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Genetic Diversity and Drug Resistance Patterns Among of HIV-1 Positive Youth with Non-Suppressed Viral Load in South Rift Valley, Kenya

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Abstract*: Background: Despite the benefits of Antiretroviral Therapy, treatment failures among the youths have been extensively reported due to non-adherence of the drug regime and viral mutations leading to drug resistant variants and data on Genetic Diversity and Drug Resistance Patterns among the youth living with HIV remains scarce. In this study, we describe the genetic diversity and drug resistance patterns among HIV-1 positive youths with non-suppressed viral load from south Rift valley region, Kenya. Objective: In this study, analyzed genetic diversity and drug resistance mutation among the youth aged 15-24 years in south rift valley region, Kenya. Methods: We obtained remnant plasma samples from the ongoing the routine viral load study and amplified HIV-1 Protease, Reverse Transcriptase and Integrase genes, using HIV-1 genotyping kit with integrase. Genotyped by Sanger sequencing and assessed HIVDR mutations using the Stanford HIV drug resistance database and HIV subtype by REGA tool. Results: From 120 samples identified, mean age was 20 years with SD of 2.6. The TDF+3TC+DTG regimen was 89.2% across the age groups, median duration on ART was >6 months to 2 years. Obtained sequenced data on 103/120 (85%) on which 88 on PRRT and 86 on Integrase. The most common DRMs were; M184V (31.4%), K103N (20.9%), G190A (15.1%), M41L (9.3%), E138A (9.3%), K101E (7.0%), Y181C (5.8%), G118R (5.7%), M46I(4.7%). The Predominance of HIV subtype A1(84.1%) circulating among the youths. Conclusion: Our study shows that HIV-subtype A1 is the dominant circulating forms among the youths and TDF+3TC+DTG (dolutegravir-containing) antiretroviral regimens will be effective in the youths. What this study adds: There is need to closely monitor profile HIV drug resistance patterns in youths from the south rift valley region, Kenya with confirmed virologic failure.

***Keywords*: Kenya, HIV-1, Genetic Diversity, Drug Resistance, Non-suppression.**

1. Introduction

HIV-1 is a human immunodeficiency virus (HIV), which has three enzymes, Integrase (IN), Protease (PR) and Reverse-transcriptase (RT) [1]. There are four phylogenetic groups of HIV-1, these are the groups M (major), O (outlier), N (non-M/non-O). The group M is majorly responsible for the global epidemic, includes more than 95% of globally sequenced HIV-1 samples and can be further classified into nine different subtypes (A–D, F–H, J, K), six A (A1–A6), and two F (F1 and F2) sub-subtypes together with 102 circulating recombinant forms. Genetic diversity has been strongly correlated with drug resistance pattern of HIV-1 strain [2]. In Northern Brazil, Transmitted Drug Resistant (TDR) HIV-1 strain has impacted negatively on the outcomes of first line of treatment with anti-retroviral therapy and failures of Highly Active Anti-Retroviral Therapy (HAART) among HIV-1 patients [3]. A phylogenetic tree is a graphic representation of the evolutionary relationships of species, and the phylogenetic distances among the species reflect the closeness of evolutionary relationships [4].

A study in Nigeria associated drug resistance of HIV-1 against common anti-retroviral therapy regimen to mutations in Integrase Strand Transfer Inhibitor (INSTI) and Circulating Recombinant Forms (CRT02) of the virus [5]. In many countries of Sub-Saharan Africa (SSA), high levels of pretreatment drug resistance to nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) have led, in 2018, the World Health Organization (WHO) to recommend a transition to dolutegravir (DTG)—an integrase strand transfer inhibitor (INSTI) with a strong resistance barrier—as a first-line ART component in resource-limited settings [5]. INSTIs, targeting the integrase (IN) enzyme crucial for HIV replication, have shown high efficacy in treatment-naïve and -experienced individuals [6]. INSTIs are now being recommended worldwide in most countries as a first-line treatment for people living with HIV (WHO 2021). Several clinical studies have shown INSTIs to be superior to NNRTI and PI comparative treatment groups [7]. Clinical trials have shown that INSTIs have excellent efficacy and favorable safety profiles compared to other ARV classes [6]. For second generation INSTIs, amino acid substitutions E92Q, G118R, S153FY, G193E, R263K decrease DTG susceptibility two- to four-fold. M50I tends to be selected in vitro by DTG and BIC in combination with R263K, contributing to reduced DTG susceptibility [8]. In Ethiopia, a study to ascertain the relationship between genetic diversity and drug resistance among HIV-1 patients on various anti-retroviral therapy regimes revealed that, 85.4% of all subjects sampled

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exhibited at least one type of Acquired Drug Resistance (ADR) mutations [9]. It was further reported that, 81% and 48.8% of all study subjects displayed resistance against Nucleoside Reverse Transcription Inhibitor (NRTI) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) respectively [10]. Previous studies have pointed out a few NRTI and NNRTI associated mutations that are responsible for drug resistance of HIV-1 among patients undergoing anti-retroviral treatment. One of the major obstacles in achieving the long-term efficacy of ART is the development of resistance [11]. In resource-limited settings, adherence is a major contributing factor to development of drug resistance and ultimately treatment failure [12]. In China, a study to determine the differences between HIV-1 drug resistance among different genotypes of the virus identified NRTI related mutants to be 184VF, 65R, Y115F, K70E, Q41L, D67N, K219N while those associated with NNRTI were E138AG, V179ED, K101EQ, 106MI, Y181C, G190A, K103N and 227L [13]. Genetic diversity of HIV-1 in Kenya has been widely reported. For instance, a cross-sectional study conducted in Teso, Western Kenya to analyze genetic diversity of HIV-1 among patients on anti-retroviral therapy reported 68% of subtype A1, 12.7% of D8 and inter-subtype recombinants A1-D3 and A1-B2 at 4.8% and 3.2% respectively [14]. In A separate study to establish prevalence and factors associated with HIV-1drug resistance mutations among treatment experienced patients in Nairobi, Kenya, NRTI related mutations, M184VI & K65REN and those of NNRTI, K103NS, G190A and Y181C were reported [15]. At the Kenyan coast, the observed prevalence of VNS among YLWH aged 18–24 years is higher than the 25% prevalence estimate reported by among the general adult population living with HIV [16]. To date, the number of studies on genetic diversity and drug resistance pattern of HIV-1 among Kenya youth undergoing anti-retroviral treatment are scarce. In this study we report a cross-sectional analysis of genetic diversity and drug resistance pattern of HIV-1 among youth of different age groups under Anti-Retroviral Therapy in the South Rift region of Kenya.

2. Methods

A. Design and Location of the Study

The study will utilize a cross-sectional study design “protocol” from the ongoing National AIDS & STI Control Program, Ministry of Health (MOH), supported by the President's Emergency Plan for AIDS Relief (PEPFAR). The study will be using the remnant coded plasma samples that will be selected on a real-time basis, of a viral load of >1000 copies/ml by the Abbott Real-Time HIV-1 m2000rt quantitative assay conducted at Walter Reed Project laboratory in Kericho and where results have been dispatched through the NASCOP database to facilities. The study will run for one year.

B. Study Population

The plasma remnant samples were identified from the youths aged between 15 – 24 years enrolled in the NASCOP for HIV-1 viral load monitoring and all ages are enrolled.

C. Sample Size and Sampling Technique

The sample size for this study was calculated using cochrans formular for sample size determination, a confidence level of 95%and a precision level of 5%. The sample size of 118 [16].

1) Extraction of DNA

Proviral DNA was extracted from each plasma sample using PureLink™ Viral RNA/DNA Mini Kit (Invitrogen by life technologies®). The HIV-1 *pol* gene (complete protease, partial reverse transcriptase and intergrase regions) was amplified and sequenced using a previously published protocol [8.] Preparation of the lysate, binding the DNA, washing the DNA with Ethanol and elute the DNA. The extracted proviral DNA will be used for polymerase chain reaction (PCR) amplification.

2) Amplification of PR/RT RT-PCR and IN RT-PCR Reactions

Both the PR/RT RT-PCR and IN RT-PCR reactions can occur in parallel on the same plate with the same thermocycler 9700(Applied Biosystems. Set up the protease/reverse transcriptase (PR/RT) RT-PCR and the integrase (IN) RT-PCR reactions. Reverse transcription at 50°C for 45 minutes, Enzyme inactivation at 94°C for 2 minute both for only one cycle, denature at 94°C for 15 sec, anneal 50°C for 20 sec, extend at 72°C for 2 minutes and repeat for 40 cycles, final extension at 72°C for 10 minutes and hold for 4°C for maximum of 18 hours

3) Amplification of PR/RT Nested PCR and IN Nested PCR Reactions

The PR/RT nested PCR and IN nested PCR reactions can occur in parallel on the same plate with the same thermocycler 9700(Applied Biosystems. Initial denaturation at 94°C for 4 minutes for only one cycle, denature at 94°C for 15 sec, anneal 53°C for 20 sec, extend at 72°C for 2 minutes and repeat for 40 cycles, final extension at 72°C for 10 minutes for one cycle and hold for 4°C for maximum of 18 hours. PCR amplicons were purified and sequenced using the Sanger sequencing platform (Applied Biosystems® 3500xL Genetic Analyzer (Thermo Fisher Scientific®, UK)

4) Set Up Cycle Sequencing Reactions for PR/RT and IN

The PR/RT and IN cycle sequencing reactions can occur in parallel on the same plate with the same thermal cycler. Set up cycle sequencing reactions for PR/RT (F1, F2, F3, R1, R2, R3) and IN (F11, F12, R11, R12), vortex, then centrifuge then add 18µL of each of the ten sequencing mixes to the appropriate wells of a chilled 96-well reaction plate ,add 2µL of ExoSAP-IT™-treated nested PCR products to each sequencing mix then set thermal cycler to run the cycle sequencing reactions , denature at 96ºC for 10 seconds, anneal 50ºC for 5 seconds, extend 60ºC for 4 minutes for 25 cycles then hold at 4ºC. Load the plate into the genetic analyzer, run capillary electrophoresis then data analysed using the Sanger ExaType software

D. Data Analysis

Collected data were entered into Microsoft Excel. Cleaning of the data was done regularly in Microsoft excel. To determine genetic diversity was by REGA HIV subtyping tool and the HIV-1 drug-resistant mutations pattern by Stanford HIV Drug Resistance Database. The HIV-1 subtype utilizes the neighbor-joining method in MEGA4 software [18].

E. Ethical Considerations

1) Data Management

The samples stored in freezer at walter reed laboratory and only the PI have access to the samples and when the samples/data by walter reed laboratory have completed their part it will be Destroyed as per WRP policy and if is retained, these samples will not be used for future research.

The laboratory results recorded in a laboratory book, kept by the PI and a soft copy saved in an access-controlled computer. Access to the data controlled using passwords.

There are human samples involved under this protocol which are being collected under another project. Even though, walter reed laboratory will be performing only samples analyses and will not have direct contact with participants or sample collection.

IRB Approvals: Ethical approval was granted by the University of Kabianga through IERC Authorization agreement between University of Kabianga and KEMRI (ISERC/2023/0004) and National commission of science and technology (NACOSTI) (ref no.455975). Permission to use remnant plasma samples was approved by WRP Lab director and PEPFAR SRV director dated 03rd august 2023 and 17th November 2023 respectively thus patients' consent was not needed.

3. Results and Discussions

A. Virological Parameters

Of all the 120-remnant plasma coded for HIV drug resistance, the ART regimen, gender, duration on ARVs distribution by age groups and Viral Load (VL) levels.

From the results distribution by ages, the mean was 20 years with SD of 2.7. Age group 23-24 was the median with total of (25.8%), ART regime of TDF+3TC+DTG 89.2% was mostly used across all the age groups. 30.8%on duration on ART for greater than six months to 2 years.

B. Genetic Diversity HIV-1 Subtyping

From the 103 samples that managed to sequenced, 88 for reverse transcriptase – protease and 86 for intergrase inhibitors. Genetic diversity analysis using regions encoding a portion of the *env-gp41* protein of HIV-1 among youth aged 15-24 years.

From the results it shows that 77.3% (68) were subtype A1, 13.6% (12) were subtype C, 9.1% and (8) were subtype D, on Integrase inhibitors while 81.4% (70) were subtype A1, 8.1% (7) were subtype C, and 10.5%, (9) were subtype D from REGA sub typing tool.

C. Drug Resistance

Sequenced DNA fragments was separated by Genetic Analyzer ABI 3500XL(Applied Biosystems). HIV-1 sequences were aligned by use of Exatype Sanger database. Mutations patterns ascertained by analyzing FASTA files using Stanford

Table 1
Comparison of ART regimen, duration on art and viral load levels by age groups among youth aged 15-24 years in south rift valley region in kenya

Virological Parameters	**(Age groups - *N*)**					
ART regimens	15-16	17-18	19-20	21-22	23-24	
ABC+3TC+ATV/r	0	0	1	0	0	0.8%
ABC+3TC+DTG	0	1	1	1	0	2.5%
ABC+3TC+LPV/r	0	1	0	1	0	1.7%
AZT+3TC+DTG	1	1	0	0	0	1.7%
AZT+3TC+LPV/r	0	1	1	0	0	1.7%
TDF+3TC+DTG	13	20	24	20	30	89.2%
AZT+3TC+ATV/r	0	0	1	0	1	1.7%
TDF+3TC+ATV/r	0	0	0	1	0	0.8%
Duration on ART						
>6 months - 2 yrs.	1	4	5	7	20	30.8%
>2 yrs. - 5yrs	4	5	7	7	7	25%
>5 yrs. - 8 yrs.	4	8	11	5	3	25.8%
>8yrs - 12yrs	5	7	5	4	1	18.3%
	14(11.7%)	24(20%)	28(23.3%)	23(19.2%)	31(25.8%)	
	Viral Load Levels(copies/ml)=*N*					
	1,000 - 9,999	10,000 - 99,999	100,000 - 999,999			
>6 months - 2 yrs.	20	15	5	33%		
>2 yrs. - 5yrs	18	7	4	16%		
>5 yrs. - 8 yrs.	18	14	4	30%		
>8yrs - 12yrs	9	4	2	13%		
VL comparison						
VL during the study	65(54.2%)	40 (33.3%)	15 (12.5%)			
VL at ARVs Initiation	71 (59.2%)	41 (34.2%)	8 (6.7%)			
Age groups	*Frequency*	x	*f*x	(x-mean)^2**f*		
15-16	14	15.5	217	289.835		
17-18	24	17.5	420	156.06		
19-20	28	19.5	546	8.47		
21-22	23	21.5	494.5	48.3575		
23-24	31	23.5	728.5	368.9775		
	120		2406	871.7		
			20	7.3		
				2.7		
mean age	20 years					
SD	2.7					

Table 2
Distribution of HIV-1 subtypes among youth aged 15-24 years in south rift valley region in Kenya

Protease-Reverse transcriptase (PR-RT)				Intergrase Inhibitors (IN)			
Pure	**assignment**	**Counts**	**%**	**pure**	**assignment**	**Count**	**%**
HIV-1 Subtype A (A1)	HIV-1 Subtype A (A1)	52	77.3	HIV-1 Subtype A (A1)	HIV-1 Subtype A (A1)	64	81.4
	HIV-1 Subtype A (A1), potential recombinant	3			HIV-1 Subtype A (A1), potential recombinant	1	
	HIV-1 Subtype A (A1)-like	7			HIV-1 Subtype A (A2)	1	
	HIV-1 Subtype A (A2)	3			Recombinant of A1, C	1	
	Recombinant of A1, C	1			Recombinant of A1, G	2	
	Recombinant of A1, G	2			Recombinant of A2, A1	1	
HIV-1 Subtype C	HIV-1 Subtype A (A2)	2	13.6	HIV-1 Subtype C	HIV-1 Subtype C	6	8.1
	HIV-1 Subtype C	7			Recombinant of C, A1	1	
	Recombinant of C, A1	2		HIV-1 Subtype D	HIV-1 Subtype D	8	10.5
	Recombinant of C, D	1			Recombinant of D, A1	1	
HIV-1 Subtype D	HIV-1 Subtype D	7	9.1				
	HIV-1 Subtype D, potential recombinant	1					

Source: REGA HIV-1 subtyping tool v3.46

HIV-1 Drug Resistance Database.

The study revealed that mutation against M184V was 31.4%, 20.9% to K103N, 15.1 % to G190A, 4.7% to M46I, 5.7% to G118R to both female and males. The major mutations were M184 V, M41L, G190A, K103N, M46I, and G118R, E138K.

Table 3
Drug mutation against gender among youth aged 15-24 years in south rift valley in Kenya

Reverse transcriptase (RT)				
NRTIs	**Mutation**	**Female**	**Male**	**%**
	M184V	17	10	31.4
	M41L	4	4	9.3
	T215Y	3	2	5.8
	M184MV	3	0	5.8
	T215F	3	2	5.8
	K219Q	2	2	4.7
	Y115F	3	0	3.5
	E44D	3	1	4.7
	L74I	3	1	4.7
	K70R	2	2	4.7
	K219E	1	1	2.3
	S68G	1	3	4.7
	D67G	1	1	2.3
	A62V	1	1	2.3
	D67N	2	1	3.5
	M41ML	1	1	2.3
NNRTIs	K103N	12	6	20.9
	G190A	9	4	15.1
	E138A	5	3	9.3
	K101E	4	2	7.0
	Y181C	4	1	5.8
	Y108I	3	1	4.7
	K103S	2	2	4.7
	A98G	3	1	4.7
Protease (PR)				
	M46I	3	1	4.7
	I54V	0	2	2.3
	I54L	1	1	2.3
	V82F	1	0	1.2
	I84V	1	0	1.2
Integrase (IN)				
	G118R	3	2	5.7
	E138K	2	2	4.5
	R263K	2	2	4.5
	T66A	1	1	2.3

Source: From the printout results

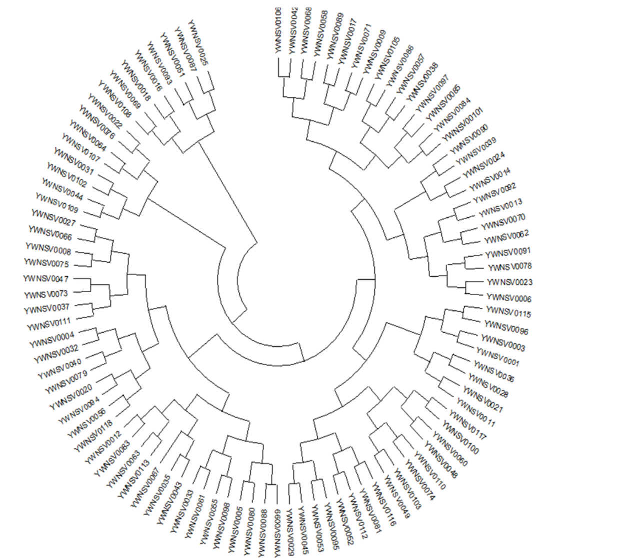

Fig. 1. Phylogenetic tree analysis among youth aged 15-24 years in south rift valley region in Kenya
Source: Molecular Evolutionary Genetics Analysis (MEGA) software

4. Discussion

Most of these guidelines recommend using INSTI-based antiretroviral therapy (ART) as a preferred option for treatment of people living with HIV [19]. Several clinical studies have shown INSTIs to be superior to NNRTI and PI comparative treatment groups [20].

5. Limitation

While this is the first report to do drug resistance on the non-suppressed viral load>1000 copies/ml among the youths aged between 15-24 years within SRV region, in this study we did not test for non-suppressed viral loan <1000copies/ml and youths on ARVs below 6months. However, the results do follow trends seen in other studies.

6. Conclusion

Our study shows susceptibility to tenofovir (TDF)+ lamivudine (3TC) + dolutegravir (DTG) regimen was high and it is current mostly used regimen across the youths age groups,

the genetic diversity of HIV-1 subtype A1 is the dominant circulating forms of HIV-1. The major mutation were M184V, K103N, G190A, M41L and G118R among the HIV-1 youths in SRV.

A considerable proportion of YLWH in this study were virally non-suppressed. Given the high frequency of VNS, there is need to closely monitor profile HIV drug resistance patterns in youths from the south rift valley region, Kenya with confirmed virologic failure. The latter will help understand whether drug resistance also contributes to poor viral suppression.

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