (SGS) of HIV-1 protease and

reverse transcriptase to longitudinal samples from a cohort of South African HIV-infected children with viraemia while receiving PI-based ART on the CHER trial. Bulk sequencing revealed NVP-selected resistance in 50 % of the study participants while SGS was revealed in 70 %. Baseline NRTI and PI mutations were detected in two of the children, suggestive of previous maternal ART use. Linked multi-class drug resistance following use of PI-based ART was detected in two of the children [25]. A South African study reported the results of resistance testing among 152 children who developed virologic failure while on first-line LPV/r-based therapy. Resistance testing was performed in about half of the children. Genotyping showed that 10.7 % had significant LPV/r-associated resistance mutations with 2 children having intermediate darunavir resistance. Half of the children remaining on LPV/r-based therapy achieved virologic suppression with 2 having significant LPV mutations. In accordance to the treatment guideline, 12/152 (8 %) children were changed to NNRTI-based therapy and only 4/12 (33 %) of these children re-suppressed, while the others did not achieve virologic suppression. The two children with lopinavir mutations were among those who did not achieve virologic suppression even after switching to second-line therapy [26].

A South African study also documented the profile of DR in 230 newly diagnosed HIV-infected children. About two-thirds had been exposed to maternal and infant PMTCT ART. Among those who were exposed to PMTCT, 56.8 % had NNRTI, 14.8 % NRTI and 1.3 % PI mutations. DRM to NNTRIs were strongly associated with younger age. One-third of the children had no exposure to PMTCT. However, 24.0 % were detected to have resistance to NNRTI, NRTI in 10.7 % and PI in 1.3 % [27]. Shao et al. [28] carried out a study to determine DRM among children under 18 months born to HIV-1-infected mothers who accessed PMTCT services in northern Tanzania. Twenty-eight percent had genotypic resistance mutations. No major mutations were detected in the protease gene region while all major mutations were found in the reverse transcriptase gene region. The prevalent mutations were Y181C and K103N, thereby imparting resistance to NNRTIs [28]. Mir et al. [29] reported about that two-third of a Pakistani HIV-infected children cohort fulfilled the criteria for treatment failure after median duration of 25 weeks on ARV. Resistance genotyping of these children revealed that all had NNRTI resistance with two of them having high grade NRTI resistance (≥4 thymidine analog mutations) [29]. A prevalence study of DR among Indian HIV-1 infected treatment-naive children reported 7.4 % (2/27) DRM among those who had VL >1000 copies/ml. NNRTI mutations, namely A98G and K103N, were observed in two separate sequences. In addition, three separate HIV-1 protease minor resistance mutations—L10I, A71T and T74S—were also detected [30]. An American paediatric cohort prevalence study showed that almost two-third of the children harboured DRMs to at least one ARV class with DRM prevalence of 54.1, 27.6 and 27.0 % for NRTIs, PIs and NNRTIs, respectively [31].

The prevalence of ARV drug resistance mutations differs across different settings, and this supported some other previous studies [31]. This is also consistent with the evidence that knowledge of patient's virus resistance profile prior to treatment will go a long way to avoid unnecessary stopping of a particular regimen and switching to others regimens. The cohorts showed resistance across the main ARV classes although in varying degrees [15, 18, 22, 24–31].

Preventing Mother-to-Child Transmission of HIV

As a measure to reduce the chance of vertical transmission of HIV infection from mothers to their infants in pregnancy, during delivery and breastfeeding periods, ARVs are administered to the mother/infant pairs. For many years, single dose or daily NVP regimen was the mainstay for the prevention of mother-to-child transmission of HIV (PMTCT) [32, 33]. There are evidences that paediatric patients who are infected despite prophylactic ARVs have higher risk of developing ARV drug resistance mutations (DRMs) [34].

International Maternal Pediatric Adolescent AIDS Clinical Trials P1097 was carried out to determine pharmacokinetics and safety of raltegravir an integrase inhibitor in neonates born to mothers who are HIV-infected and were on raltegravir-based ART during pregnancy. The focus was on the effects of exposure to the drug by the neonates during pregnancy and delivery. The findings showed that sub-optimal concentration levels may lead to inadequate viral suppression and development of raltegravir resistance among the children later on in life [35]. Luo et al. [36] in a study aimed at determining the influence of human leukocyte antigen (HLA) class II genes and to understand the risk/protective factors in perinatal HIV transmission in an ART naïve, mother–child pair cohort during the perinatal period in Kenya. The study also looked at way of developing alternative methods to ARV for the prevention of transmission in the event of development of drug resistance. The findings of the study showed that DRB concordance/discordance between mother–child pairs, DRB1*15:03, DRB3 phenotype of the children and their various interactions play an important role in perinatal HIV transmission [36].

ART regimens used for PMTCT and during perinatal period by both mother and infants should be monitored for drug resistance [36]. Identification of genetic factors linked with susceptibility, protection and risk in perinatal transmission will go a long way in understanding declining antiviral effectiveness of ARV due to development of drug resistance in the children.

Monitoring of HIV Drug Resistance

In monitoring ART treatment response, WHO recommended routine yearly VL testing for children on antiretroviral therapy. Those having 'non-suppressed' VL after using ARVs for at least 6 months can be said to have therapeutic failure which could be due to a number of factors such as ARV resistance, non-adherence to ART or both [37]. Children whose viral loads are not suppressed at retesting can be then be termed as having treatment failure possibly due to ARV drug resistance and this group of patients must be started on the second-line ART [37].

Jobanputra et al. [38] explored factors associated with virological detectability, subsequent virologic failure and also established whether receiving enhanced adherence counselling is associated with likelihood of viral re-suppression in children, adolescents and adults with initial detectable viral load in Swaziland. Children, adolescents, patients with advanced disease and those on ART for longer duration were found to be more likely to have detectable VLs. At retesting, about half of the study population who were children and adolescents were less likely to achieve viral re-suppression. These findings suggested that those who did not re-suppressed are more likely to have resistance mutations [38–40]. A research carried out on children living with HIV in Rhodes Island, USA, reported high HIV-1 diversity with widespread drug resistance among ART-experienced participants [41]. There was a case of resistance transmission from a perinatally infected teen to a newly infected teen via sexual contact. The risk of horizontally transmitted DR is amplified as children living with HIV grow into adulthood and start engaging in sexual activities [41].

Pharmacokinetic data obtained on children from low- and middle-income countries who were on NVP containing regimens combined adherence data using individual pharmacokinetic model and identified that many children were at risk for viral resistance because of insufficient NVP exposure [42]. The impact of ART adherence on sub-therapeutic concentrations of NVP regimens in the long run may likely lead to viral resistance in these children [42]. A multi-centre research conducted in three Latin American countries measured ART adherence levels and assessed the ability of the adherence measures to predict viral suppression in HIV-infected children. Many of the fully adherent children had detectable VLs, perhaps as a result of previous sub-therapeutic concentrations that led to ARV resistance and sub-optimal suppression of HIV replication while some of the children who were non-adherent had undetectable VLs [43]. ART adherence findings showed the association with VL in the study participants. However, this association may not be sufficient to clearly identify non-adherence and those at risk of developing viral resistance [43].

Monitoring of drug resistance is important and useful for planning and developing ARV treatment services. Empowerment and active involvement of local teams in abstraction, analysis and use of these data are considered important next steps.

Diagnosis

In ideal setting, HIV DR testing is recommended at ART initiation of children infected with HIV [37]. The preferred resistance testing is the genotypic testing to guide in the management of ARV treatment-naive patients. The standard genotypic DR testing involves testing for mutations in the reverse transcriptase and protease genes. DR testing is also recommended when managing patients with suboptimal VL reduction and guide in the treatment of those with suboptimal virologic responses or virologic failure while on first or second ARV regimens [37]. Minor variant DRMs to NVP is quite important in situations where LPV/r paediatric formulations are in limited supply, not in use or when NNRTIs are part of second-line regimens [44]. In order to detect minor variant DRMs after NVP exposure as a result of PMTCT prophylaxis, diagnostic test such as allele-specific real-time PCR and oligonucleotide ligation assays (OLA) are employed [44]. However, these methods were limited by mismatches in primer binding [45] and the number of reactions that can be investigated. A newer alternative diagnostic method, known as next-generation sequencing (NGS) with potential for detection of various DRMs across the HIV-1 reverse transcriptase coding region, was used to compare major and minor variant HIV DRMs with both Illumina MiSeq and Life Technologies Ion Personal Genome Machine (Ion PGM) in South African infants [46]. These infants had already failed dual AZT and NVP PMTCT regimen. The study compared bulk sequencing to Ion PGM and MiSeq. Only 13 % NNRTI DRMs (K103N and Y181C) were detected by bulk sequencing, whereas PGM detected 26 % and MiSeq 30 %. Furthermore, NGS allowed detection of extra minor variant DRMs in the children with K103N. The instrument quality and coverage scores were higher with MiSeq, thereby increasing the confidence of minor variant detection [46].

de Mulder et al. [47] evaluated the prevalence of DRMs among 47 American perinatally infected HIV-1 paediatric cohort. The study made use of paired plasma and dried blood spots (DBSs) specimens obtained from the patients. Most of the patients had received combined ART, and it was found that the prevalence of DRM was 54.1, 27.6 and 27.0 % for NRTIs, PIs and NNRTIs, respectively. About two-thirds of this group harboured DRMs to at least one of the main ARV classes and 5.4 % had triple resistance. The plasma and DBS sequences had 97.9 % mean nucleoside similarity [47].

The newer diagnostic method, namely NGS and the use of DBS samples for diagnostic purpose, has been proven to provide reliable data needed for the monitoring of HIV infection among HIV-infected children especially in constrained settings [46, 47].

Treatment Regimen Options

Some HIV-infected children on first-line ART may develop treatment failure due to drug resistance, poor adherence to treatment, poor drug absorption or sub-optimal ART combination. Establishment of treatment failure signifies the need to switch to second-line ART regimen. The switching may involve just one drug or more among the combination ART regimens.

WHO recommended the use of efavirenz (EFV) as one preferred NNRTI for the first-line treatment of HIV-1 infection from children above 3 years [2]. The minimal interactions of EFV with antituberculous drugs in HIV/TB co-infected patients make it the NNRTI of choice for this category of patients [48]. However, EFV has a limitation because it is susceptible to single point mutation in the reverse transcriptase enzyme, thereby resulting in a high-level DR against HIV-1 virus [49]. Reverse transcriptase genotypes and NNRTI resistance mutations such as K101E and G190S are resistant to EFV and may develop while a patient is failing on EFV-containing regimens [49]. Larru et al. [50] found that newer NNRTI, namely etravirine (ETV) and rilpivirine (RPV) which were designed primarily to overcome NNRTI resistance mutations that affect other first-line choices, may be a more effective alternative as part of a second-line regime especially after development of resistance to EFV [50]. However, ETV use in children is presently limited to those aged 6 years and above while RPV is not yet recommended for paediatric use [50]. Some studies on the newer NNRTIs showed that about half of NNRTI pre-treated children also had significant ETV resistance [51].

PI-based ART was recommended for children who were vertically infected with HIV after failing on NVP prophylaxis during pregnancy and breastfeeding periods [52]. Development of PI resistance in the same patient with established NNRTI resistance limits the choices that are available for the inevitable long-term treatment options in HIV-infected children [25]. The Children with HIV Early Antiretroviral (CHER) study looked at children who had viraemia while on PI-based regimens and were characterised as having multi-class drug resistance by the use of single genome sequencing [25]. The study showed that initiating PI-based ART immediately after child delivery as a follow-up to single dose NVP prophylaxis can lead to development of resistance to other classes of ARVs such as PIs and NRTIs. This finding brings up the issue of potential future therapeutic challenge for children who were infected via mother-to-child transmission and were exposed to single NVP doses. It also poses a big challenge to children who are in limited resource settings where ART choices are very few [53]. Extensive triple-class drug-resistant mutations (TC-DRM) in HIV-infected paediatric patients often results to phenotypic or genotypic resistance in at least one drug from all the three main classes of ARVs [54]. Rojas Sanchez et al. [55] reported the virologic and clinical follow-up of a Spanish paediatric cohort after the detection of selected triple-class drug-resistant viruses. About one-fourth of the HIV-1 infected children and adolescents with resistance data had TC-DRM and were found to be extensively exposed to the three main classes of ARVs. Most of these patients were exposed to sub-optimal concentrations and inadequate doses. Majority of these children and adolescents were previously on monotherapy or dual therapies and subsequently developed treatment failure as a result of incomplete viral suppression. There was reduction in the antiretroviral efficacy after DRM selection [55]. However, with the use of newer ARVs in the treatment of the children such as enfuvirtide, etravirine, atazanavir, darunavir, fosamprenavir, tipranavir, raltegravir and maraviroc, a reduction in the number of children carrying TC-DRM was noted. DRMs can revert with newer and potent ARV regimens in children with TC-DRM, thereby leading to susceptibility to formerly affected ARVs [55].

There is a need to encourage research in developing more suitable alternative regimen in case of development of drug resistance as seen in the usage of EFV. As it is already a practice to initiate most infants on LPV/r containing combination in most resource-limited settings where resistance testing is not possible, the use of EFV may become less preferred due to the issue of resistance development and concern about toxicity during pregnancy [2, 55].

Conclusions

From public health perspective, ARV drug resistance remains a limiting factor in the effective management of HIV infection in children. This review is instructive for paediatricians engaged in the clinical management of HIV-infected children who are at risk of developing DR. It is also informative for health professionals and researchers who are involved in treatment guideline development and policy making especially in the area of ARV treatment in children and adolescents. Categorisation of ART in children needs to prioritise the issue of DRMs resulting from exposure to ARVs during pregnancy and breastfeeding period as part of PMTCT intervention and as part of day-to-day therapy during childhood. Extra efforts should be taken to address adherence issues, develop routine adherence monitoring and take into account the children's age and disease status in order to avoid making unsuitable switch to second-line therapy especially when interpreting VL. In

settings where feasible, consistent VL measurements and resistance genotyping are advised when dealing with suspected DR cases [38, 42]. Newly developed diagnostic methods are platforms for improved quality of detections especially for minor variant DRMs [46]. In limited resource settings, DBS specimens are also reliable just as plasma specimens for DRMs diagnosis in infants [47]. There should be prioritisation of resources for the screening of ART experienced and naïve children for HIV DRM genes, before and after initiation of ART in order to achieve the desirable efficacy and treatment outcomes [19]. It should be noted that the use of PMTCT history to rule out ART pre-initiation DR is not reliable because many HIV-infected children without PMTCT history do harbour DRMs. For children who had history of PMTCT and were infected, NNRTI mutations remain the most prevalent. Most adherent patients that had treatment failures tend to have underlined DRMs [27]. There is also need to do further work on developing alternative prevention and treatment methods peradventure there are increased cases of DRMs taking into account scarcity of resources and limited ARV choices for those readily failing on second-line regimens [18, 36].

Compliance with Ethics Guidelines

Conflict of Interest Olatunji Adetokunboh, Oluyemi Atibioke, Tolulope Balogun and Mojisola Oluwasanu declare no conflict of interest.

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