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Determinants of Virologic Failure and Prevalence of Resistance Mutation among HIV Infected Children on Antiretroviral Therapy at University Teaching Hospital, Lusaka, Zambia

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Abstract: ***Introduction: Viral suppression among HIV infected children is estimated at 54.3% compared to 95% UNAIDS target for viral suppression. This implies that 45.7% of children have virologic failure. An assessment of 2018 viral load results indicated a 21% cumulative incidence of virologic failure at Paediatrics Center of Excellence, University Teaching Hospital (PCOE-UTH). We aimed to investigate the determinants of virologic failure and prevalence of antiretroviral (ARV) drug resistance mutation among HIV infected children on antiretroviral therapy at PCOE-UTH, Lusaka. Methods: Retrospective cohort data was extracted from SmartCare Electronic Data Management System generated between January 2016 and December 2018, for children aged between 18 months and 14 years with valid record of viral load results at PCOE-UTH. Analytical cross sectional design was used for this study. A stepwise multivariable logistic regression was performed to identify determinants of virologic failure in children. All analyses were done using Stata software version 14.0 (Stata Corporation, College Station, Texas, USA). Results: Out of 415 participants, 91 [21.9%] had virologic failure [>1000 copies/ml]. Prevalence of ARV drug resistance mutation as sequelae of virologic failure was 16 [17.6%, CI: 10.4%−26.9%]. The 5 – 9 years age group had 2.3 times the odds of developing virologic failure compared to 18 months to 4 years age group [AOR= 2.3; 95% CI 1.06−5.88; P= 0.037]. Household income >k3000 showed a protective effect against development of virologic failure [AOR= 0.48; 95% CI 0.23−0.99; P= 0.047]. Children on Nucleoside Reverse Transcriptase Inhibitors and Protease Inhibitor (NRTI+PI)antiretroviral combination had 2.6 times the odds of developing virologic failure compared to Nucleoside Reverse Transcriptase Inhibitor an Integrase Strand Transfer Inhibitor (NRTI+INSTI) antiretroviral combination [AOR= 2.6; 95% CI 1.27−5.14; P= 0.009]. Conclusion: Virologic failure and antiretroviral resistance mutation are still evident despite initiation of antiretroviral therapy among HIV infected children at University Teaching Hospital. Determinants such as household income, adherence to antiretroviral therapy and antiretroviral drug combination were associated with virologic failure. Assessment of children with virologic failure showed evidence of resistant mutation to Nucleoside Reverse Transcriptase Inhibitors (NRTI) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI). Therefore, guidelines for HIV management in children may need to address highlighted socio-economic and clinical related factors besides testing for drug sensitivity for children with antiretroviral pre-exposure before antiretroviral therapy initiation***

Keywords: Antiretroviral therapy, ARV drug resistance mutation, Determinants, Paediatrics HIV, Virologic failure

1. Introduction

Antiretroviral (ARV) treatment failure is progression of disease after initiation of antiretroviral therapy due to failure of medication to control Human Immuno-deficiency Virus (HIV) infection. Treatment failure in HIV takes the following forms: clinical failure, immunological failure, virologic failure and a combination of the three (Zambia-MOH, 2018). Virologic failure may result from drug resistance, drug toxicity and sub-optimal adherence to treatment. HIV drug resistance mutation is a crucial determinant of antiretroviral therapy outcome. Studies have demonstrated that infants exposed to antiretroviral medication perinatally had increased chances of developing HIV drug resistance and treatment failure. According to a study, comparing infants with prior exposure to ARVs against those not exposed, the prevalence of HIV drug resistance was 21.6% and 8.3% in the respective groups. Similarly, the results from a five-year-long study observing the efficacy of treatment in Zambia found that 40% of infants diagnosed with HIV in Lusaka had developed resistance to at least one ARV drug by 2014 compared to 21.5% in 2009 (WHO, 2017). These studies suggested that virologic failure was a problem among HIV infected children on antiretroviral therapy.

The goal of antiretroviral therapy is to suppress HIV replication to a level below risks for drug resistance and mutation. In Zambia, about 92.3% of HIV infected children aged 0 to 14 years initiated combination antiretroviral therapy (cART) to attain viral suppression. Despite the interventions, the proportion of virologic failure has remained high. In Zambia, viral load suppression in children was about 54.3% compared to 95% UNAIDS target for viral suppression. This implies that 45.7% of children have virologic failure. Furthermore, an assessment of 2018 viral load results indicated a 21% cumulative incidence of virologic failure at Paediatrics Center of Excellence, University Teaching Hospital (PCOE-UTH, 2019). These children with failure have a high risk of HIV related morbidity and mortality that would in turn deny them a chance to perform daily activities and live to adulthood. Furthermore, children with non-suppressed viral load can easily transmit HIV infection to other susceptible individuals, besides developing HIV drug resistance compromising subsequent regimens (Boender et al., 2016).We aimed to investigate the determinants of virologic failure and prevalence of ARV drug resistance mutation among HIV infected children aged 18 months to 14 years at the University Teaching hospital, Lusaka, Zambia.

2. Material and Method

The study design was analytical cross sectional, analyzing data between January 2016 and December 2018 of children who had filed viral load results. We extracted data from SmartCare database and patient's ART medical records for children aged 18 months to 14 years. We extracted data generated between January 2016 and December 2018 using data extraction checklist. The researcher checked for data completeness and accuracy on Microsoft Excel sheet after importation from epi-info software version 7.2.2.6. We coded the variables systematically for efficient electronic analysis in Stata version 14 (Stata Corporation, College Station, Texas, USA).

Statistical analysis

Categorical characteristics such as virologic failure and CD4 count were presented as numbers and percentage. For the crude assessment of association, we used Chi-squared or Fisher's exact test depending on expected frequency values in all the cells (Chi-squared assumption). The researcher repeated this process for variables including immunologic status, adherence, age, WHO clinical staging, history of tuberculosis.

For continuous variables such as time on ART, time lag between diagnosis and treatment initiation, we tested for normal distribution using the Shapiro Wilk test and histogram. We presented data using mean and standard deviation for normally distributed data or alternatively the median and interquartile range for skewed data. To check for a difference in the means/median of continuous variables stratified by viral load outcome, we used either t-test or Wilcoxon rank sum/Manny-Whitney depending on distribution.

For inferential statistical analysis, we used Logistic regression model to determine predictors of virologic failure while adjusting for other factors. We adopted a backwards stepwise investigator led approach to select the best predictors of virologic failure. Variables with the least contribution basing on the threshold p-value >0.05 were dropped; only statistically significant predictors remained in the model.

Study outcome

The outcome variable for the study was Viral load outcome (Viral suppression: <1000 copies/ml or virologic failure: ≥1000 copies/ml).

3. Results

Participants' Baseline Characteristics

We aimed to investigate determinants of virologic failure and prevalence of drug resistance mutation among HIV infected children on antiretroviral therapy at Paediatrics Center of Excellence, University Teaching Hospital. The data depicts virologic failure of HIV infected children from January 2016 to December 2018. Overall, the proportion of virologic failure among HIV Infected children receiving antiretroviral therapy at UTH between January 2016 and December 2018 was 21.9 % and the prevalence of ARV drug resistance mutation as a sequela of virologic failure was 16 [17.6%, CI: 10.4−26.9]. Resistance mutation to ARV medication was highest among the 5 years to 9 years children standing at 9 [56%]. About 10 [63%] of resistance mutation was associated with Nucleoside Reverse Transcriptase Inhibitors (NRTI) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) ARV class combination.

Four hundred and fifteen (415) participants were included in the study, and table 1.1 showed the cross tabulation of characteristics of the participants. At univariable analysis participants' characteristics such as adherence to antiretroviral medication [p-value=0.001], Antiretroviral (ARV) class combination [p-value =0.020], baseline CD4 count [p-value =0.035], WHO clinical staging [p-value =0.007] and household income [p-value =0.016] showed a significant difference between virally suppressed and virologic failure children. Whereas characteristics such as age, place of residence, caretaker's occupation, weight of child, time lag between diagnosis and initiation, history of TB and gender showed no significant difference between virally suppressed and virologic failure children.

Table 1.1: Baseline Characteristics of participants who were receiving antiretroviral therapy with viral load results at Paediatrics Centre of Excellence UTH between January 2016 and December 2018: *Univariable comparison*

Characteristic	Viral Suppression (n=324)	Virologic Failure (n=91)	P-value
Age, Number (%)			
18 Months to 4 years	71 (22%)	17 (18%)	0.800[P]
5 years to 9 years	126 (39%)	37 (41%)	
10 years to 14 years	127 (39%)	37 (41%)	
Gender, Number (%)			
Female	152 (47%)	43 (47%)	0.954[P]
Male	172 (53%)	48 (53%)	
Household income, Number (%)			
Less than K3000	202 (65%)	68 (79%)	0.016[P]
More than K3000	107 (34%)	18 (21%)	
WHO Clinical stage, Number (%)			
Stage I & II	272 (84%)	65 (71%)	0.007[P]
Stage III & IV	52 (16%)	26 (29%)	
CD_4 Count, Number (%)			
Less than 350 cells/µl	63 (19%)	29 (32%)	0.035[P]
More than 350 cells/µl	261 (81%)	62 (68%)	
ARV class combination, Number (%)			

Characteristic	Viral Suppression (n=324)	Virologic Failure (n=91)	P-value
NRTI+NNRTI	112 (35%)	26 (29%)	0.020[E]
NRTI+PI	99 (30%)	42 (46%)	
NRTI+INSTI	113 (35%)	23 (25%)	
Duration on ART in months, Median (IQR)	35 (21, 49.5)	33 (20, 46)	0.324[M]
Adherence to ART, Number (%)			
No	21 (6%)	18 (20%)	0.001[P]
Yes	303 (94%)	73 (80%)	

E: Fisher's Exact, **INSTI**: Integrase Strand Transfer Inhibitor, **M**: Mann-Whitney, **NRTI**: Nucleoside Reverse Transcriptase Inhibitor, **NNRTI**: Non-Nucleoside Reverse Transcriptase Inhibitor, **P**: Pearson Chi-squared, **PI**: Protease Inhibitor.

Determinants Associated with Virologic Failure

Table 1.2 showed logistic regression results at crude and adjusted levels of socio-economic determinants. After adjusting for other factors like gender, caretaker's occupation, place of residence and household income, the age group of child was associated with virologic failure. The odds of developing virologic failure was 2.3 times in the age group 5 years to 9 years [AOR=2.3; 95% CI 1.06−5.8; P=0.037] compared to the age group 18 months to 4 years.

Household income was another socio-economic determinant associated with virologic failure. Household income above K3000 reduced the chance of developing virologic failure by 52% [AOR=0.48; 95% CI 0.23−0.99; P=0.047] compared to household income less than K3000 while adjusting for other factors like age, gender, place of residence, caretaker's occupation and disclosure of HIV status to the child.

Table 1.2 showed logistic regression results for clinical related determinants at crude and adjusted levels. Weight of a child was significantly associated with virologic failure; a unit increase in child's weight (kilograms) predicted less chance of developing virologic failure [AOR=0.95; 95% CI 0.91−0.99; P=0.022], while adjusting for other clinical determinants like duration on ART, adherence, WHO clinical stage and baseline CD_4 count. Adherence to intake of ARV medication reduced the chance to develop virologic failure by 74% [AOR=0.26; 95% CI 0.12−0.53; P<0.001] compared to non-adherence to intake of ARV medication, while accounting for other factors such as ART class combination, duration on ART, WHO clinical staging, baseline CD_4 count and time lag between diagnosis and initiation.

Baseline immunological status marked by CD_4 cell count was associated with virological failure [AOR= 0.38; 95% CI 0.21−0.67, P= 0.032]. Baseline CD_4 count above 350 cells/ml predicted a reduced chance of developing virologic failure by 62%, while adjusting for other determinants like ART class combination, duration on ART, WHO clinical staging, adherence and time lag between diagnosis and initiation. Nucleoside Reverse Transcriptase Inhibitors and Integrase Strand Transfer Inhibitor combination showed a decreased chance of developing virologic failure [AOR=0.80; 95% CI 0.40−1.61; P=0.536], while accounting for other determinants like duration on ART, adherence, WHO clinical staging, baseline CD_4 count and time lag between diagnosis and initiation.

Table 1.2: Unadjusted and adjusted Determinants of Virologic failure

	Crude			Adjusted		
Determinant	**Odds ratio**	**95% CI**	**P-value**	**Odds ratio**	**95% CI**	**P-value**
Age of child						
18 months to 4 years	Ref	n/a	n/a	Ref	n/a	n/a
5 years to 9 years	1.22	0.62−2.33	0.534	2.29	1.06−5.88	0.037
10 years to 14 years	1.21	0.64−2.31	0.550	2.50	0.66−9.42	0.175
Gender						
Female	Ref	n/a	n/a	Ref	n/a	n/a
Male	0.99	0.62−1.57	0.954	1.19	0.70−2.02	0.514
Household income						
Less than K3000	Ref	n/a	n/a	Ref	n/a	n/a
More than K3000	0.53	0.31−0.92	0.024	0.48	0.23−0.99	0.047
Disclosure of HIV status to child						
n/a	Ref	n/a	n/a	Ref	n/a	n/a
No	1.15	0.64−2.06	0.636	1.53	0.65−3.58	0.331
Yes	1.31	0.76−2.27	0.326	2.53	0.81−7.91	0.109
Place of residence						
High-density pop.	Ref	n/a	n/a	Ref	n/a	n/a
Medium density pop.	0.66	0.41−1.09	0.105	0.92	0.49−1.74	0.811
Low-density pop.	0.92	0.25−3.45	0.902	1.67	0.34−8.33	0.529
Time-lag between diagnosis and treatment initiation (Weeks)	0.99	0.99−1.00	0.653	0.99	0.99−1.00	0.342
Weight of a child (Kg)	0.99	0.97−1.01	0.523	0.95	0.91−0.99	0.022
Adherence to ART						

INSTI: Integrase Strand Transfer Inhibitor, **NRTI**: Nucleoside Reverse Transcriptase Inhibitor, **NNRTI**: Non-Nucleoside Reverse Transcriptase Inhibitor, **PI**: Protease Inhibitor, **Ref**: Reference group, **n/a**: not applicable

Crude				Adjusted		
Determinant	**Odds ratio**	**95% CI**	**P-value**	**Odds ratio**	**95% CI**	**P-value**
No	Ref	n/a	n/a	Ref	n/a	n/a
Yes	0.28	0.14−0.55	<0.001	0.21	0.01−0.45	<0.001
WHO clinical staging						
Stage I&II	Ref	n/a	n/a	Ref	n/a	n/a
Stage III&IV	2.09	1.22−3.60	0.008	2.50	1.34−4.58	0.004
Baseline CD_4 count						
Less than 350 cells/µl	Ref	n/a	n/a	Ref	n/a	n/a
More than 350 cells/µl	0.51	0.31−0.87	0.013	0.51	0.28−0.94	0.032
ARV class combination						
NRTI+NNRTI	Ref	n/a	n/a	Ref	n/a	n/a
NRTI+PI	1.83	1.05−3.19	0.034	2.55	1.27−5.14	0.009
NRTI+INSTI	0.88	0.47−1.63	0.677	0.80	0.40−1.61	0.536

INSTI: Integrase Strand Transfer Inhibitor, **NRTI**: Nucleoside Reverse Transcriptase Inhibitor, **NNRTI**: Non-Nucleoside Reverse Transcriptase Inhibitor, **PI**: Protease Inhibitor, **Ref**: Reference group, **n/a**: not applicable

4. Discussion

We found that resistance mutation to antiretroviral medication was highest among the 5 years to 9 years children. This age group also had a majority of children with virologic failure. Much of resistance mutation was associated with Nucleoside Reverse Transcriptase Inhibitors (NRTI) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) antiretroviral class combination. This could be attributed to common usage of the above antiretroviral combination in Elimination of Mother-to-Child Transmission (EMTCT) interventions. These findings were similar to a study done at HIV clinic at Hasbro Children's Hospital in Rhode Island, where children developed virological failure coupled with NNRTI resistance mutation. In the Rhode Island study, Protease Inhibitors (PI) resistance mutation was also evident. Most children with drug resistance were younger with history of perinatal HIV infection (Rogo et al., 2015).Similarly, a study done in Uganda the prevalence of resistance mutation among children less than 3 years was documented with NRTI and NNRTI mutation detected among naïve patients and EMTCT exposed children (Kityo et al., 2016).

Our findings show an association between virologic failure and household income. A child coming from a home with a household income equal and above K3000 had reduced chance of developing virologic failure. The findings predicted that children coming from financially stable homes had a reduced chance of failing on antiretroviral therapy, and the opposite in terms of financial stability predicted a higher chance of virologic failure. This could be as result of accessibility to material requirements and desirable health service by a child. Flynn et al. (2017), found the exact opposite of our findings. In his study household income above US $1 per day was associated with higher risk of virologic failure among clients on ART in Uganda.

We found that children on Nucleoside Reverse Transcriptase Inhibitors (NRTI), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) and Protease Inhibitor (PI) had increased chance of developing virologic failure compared to their counterparts on Nucleoside Reverse Transcriptase Inhibitors (NRTI) and Integrase Strand Transfer Inhibitor (INSTI) combination. This was consistent with findings on resistance mutation that was also highest in NRTI and NNRTI ARV class combination. NRTI and INSTI combination was superior in suppression of viral load. Similarly, a study conducted at Regional Referral Centre for Paediatrics HIV of the University of Naples Federico II-Italy, found considerable viral suppression following use of INSTI (Dolutegravir) in adolescents between 12 and 18 years (Bruzzese et al., 2018).

The explanation to virologic failure for children on NRTI, NNRTI and PI class combination could be due to prolonged exposure to these drug classes among children from a younger age and possible acquisition of pre-treatment drug resistant virus to NRTI and NNRTI that has been a backbone in ART regimen. This could be the case among children whose mother could have used this regimen during antenatal. The other point could be due to unpleasant taste associated with PI based drugs (Lopinavir/ritonavir) contributing to irregularities in intake of medication. In one clinical trial conducted in Ouagadougou and Abidjan West Africa, the findings revealed drug resistance to Lamivudine with mutation to other Non-Nucleoside Reverse Transcriptase Inhibitors. Resistance mutation was also evident to Zidovudine and Lopinavir. On the contrary, the study established that more clients on Protease Inhibitor based regimen achieved viral suppression after 12 months on medication despite the minor recorded of resistance to Lopinavir (Amani-Bosse et al., 2017).

Baseline WHO clinical stage and CD_4 count were significantly associated with virologic failure. Baseline clinical stage III and IV predicted a high chance of developing virologic failure at later time while on treatment. Similarly, CD_4 count above 350 cells/µl predicted reduced chance of developing failure signifying the importance of having a competent immune system in order to achieve viral suppression. This evidence emphasizes the significance of monitoring the clinical and immunological parameters at regular clinical interaction with HIV infected children receiving antiretroviral therapy.

Costenaro et al. (2015), found that HIV infected children with TB and those with WHO stage IV defining illness were significantly more likely to experience treatment failure compared to children with WHO stage III disease without TB. Furthermore, starting cART with an unconventional regimen (not containing an NRTI backbone in combination with EFV, NVP, lopinavir/ritonavir (LPV/r), or ABC) was also significantly associated with risk of treatment failure.

The findings of CD_4 count above 350 cells/µl, and WHO clinical stage I/II reducing the chance of virologic failure were consistent with findings of a study conducted in the Myanmar regions Southeast Asia. The study reported that patients with severe to moderate immune suppression with CD_4 count less than 350 cells/µl and those with stage III and IV had a higher risk of developing virologic failure (Kyaw et al., 2017). It was evident that CD_4 count had an inverse relationship with viral replication although not in all the patients. The rate of viral replication was highest in a host with compromised immunity (Bayu et al., 2017).

We found that adherence to antiretroviral therapy is an important factor with huge bearing on HIV treatment outcome. Children not adhering to ARV medication had a higher chance of developing virologic failure compared to their counterparts who were adherent. These findings were consistent with results at University of Gondar Referral Hospital in Ethiopia. The study predicted that poor medication adherence were 16 times as likely to develop virologic failure. In other studies it was documented that poor medication adherence was the main risk factor for virologic failure. Missing three (3) doses per week was associated with an increased risk of drug resistance and reduced immunity (Bayu et al., 2017).

5. Conclusion

Virologic failure and antiretroviral resistance mutation are still evident despite initiation of antiretroviral therapy among HIV infected children at University Teaching Hospital. Determinants such as: household income, adherence to antiretroviral therapy, clinical and immunologic status and antiretroviral drug combination were associated with virologic failure. Assessment of children with virologic failure showed evidence of resistant mutation to Nucleoside Reverse Transcriptase Inhibitors (NRTI) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI).Therefore, guidelines for HIV management in children, may need to address highlighted socio-economic and clinical related factors besides testing for drug sensitivity for children with antiretroviral pre-exposure before initiation of antiretroviral therapy.

6. Limitations

The study data used was generated from medical routine visits with varying clinicians, who would not have had adhered to the same rigorous quality control standards involved in history taking and medical examination. This was a source of information bias. The study used sample size based on available data at the time of the study as such; the sample size was small despite doing a complete enumeration. The other limitation was non-availability of resistance mutation results for children who manifested with virologic failure.

7. Ethical issues

During the study we extraction of information on HIV infected children accessing ART services at University Teaching Hospital, this process involved invasion of children's privacy. In order to uphold respect for persons involved in the study, we sought for permission from the hospital authorities. We upheld to confidentiality by de-identifying records on extraction of data for patients to promote privacy. We also sought for permission from the Institutional Research Board (IRB)/UNZABREC to conduct the study.

References

[1] Amani-Bosse, C,. et al. 2017. Virological response and resistances over 12 months among HIV-infected children less than two years receiving first-line lopinavir/ritonavir-based antiretroviral therapy in Cote d'Ivoire and Burkina Faso: the MONOD ANRS 12206 cohort. *J Int AIDS Soc*, 20**,** 21362.

[2] Bayu, B., et al. 2017. Determinants of virological failure among patients on highly active antiretroviral therapy in University of Gondar Referral Hospital, Northwest Ethiopia: a case-control study. *HIV AIDS (Auckl)*, 9**,** 153-159.

[3] Boender, T. S., et al. 2016. Accumulation of HIV-1 drug resistance after continued virological failure on first-line ART in adults and children in sub-Saharan Africa. *J Antimicrob Chemother*, 71**,** 2918-27.

[4] Bruzzese, E., et al. 2018. Dolutegravir-based anti-retroviral therapy is effective and safe in HIV-infected paediatric patients. *Ital J Pediatr*, 44**,** 37.

[5] Costenaro, P., et al. 2015. Predictors of Treatment Failure in HIV-Positive Children Receiving Combination Antiretroviral Therapy: Cohort Data From Mozambique and Uganda. *J Pediatric Infect Dis Soc*, 4**,** 39-48.

[6] Flynn, A. G., et al. 2017. Socioeconomic position and ten-year survival and virologic outcomes in a Ugandan HIV cohort receiving antiretroviral therapy. *PLoS One*, 12**,** e0189055.

[7] Ginwalla, R., et al. (2012). "Prevalence of clinical, immunological and irological failure among children on Haart at the university teaching hospital, Lusaka, Zambia." Medical Journal of Zambia 39 (3): 1-5.

[8] Kazooba, P., Billy Nsubuga Mayanja, Jonathan Levin, Ben Masiira, Pontiano Kaleebu (2018). "Virological failure on first-line antiretroviral therapy; associated factors and a pragmatic approach for switching to second line therapy–evidence from a prospective cohort study in ruralSouth-Western Uganda, 2004-2011." Pan African Medical Journal. 2018;29:191. doi:10.11604/pamj.2018.29.191.11940.

[9] Kyaw, N. T., et al. 2017. High rate of virological failure and low rate of switching to second-line treatment among adolescents and adults living with HIV on first-line ART in Myanmar, 2005-2015. *PLoS One*, 12**,** e0171780.

[10] Rogo, T., Delong, A. K., Chan, P. & Kantor, R. 2015. Antiretroviral treatment failure, drug resistance, and subtype diversity in the only pediatric HIV clinic in Rhode Island. *Clin Infect Dis*, 60**,** 1426-35.

[11] Sisay, M. M., et al. (2018). "Incidence and risk factors of first-line antiretroviral treatment failure among human immunodeficiency virus-infected children in Amhara regional state, Ethiopia: a retrospective follow-up study." BMJ Open 8 (4): e019181.

[12] Suaysod, R., et al. (2015). "Treatment Failure in HIV-Infected Children on Second-line Protease Inhibitor-Based Antiretroviral Therapy." Clin Infect Dis 61 (1): 95-101.
[13] Tran, B. X., et al. (2016). "Impact of Socioeconomic Inequality on Access, Adherence, and Outcomes of Antiretroviral Treatment Services for People Living with HIV/AIDS in Vietnam." PLoS One 11 (12): e0168687.
[14] UNAIDS (2019). "Global AIDS monitoring 2019: Indicators for monitoring the 2016 United Nations Political Declaration on HIV and AIDS." (UNAIDS (2019) Global AIDS monitoring 2019: Indicators for monitoring the 2016 United Nations Political Declaration on HIV and AIDS. UNAIDS, Geneva.).
[15] WHO 2017. 2017 HIV drug resistance report.
[16] ZAMBIA-MOH 2018. Zambia Consolidated Guidelines for Prevention and Treatment of HIV Infection.
[17] ZAMPHIA (2016). Zambia Population-based HIV Impact Assessment 2016. Lusaka, Ministry of health. 1.