24/04/2018

Viral Dynamics & Basics of Drug Resistance

By Dr. Clarice Ambale
Alarm as children and youth develop resistance to ARVs
Unpalatable tablets and difficulties in administering medication to young children could reverse gains

BY ELIZABETH MERAB
More by this Author
18 hours ago

Kenyan youth and children may be developing resistance to antiretroviral medicines, researchers have warned.

Among infants and young children, the researchers say, under-dosing and resistance to a group of HIV drugs (nevirapine-based regimens) are the leading hindrance to viral load suppression. Bitter
combinations for children are also making it difficult for children to swallow the antiretrovirals (ARVs).

In adolescents, on the other hand, high viral loads resulted from non-adherence because of stigma, poor transition to adult care, and lack of social support.

For instance, the study results show that about four in every 10 children under three years (43 per cent) and adolescents under 20 years (36 per cent) have high viral loads measuring more than 1,000 copies per millilitre.

This, compared to adults between 30 and 60 years whose viral load was much suppressed such that only one in 10 had a high viral load (13 per cent), is quite high.

**HIGH VIRAL LOADS**

"Such high viral loads mean that we may not be able to reach the 90:90:90"
• Children not taking their ARVs well due to unpalatable drugs, large size of tablets etc
• Under dosing is also leading to high viral loads amongst children
• Adolescents are not taking their drugs due to stigma, poor transition to adult care and lack of social support
• $3 \%$ and $36 \%$ of children and adolescents respectively have high viral loads ($>1000$)
Goal and Learning Objectives

- Origin of HIV drug resistance mutations
  I. Normal viral replication
  II. Mutations due to ARV pressure

- Explain the ARV genetic barriers
What causes treatment failure?
Factors contributing to ARV failure & resistance

- High replication rate
- Error prone RT enzyme & high mutation rate
- Latent reservoirs of HIV

Pharmacoecologic factors (day-to-day of drugs (adherence))
- Pharmacogenetic

Provider

Virus

Drug

Host

Inadequate potency
Inadequate durability
Drug-drug interactions
Poor tolerability
Inconvenience

NOTE: One or more of these factors can lead to ARV resistance in a given patient
Basic HIV Biology

Question:
Is HIV (Retrovirus) a RNA or a DNA virus?
Life Cycle of HIV

Steps after virus attachment to cell:
- Entry
- Reverse Transcription (RNA to DNA)
- Integration
- Transcription & Translation
- Assembly
- Maturation and budding

Many steps during the life cycle of HIV virion
How DNA works

• Stores genetic information (genes)
• DNA code is in triplet codons (3 nucleotides)
• Codes are translated into amino acids
• Sequence of amino acids indicates the sequence of proteins
# Names of Nucleosides and Nucleotides

<table>
<thead>
<tr>
<th>Base</th>
<th>Nucleosides</th>
<th>Nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine (A)</td>
<td>Adenosine (A)</td>
<td>Adenosine 5’-monophosphate (AMP)</td>
</tr>
<tr>
<td>Guanine (G)</td>
<td>Guanosine (G)</td>
<td>Guanosine 5’-monophosphate (GMP)</td>
</tr>
<tr>
<td>Cytosine (C)</td>
<td>Cytidine (C)</td>
<td>Cytidine 5’-monophosphate (CMP)</td>
</tr>
<tr>
<td>Uracil (U)</td>
<td>Uridine (U)</td>
<td>Uridine 5’-monophosphate (UMP)</td>
</tr>
<tr>
<td>DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine (A)</td>
<td>Deoxyadenosine (A)</td>
<td>Deoxyadenosine 5’-monophosphate (dAMP)</td>
</tr>
<tr>
<td>Guanine (G)</td>
<td>Deoxyguanosine (G)</td>
<td>Deoxyguanosine 5’-monophosphate (dGMP)</td>
</tr>
<tr>
<td>Cytosine (C)</td>
<td>Deoxycytidine (C)</td>
<td>Deoxycytidine 5’-monophosphate (dCMP)</td>
</tr>
<tr>
<td>Thymine (T)</td>
<td>Deoxythymidine (T)</td>
<td>Deoxythymidine 5’-monophosphate (dTMP)</td>
</tr>
</tbody>
</table>
Mutations

- **Molecular definition**: Change in nucleic acid sequence compared to a reference sequence

- **Biological definition**: Change in nucleic acid sequence that results in a change in structure or function of the nucleic acid or a resulting protein

**Codon**

AAA GAC AGT

---

AAA GAC AGT

---

AAC GAC AGC

---

Silent Mutation

Lys (K) Asp (D) Ser (S)

Asn (N) Asp (D) Ser (S)
Mutational Nomenclature

M184V

Wild-type (wt) amino acid (consensus or reference)

Mutant amino acid

Codon position
PR: 1-99 amino acids
RT: 1-540 amino acids (~200 aa)

M184M/V (mix of wt and mutant)

M184V/I (mix of 2 mutants)
HIV Mutations May Affect:

- **Virulence** - the ability of the virus to invade a cell

- **Viral fitness** - the ability of the virus to compete with wild type virus

- **Immune response**: HIV may escape antibody and CD8 immune control

- **Transmission Efficiency & Hypersusceptibility**: M184V resistant to 3TC/FTC but highly susceptible to TDF and ZDV
HIV Treatment
Antiretrovirals (ARVs)
ARVs available

Different classes of ARVs:

**Six** classes of antiretroviral agents currently exist, as follows:

1. Nucleoside reverse transcriptase inhibitors (NRTIs)
2. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
3. Protease inhibitors (Pis) - **Maturation inhibitor**
4. Integrase inhibitors (INSTIs)
5. Fusion inhibitors (Fis)
6. Chemokine receptor antagonists (CCR5 antagonists)
HIV life cycle and action of ARVs

1. Entry inhibitors
2. RT inhibitors
3. Integrase inhibitors
4. Maturation inhibitors
How Drug Resistance Arises

Population Dynamics DR strains of HIV within an individual on therapy

Drug resistance can be:
- **Acquired** – occurs within infected individual through drug selection process
- **Transmitted** (from person to person)

Adherence-resistance relationship

- Drug-susceptible Virus
- Drug-resistant Virus
- Not taking medicines properly
- Taking medicines properly
Consequence of Mutation

- Once mutations make HIV resistant to one ARV drug, it can then quickly develop other mutations which can cause resistance to related ARV drugs, including an entire class of drugs.
  - e.g., if replication is allowed to persist, resistance to AZT can extend to other NRTIs and thus limit future treatment options.
Latent Reservoirs and Resistance

- ARV resistance, once it develops, is probably life-long, since resistant HIV can hide in latent cellular reservoirs, which can be activated many years later.
- Once a patient is resistant to an ARV drug, that drug will probably be ineffective in the future.

*HIV does not “forgive” treatment errors or the nonadherence*
- **VL <1000 copies/ml:** “blip”
  - Low risk of acquired drug resistance mutations (DRMs)
  - Likely to suppress with improved adherence

- **VL 1000-5,000 copies/ml**
  - Higher risk of DRMs
  - Ability to suppress with improved adherence dependent on presence of DRMs

- **VL >5,000 copies/ml**
  - Highest risk of DRMs
  - Ability to suppress with improved adherence dependent on presence of DRMs
How quickly resistance occurs depends on viral load

<table>
<thead>
<tr>
<th>Viral Load</th>
<th>Days Before Mutation Arises</th>
</tr>
</thead>
<tbody>
<tr>
<td>300,000</td>
<td>0.1</td>
</tr>
<tr>
<td>30,000</td>
<td>1</td>
</tr>
<tr>
<td>3,000</td>
<td>10</td>
</tr>
<tr>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td>30</td>
<td>1,000</td>
</tr>
</tbody>
</table>
ARV Genetic Barriers
Single Mutations and Resistance

- Certain single mutations will cause the HIV to be completely resistant to a drug, or even to an entire class (e.g., NNRTIs).

- Drugs in which such single mutations cause complete resistance are said to have a LOW GENETIC BARRIER to the development of resistance.
Multiple Mutations and Resistance

- Some drugs require multiple, step-wise mutations for HIV to become resistant.
- Drugs which require multiple mutations for resistance have a High Genetic Barrier, e.g. Protease inhibitors.
- The longer a failing regimen is continued, the greater the number of mutations which will occur and which will lead to greater resistance, including cross resistance.
Mutations and Resistance

- For certain ARVs, only one mutation is needed to stop the drug from working; example: K103N a NNRTI mutation:
  
  \[
  \begin{array}{c@{}c@{}c@{}c}
  \text{AAA} & \text{AAG} & \rightarrow & \text{AAC} \\
  \text{Lys} (K) & \text{Lys} (K) & & \text{Asn} \quad \text{Asn}
  \end{array}
  \]

  LOW GENETIC BARRIER

- For other ARVs, multiple, step-wise mutations must occur before the drug loses affect; example: I47A Protease inhibitor mutation (2 base mutation change):
  
  \[
  \begin{array}{c@{}c@{}c@{}c}
  \text{ATT} & \text{ATC} & \text{ATA} & \text{GC} \\
  \text{Ile} & \text{Ile} & \text{Ile} & \text{GCT} \\
  \downarrow & \downarrow & \downarrow & \downarrow \\
  \text{Ile} & \text{Ile} & \text{A} & \text{GC} \\
  \downarrow & \downarrow & \downarrow & \downarrow \\
  \text{Ile} & \text{Ile} & \text{A} & \text{GC} \\
  \end{array}
  \]

  HIGH GENETIC BARRIER
Genetic barrier & Potency of some ARVs

(HIV-1 Antiretroviral Resistance. Scientific Principals and Clinical Implications. Drugs 2012)

Genetic Barrier to 6 classes of ARVS

NRTIs, NNRTIs, PIs, Integrase Inhibitor, CCR-5 inhibitor, Fusion inhibitor
# Genetic barrier & Cross-resistance

<table>
<thead>
<tr>
<th>Class</th>
<th>ARVs</th>
<th>Genetic barrier</th>
<th>X-resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRTIs</td>
<td>ZDV/3TC, d4T/3TC</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>ABC/3TC, TDF/3TC</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>TDF/FTC</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>NNRTIs</td>
<td>EFV, NVP, RPV</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>ETV</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>PIs</td>
<td>Unboosted</td>
<td>++</td>
<td>++/+++</td>
</tr>
<tr>
<td></td>
<td>Boosted</td>
<td>+++/+++++</td>
<td>+/+</td>
</tr>
<tr>
<td>Fusion inhibitors</td>
<td>T20</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CCR5 antagonists</td>
<td>MVC</td>
<td>++/+++</td>
<td>-</td>
</tr>
<tr>
<td>Integrase inhibitors</td>
<td>RAL, EVG</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>DTG</td>
<td>++/+ +++</td>
<td>++</td>
</tr>
</tbody>
</table>
Nevirapine Resistance in Previously Nevirapine-Unexposed HIV-1-Infected Kenyan Infants Initiating Early Antiretroviral Therapy

Bhavna H. Chohan,1,3,* Kenneth Tapia,2,* Sarah Benki-Nugent,2 Brian Khasimwa,4 Musa Ngayo,3 Elizabeth Maleche-Obimbo,4 Dalton Wamalwa,4 Julie Overbaugh,5 and Grace John-Stewart2,6–8

Abstract

Nevirapine (NVP) resistance occurs frequently in infants following NVP use in prevention of mother-to-child transmission (PMTCT) regimens. However, among previously NVP-unexposed infants treated with NVP-antiretroviral therapy (ART), the development and impact of NVP resistance have not been well characterized. In a prospective clinical trial providing early ART to HIV-infected infants <5 months of age in Kenya (OPH03 study), we followed NVP-unexposed infants who initiated NVP-ART for 12 months. Viral loads were assessed and resistance determined using a population-based genotypic resistance assay. Of 99 infants screened, 33 had no prior NVP exposure, 22 of whom were initiated on NVP-ART. Among 19 infants with follow-up, seven (37%) infants developed resistance: one at 3 months and six at 6 months after ART initiation. The cumulative probability of NVP resistance was 5.9% at 3 months and 43.5% at 6 months. Baseline HIV RNA levels (p = 0.7) and other characteristics were not associated with developing resistance. Post-ART, higher virus levels at visits
HIV type 1 drug resistance patterns among patients failing first and second line antiretroviral therapy in Nairobi, Kenya

Peter Koigi¹, Musa Otieno Ngayo², Samoel Khamadi³, Caroline Ngugi⁴ and Anthony Kebira Nyamache⁵*

Abstract

Background: The ever-expanding rollout of antiretroviral therapy in poor resource settings without routine virological monitoring has been accompanied with development of drug resistance that has resulted in limited treatment success.

Methods: A cross-sectional study with one time viral load was conducted during the period between 2012 and 2013 to determine treatment failure and drug resistance mutations among adults receiving first-line (44) (3TC_d4T/AZT_NVP/EFV) and second-line (20) (3TC/AZT/LPV/rt) in Nairobi, Kenya. HIV-1 pol-RT genotyping for drug resistance was performed using an in-house protocol.

Results: A total of 64 patients were recruited (mean age 36.9 yrs.) during the period between 2012 and 2013 of the 44 adult patients failing first-line 24 (40.9%) had drug resistance mutations. Eight (8) patients had NRTI resistance mutations with NAMS M184V (54.2%) and K65R (8.4%) mutations being the highest followed by TAMs T215Y and K70R (12.5%). In addition, among patients failing second-line (20), six patients (30%) had NNRTI resistance; two patients on K103N and G190A mutations while V106A, Y184V, A98G, Y181C mutations per patient were also detected. However, for NRTI two patients had TAM T215Y, M184V mutation occurred in one patient.

Conclusions: The study findings showed that HIV-1 drug resistance was significantly high in the study population. The detected accumulated resistance strains show that emergence of HIV drug resistance will continue to be a big challenge and should be given more attention as the scale up of treatment in the country continues.
Transmitted HIV-1 Drug resistance and the Role of Herpes Simplex Virus-2 Co-infection among Fishermen along the Shores of Lake Victoria, Kisumu, Kenya

Victor Mburu Macharia¹, Caroline Ngugi¹, Raphael Lihana² and Musa Otieno Ngayo³

¹College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya
²Centre for Virus Research, Kenya Medical Research Institute, Nairobi, Kenya
³Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya

Corresponding author: Musa Otieno Ngayo, Centre for Microbiology Research Kenya Medical Research Institute: Nairobi, Kenya, Tel: 254720607890; Email: musaotieno@yahoo.com

Received date: October 17, 2016; Accepted date: October 24, 2016; Published date: October 29, 2016

Citation: Macharia VM, Ngugi C, Lihana R, et al. Transmitted HIV-1 drug resistance and the role of herpes simplex virus-2 co-infection among fishermen along the shores of lake Victoria, Kisumu, Kenya, J HIV Retrovirus. 2016, 2:3.

Copyright: © 2016 Macharia VM, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Introduction: Herpes simplex virus type 2 (HSV-2) infection has been associated with a 3-fold risk of HIV-1 acquisition. The prevalence of HIV-1 and HSV-2 in the fishing communities along the shores of Lake Victoria in Kisumu have been reported to be high. This may contribute to the growing HIV epidemic in Kenya including the spread of transmitted drug resistance (TDR). We report data on the association of HSV2/HIV-1 co-infection and TDR in this antiretroviral (ARV)-naive population.

Methods: Blood samples were obtained from 249 consenting fishermen from 5 beaches and a detailed sociodemographic questionnaire was administered. Blood samples were analyzed for HIV-1/HSV2 co-infection. The HIV-1 genotypes were classified into drug resistant genotypes (GR) and drug sensitive genotypes (DS) according to World Health Organization (WHO) criteria. The HSV-2 genotypes were classified into drug resistance; K103N, G190A and Y181C mutations each. In the regression model, HIV/HSV-2 co-infection was independently associated with TDR (OR 4.1 [95% CI 1.4 to 11.9]).

Conclusion: The level of TDR to NNRTIs in these ARV-naive fishermen was significantly high especially among those co-infected with HSV-2. HSV-2 infection may increase the risk of TDR in this population.

Keywords: HIV; AIDS; HIV-1; Drug resistance

Introduction

Fishing communities along the shore of Lake Victoria in Kenya comprise young, highly migratory men who spend long periods away from home. In this context, fishing provides means of income and sustenance for many and is a major contributor to the local economy. High HIV infection rates have been reported among fishermen in this area [1]. HIV and HSV-2 transmission has been shown to increase the risk for HIV drug resistance [2]. Given the increasing rate of co-infection between HSV-2 and HIV-1 in the fishing communities along the shores of Lake Victoria, this study was designed to determine the association of HSV2/HIV-1 co-infection and TDR in this ARV-naive population.
Cytochrome P450 2B6 genetic variants are associated with plasma nevirapine levels and clinical response in HIV-1 infected Kenyan women: a prospective cohort study

Margaret Ngwono Oluka¹, Faith Apolot Okalebo¹, Anastasia Nkatha Guantai¹, R Scott McClelland² and Susan M Graham²

Abstract

Background: Polymorphisms in cytochrome P450 2B6 (CYP2B6) affect the steady state plasma concentration of nevirapine. CYP2B6 516G>T and 983T>C are common in African populations, but data on their influence on plasma nevirapine concentration and clinical response in African women are limited. We investigated the impact of CYP 516G>T and 983T>C on plasma nevirapine concentration and clinical outcomes in a prospective cohort study of HIV-infected Kenyan women.

Methods: Study subjects were 66 HIV-1-seropositive women taking nevirapine-based antiretroviral therapy. Plasma collected at week 12 was analyzed for nevirapine concentration by high performance liquid chromatography. Baseline samples were genotyped for CYP2B6 516G>T and 983T>C single nucleotide polymorphisms by real-time polymerase chain reaction. CD4 cell count, plasma viral load, and genotypic drug resistance in plasma and genital
Acknowledgement

- NAHCC
- CHS
- CDC
- KPA
You don’t have to be a genius to do quality work.
All that you need is a good work ethic and consistency.