Socio-Demographic, Nutritional and Adherence as Determinants of Nevirapine Plasma Concentration among HIV-1 Patients from Two Geographically Defined Regions of Kenya

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Abstract

Background: Data are skewed on the role of Socio-demographic, nutritional and adherence related factors on the influence of nevirapine plasma concentrations among Kenyan population. This study rigorously determined these three factors on nevirapine plasma concentrations among HIV patients receiving HIV treatment in two regions known for high prevalence of HIV and long duration of ART uptake. Methods: Blood samples were collected from 377 consenting HIV adult patients receiving an NVP-based first-line ART regimen. A detailed sociodemographic questionnaire was administered. NVP plasma concentration was measured by liquid chromatography - tandem mass spectrometry (LC-MS/MS). Results: The majority (59.2%) of the patients were female, 72.2% were from western Kenya (predominantly Nilotic speaking community). The patients’ mean age was 41.6 (SD ± 11.5) years and the mean duration of ART was 5.1 (SD ± 4.8) years. The median BMI of the patients was 25 kg/m² (IQR = 22.2 - 28.7 kg/m²). The majority 81.2% were receiving 3TC/NVP/TDF ART regimen, 30% had changed their initial ART regimen with 54.4% reporting missing taking current ARVs. Overall NVP plasma levels ranged from 4-44207 ng/mL (median 6213 ng/mL, IQR 3097–8606.5 ng/mL). There were 105 (25.5%) participants with NVP levels of <3100 ng/mL, associated with poor viral suppression. Multivariate linear regression analysis showed region of origin (adjusted \( \beta \) 976, 95% CI, 183.2 to 1768.82; \( p = 0.016 \)), gender (adjusted \( \beta \) 670, 95% CI, 293.6 to 1634.2; \( p = 0.047 \)), education level (adjusted \( \beta \) -39.0779, 95% CI, -904.2 to -192; \( p =0.003 \)) and ARV uptake in the past 30 days (adjusted \( \beta \) = -1109, 95% CI = -2135 to -83; \( p =0.034 \)) remained independently associated with NVP plasma levels.

Conclusion: Nevirapine plasma concentration is highly heterogeneous among Kenyan population with a significant proportion of patients reporting levels of <3100 ng/ml, correlated with poor viral suppression. The host pharmacoeologic factors, such as gender, age, weight, education level, region of origin (ethnicity), ART regimen type and adherence, are key in influencing NVP plasma concentration. Taking these factors into consideration, HIV treatment may be personalized to achieve optimal treatment success.

Keywords: Nevirapine plasma concentration, host pharmacoeologic factors, HIV-1 patients in Kenya

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Background

Globally, the introduction of antiretroviral therapy (ART) as treatment for HIV infections has greatly reduced mortality and morbidity among patients living with HIV (UNAIDS, 2020). Kenya is steadily on the track for scale up of ART uptake in line with the 2015 World Health Organization recommendations requiring immediate initiate ART treatment to people testing HIV positive regardless of their CD4 or viral load (UNAIDS, 2016). By the end of 2019, about 74% adults and 73% children in Kenya needing ART were receiving ART treatment (UNAIDS, 2020). A remarkable proportion of these patients (68%) were virally suppressed (UNAIDS, 2020). At the time of the study, the first-line ART guidelines for children, youth and adults in Kenya typically contained a backbone of two nucleoside reverse transcriptase inhibitors (NRTIs; zidovudine [AZT], stavudine [d4T], tenofovir [TDF] or lamivudine [3TC]), plus one non-nucleoside reverse transcriptase inhibitor (NNRTI): either nevirapine (NVP) or efavirenz (EFV) (NASCOP, 2016).

Nevirapine has been widely used in Kenya just like in many developing countries. Efficacy, availability, low cost and use in prevention of vertical HIV transmission are the key reasons for this choice (WHO, 2016). Unfortunately, NVP is associated with a higher incidence of rashes (van Leth et al., 2004), hepatotoxicity (Aizire et al., 2012) and a low genetic barrier to resistance limiting the use of this drug (Clutter et al., 2016). In some countries especially, the developed countries the drug is no longer dispensed. Several factors are pointed out affecting NVP plasma concentration. Pharmacogenetic factors such as the occurrence of single-nucleotide polymorphisms (SNPs) in the P450 2B6 (CYP2B6) and minorly by CYP3A have been extensively documented affecting NVP plasma concentration (Xu et al., 2012). The association between CYP2B6 SNPs and NVP plasma concentration has also been widely published in Kenya (Oluka et al., 2015); including among the population in the current study (Mungiria et al., 2020). Other factors affecting NVP plasma levels identified in other settings
but yet to gain much attention especially among the Kenyan population includes holistic socio-demographic, nutritional and adherence related factors (Bennett et al., 2008; Wang et al., 2011).

Data aimed at individualization of ART treatment in Kenya are key to successful ART treatment programs. This study rigorously evaluated the relationship between socio-demographic, nutritional and adherence related factors with NVP concentrations among HIV-1 patients receiving ART treatment in western and coastal Kenya.

**Methods**

**Design and study population**

A cross-sectional study approved by the Kenya Medical Research Institutes’ (KEMRI) Scientific Review Unit (SERU) (KEMRI/SERU/CVR/002/3214) and NACOSTI (NACOSTI/P/19/11747/28173) was conducted between August, 2018 and January 2020. Patients recruited in this study were part of an ongoing study designed to establish a cost-effective laboratory method to monitor antiretroviral adherence in HIV-1 infected individuals on treatment in Kenya. The patients recruited in this study were those: (i) HIV-1 infected adults (aged above 18 years), attending the two HIV treatment programs, (ii) willing to voluntarily give written informed consent, (iii) able to read either English or Kiswahili, (iii) be on ARV treatment for 12 months, (iv) be on nevirapine based first line ARV treatment regimen and (v) HIV patients with viral load results at month 12 of treatment. All patients filled in a written informed consent for study participation.

**Data collection**

Detailed description of the population of the study has been described previously (Mungiria et al., 2020). The study recruited 272 patients from Kisumu (western region of Kenya) and 105 patients from Malindi (coastal region of Kenya). An exhaustive structured interview (including demographic data, clinical history, adherence, HIV stigma and medical history) was used to collect patient-related information. From each patient, about 5 ml of intravenous blood was collected into ethylenediaminetetraacetic acid (EDTA)-containing Vacutainers® (BD, US) for determination of NVP plasma concentration measurement using a tandem quadrupole mass spectrometer designed for ultra-high performance: Xevo TQ-S (Waters Corporation, U.S.A) as described by Reddy et al. (2016). The NVP plasma concentration was categorized as <3100 ng/mL (below therapeutic range), 3100–4300 ng/mL (therapeutic range) and >4300 ng/mL (above therapeutic range) as previously defined (Gopalan et al., 2017; Mungiria et al., 2020).

**Data analysis**

Frequencies and percentages were used to present the sociodemographic data. The effect of socio-demographic, nutritional and adherence on NVP plasma concentrations was determined using Kruskal-Wallis test and Dunn’s test at $\alpha = 0.05$. Univariate and multivariate linear regression analyses were performed to determine the relationship between NVP plasma concentration and Socio-demographic, nutritional and adherence and other clinical characteristics at 5% significance level. All statistical analyses were performed using STATA v 13 (StataCorp LP, Texas, USA).

**Results**

**Baseline characteristics**

The results from the all the 377 patients were assessed, of whom 272 (72.2%) were from Kisumu county (western region of Kenya), 223 (59.2%) were female and 114 (30.2%) had a HIV viral load of >1000 copies/mL. The median age of the patients was 41 years (IQR = 34–49 years), with a median duration of living with HIV infection of five years (IQR = 1–11years) and a median duration since ART initiation of three years (IQR = 1–8 years). The median BMI of the patients was 25 kg/m² (IQR = 22.2 - 28.7 kg/m²). There were 988(26%) of the patients whose staple food stuff was protein and carbohydrates with 264(70%) reporting lacking staple foods during the study. At the time of the study, there were 169 (44.8%) patients taking lamivudine, nevirapine, tenofovir regimen, with 205 (54.4%) reporting missing taking current ART at least once (Table 1).
Table 1. Summary of patient demographics and clinical characteristics of patients

| Variables                                      | Total (N = 377) |
|------------------------------------------------|-----------------|
| Region - Kisumu, n (%)                         | 272             |
| Female, n (%)                                  | 223             |
| Age (years), Median (IQR)                      | 41              |
| HIV-RNA <1000 copies/mL copies/mL, n (%)       | 263             |
| Time to HIV clinic (Minutes) Median (IQR)       | 60              |
| Duration with HIV (Years), Median (IQR)        | 5               |
| Age of sexual debut (Years), Median (IQR)      | 18              |
| Duration post ART initiation (Years), Median (IQR) | 3               |
| BMI (kg/m2), median (IQR)                      | 25              |
| Staple food stuff (Proteins/Carbohydrates) n (%) | 98             |
| Missed staple food n (%)                       | 264             |
| Porridge consumption (≥ 2 times) n (%)         | 147             |
| Initial ARV Type                               |                 |
| Lamivudine, Nevirapine, Stavudine              | 131             |
| Lamivudine, Nevirapine, Zidovudine             | 134             |
| Lamivudine, Nevirapine, Tenofovir              | 112             |
| Changed ARV, yes n (%)                         | 113             |
| Current ARV Type                               |                 |
| Lamivudine, Nevirapine, Stavudine              | 83              |
| Lamivudine, Nevirapine, Zidovudine             | 125             |
| Lamivudine, Nevirapine, Tenofovir              | 169             |
| Missed taking current ART n (%)                | 205             |
| 76 - 100 % uptake of current ART n (%)         | 345             |
| Missed taking ART n (%)                        | 27              |

NVP plasma concentration
The steady-state NVP plasma concentrations ranged from 4 to 44,207 ng/mL (median 5179, IQR 2557–7453 ng/mL) varying widely among patients. There were 96 (n = 377, 25.5%) patients with NVP concentration <3100 ng/mL and 26 (n = 377, 6.9%) with NVP concentration of 3100-4300 ng/mL, with the majority, 255 (n = 377, 67.6%), of the patients having an NVP concentration >4300 ng/mL. The steady-state plasma nevirapine concentrations was tested for normality by the Shapiro- Wilk test, and subsequently log10-transformed due to lack of normality (Shapiro- Wilk test = 0.86194; v = 36.048; p = 0.000001).

Linear regression model
Socio-demographic characteristics
In the final linear regression model, sociodemographic factors significantly associated with a higher NVP concentration included region of origin (adjusted $\beta$ 976, 95% CI, 183.2 to 1768.82; $p = 0.016$) and gender (adjusted $\beta$ 670, 95% CI, 293.6 to 1634.2; $p = 0.047$). Education level on the other hand was associated with a lower NVP were education level (adjusted $\beta$ -39.0779, 95% CI, -39.07 to 1085.7; $p = 0.068$, (Table 2)
Table 2. Univariate and multivariable regression analyses of socio-demographic factors associated with NVP plasma concentrations.

| Variable       | Unadjusted β | Bivariate (95% CI) | P-value | Adjusted β | Multivariate (95% CI) | P-value |
|----------------|--------------|-------------------|---------|------------|------------------------|---------|
| Age            | 282.5        | -86.9  651.9      | 0.133   | 393        | -168.7  954.0         | 0.17    |
| Gender         | 612          | -329.1  1553.1    | 0.089   | 670        | 293.6   1634.2        | 0.047   |
| Religion       | -769         | -3173.9  1635.9   | 0.53    | -648       | -2731.8  1435.1       | 0.541   |
| Education      | 565          | -18.4   1148.4     | 0.058   | 502        | 60.6    943.4         | 0.026   |
| Ever been married | 60       | -1187.6  1307.6    | 0.925   | -335       | -2299.5  1628.9       | 0.737   |
| Currently Married | 250      | -935.6   1435.6    | 0.679   | 251        | -698.1  1200.7        | 0.603   |
| Region         | 670          | -218.2   1558.2    | 0.139   | 976        | 183.2   1768.8        | 0.016   |
| Ethnicity      | -412.5       | -862.9   37.9      | 0.073   | -333       | -845    179           | 0.202   |
| Alcohol use    | -18.5        | -1145.2  1108.2    | 0.974   | 25.6       | -953.2  1004.4        | 0.959   |
| Smoking        | -97          | -3126.2  2932.2    | 0.95    | 127.1      | -2167.4  2421.6       | 0.913   |

Nutritional factors
None of the nutritional related factors were found associated with NVP plasma concentration (Table 3).

Table 3. Univariate and multivariable regression analyses of nutritional factors associated with NVP plasma concentrations.

| Variable                          | Unadjusted β | Bivariate (95% CI) | P-value | Adjusted β | Multivariate (95% CI) | P-value |
|-----------------------------------|--------------|-------------------|---------|------------|------------------------|---------|
| Body Mass Index (Kg/M2)           | -51.5        | -358   255         | 0.741   | -92.2      | -589.1  404.7         | 0.715   |
| Types of stable food stuff        | -132         | -428.3  164.3      | 0.382   | -120.6     | -319.6  78.4         | 0.234   |
| Access to staple food             | 1958         | -2139.8  6055.8    | 0.348   | 1826.6     | -1683.2  5336.4       | 0.307   |
| Missed staple food                | 435          | -141.1  1011.1     | 0.138   | 351        | -507.1  1209.1        | 0.422   |
| No of times missed staple food    | -125         | -1512.8  1262.8    | 0.86    | -441.1     | -1646.9  764.6        | 0.472   |
| Meal preparation                  | 290          | -659.6  1239.6     | 0.549   | 196.7      | -436.4  829.7         | 0.542   |
| Porridge consumption              | 49.5         | -491.2  590.2      | 0.857   | -40.4      | -677.5  596.6         | 0.901   |

ART treatment and adherence related factors
Table 4 describes linear regression analysis estimating the relationships between NVP and plasma levels and ART history and adherence factors. In multivariate analysis, initial ART regimen type (adjusted β = -548.1, 95% C = -904.2 to -192; p = 0.003) and ARV uptake in the past 30 days (adjusted β = -1109, 95% C = -2135 to -83; p = 0.034) remained independently associated with NVP plasma levels.
Table 4. Univariate and multivariable regression analyses of ART treatment and adherence related factors associated with NVP plasma concentrations.

| Variable                                      | Unadjusted $\beta$ | (95% CI)   | P-value | Adjusted $\beta$ | (95% CI)   | P-value |
|-----------------------------------------------|--------------------|------------|---------|------------------|------------|---------|
| Time taken to ARV clinic (Minutes)            | 3.1                | -6.4, 12.5 | 0.528   | 191.4            | -1175.4, 1558.2 | 0.783   |
| Fare to ARV clinic (Ksh)                      | 121                | -849.2, 1091.2 | 0.806 | -1084.5, 3149.4 | 980.5, 1558.2 | 0.302   |
| Hospital Admission                            | -1279              | -2622.7, 64.7 | 0.062 | 1100.6, -3033.7 | 1294.9, 980.5 | 0.43    |
| No of times missed HIV medical visits         | 602.5              | -74.3, 1279.3 | 0.081 | 393.3, -415.5    | 1131.3, 1294.9 | 0.363   |
| Missed ART scheduled visit                   | 111                | -934.6, 1156.6 | 0.835 | 605.4, -854.1    | 1526.8, 980.5 | 0.579   |
| Duration post ART initiation (Years)         | -39                | -615.6, 537.6 | 0.894 | 435.7, -1429.6   | 914.6, 1294.9 | 0.77    |
| Initial ARV Type                              | -506.8             | -778.9, -234.6 | 0.0001 | -548.1, -904.2   | -192, 1294.9 | 0.003   |
| Changed ARV                                   | -1238              | -1895.9, -580.1 | 0.0001 | -159.6, -1233.7  | 914.6, 1294.9 | 0.77    |
| Current ARV Type                              | 87                 | -894.1, 1068.1 | 0.862 | 523.5, -651.3    | 1698.2, 980.5 | 0.381   |
| Missed taking current ART                     | -986               | -1661.1, -310.9 | 0.004 | -192.9, -1173.5  | 787.7, 1294.9 | 0.304   |
| Percent uptake of current ART (%)             | -43.8              | -81.5, -6.1 | 0.023 | 68.5, -2295.2    | 2432.3, 980.5 | 0.955   |
| Missed taking past ART                        | -906               | -1861.1, 49.1 | 0.063 | -602.4, -3362.2  | 2157.3, 980.5 | 0.687   |
| Missed ARV uptake 4 to 14 days                | -441               | -1262.4, 304.2 | 0.292 | 67, -1097.0      | 1231.0, 980.5 | 0.91    |
| Missed ARV uptake ≥ 14 days                   | -447               | -1476.3, 582.3 | 0.394 | -613.5, -1954.3  | 727.3, 980.5 | 0.369   |
| ARV dose missed yesterday                     | -2959              | -4795.1, -1122.9 | 0.002 | -2097.5, -4825.7 | 630.7, 980.5 | 0.131   |
| ARV dose missed in the past 2 days            | -1457.5            | -4089.8, 1174.8 | 0.277 | 1183.5, -936.5   | 3303.5, 980.5 | 0.273   |
| ARV dose missed in the past 3 days            | -2571.7            | -4070.9, -964.1 | 0.002 | -2059.5, -4696.9 | 577.9, 980.5 | 0.126   |
| ARV uptake in past 30 days                    | -822               | -1625.5, -18.5 | 0.045 | -1109, -2135     | -83, 980.5 | 0.034   |
| ARV dose missed in the past 30 days           | -179               | -1048.4, 690.4 | 0.686 | -176, -939.9     | 587.9, 980.5 | 0.651   |

Discussion

Efforts contributing to the personalization of ART treatment aimed at prolonging life of persons living with HIV marks huge component of HIV treatment programs in many countries. The recommendation by WHO requiring immediate initiate ART treatment to people testing HIV positive regardless of their CD4 or viral load (UNAIDS, 2016), must also take cognizant of the fact that optimal ART outcomes inevitably requires an understanding of the individual variation in response to ART, both efficacy and toxicity. ART treatment outcomes are influenced in part by host pharmacoecologic factors (Phillips and Mallal, 2009). The pharmacoecologic factors include factors relating to lifestyle and adherence of the patient, drug interactions or pregnancy (Pavlos and Phillips, 2012). Patients demographic factors such as age, gender (Wyen et al., 2008; Swaminathan et al., 2011), nutritional status such as body weight, growth rate (Schipani et al., 2011) are also shown to influence the treatment outcomes attributed (Gopalan et al., 2017). This study evaluated the influence of socio-demographic factors (such as age, sex, education level), nutritional status and ARV treatment and adherence on the steady-state plasma concentrations of nevirapine among HIV patients receiving treatment in two regions of Kenya known for high prevalence of HIV as well as uptake of ART treatment in Kenya.

We reported wide interindividual variability in NVP plasma levels ranging from 4ng/mL to 44,207 ng/mL (median 5179 ng/mL, IQR 2557–7453 ng/mL). This range is far broader than those reported in other studies (Oluka et al., 2015; Giacomelli et al., 2018; Mazanderani et al., 2019). There were 255(67.6%) patients with NVP plasma of 3000 to 8000 ng/mL (la Porte et al., 2006) considered within therapeutic range. Further, there were 25.5% patients who had NVP plasma levels of <3100 ng/ml, correlated with poor viral suppression (la Porte et al., 2006). Previous studies in Kenya and in other countries such as Italy have reported fewer cases of persons with NVP plasma level of levels of <3100 ng/ml (Oluka et al., 2015; Giacomelli et al., 2018).

Region of origin contributed significantly to NVP plasma concentration with the patients from coastal...
Kenya region having higher median NVP plasma concentration than those from Western Kenya region. A similar observation was made among the Bantu-related communities from Serowe/Palapye and Chobe districts, and the San-related communities of the Ghanzi area in Botswana (Tawe et al., 2018). Kenya’s ethnic groups can be divided into three broad linguistic groups: Bantu (mostly in the Central Kenya), Nilotic (western Kenya) and Cushite (Coastal and Northern Kenya). Although these ethnic groups reside in close proximity to each other, there is wide environmental and cultural diversity between these populations (Kenya National Bureau of Statistics, 2019). Consequently, there exist wide host genetic and environmental diversity which may result in different efficacy and adverse event profiles or treatment outcome between different African populations treated with same ART regimen (Ngaimisi et al., 2013). The human cytochrome P450 2B6 enzyme (CYP2B6) plays a pivotal role in the metabolism of different drugs used for HIV life-long therapy (non-nucleoside reverse-transcriptase inhibitors such as efavirenz and nevirapine). CYP2B6 is a highly polymorphic enzyme that affects the therapeutic response including drug interactions in individuals (Hedrich et al., 2016). Importantly, African populations show a high degree of variation in the CYP2B6 gene (Čolić et al., 2015).

Males in this study tended to have elevated median NVP plasma concentration than female contrary to several independent studies (Gonzalez et al., 2005; Shiau et al., 2014; Giacomelli et al., 2018). Generally, the difference in ARV plasma levels by gender has been attributed to the difference in body size and drug clearance between males and females. Although not significant, the older patients had higher median NVP plasma level than younger ones. This is consistent with other studies which have indicated that nevirapine metabolism in younger population is generally more rapid than older population, and that younger population including children require higher doses of nevirapine to achieve therapeutic concentration (Swaminathan et al., 2011; Gopalan et al., 2017).

The observed association between education level and NVP plasma levels suggests that literacy, formal education and possibly Koranic education may impact favorably on adherence to ART with better treatment outcome (Hegazi et al., 2010).

We did not observe an association with NVP plasma concentration and nutritional related factors such as body mass index, regular uptake of porridge, availability of balanced diet and access to staple food. However, patients with BMI of >30kg/m² (obese), accessed balanced diet and regularly consumed porridge tended to have higher NVP plasma concentration. Studies have associated body composition, nutritional status, food security with NVP plasma concentration (Vreeman et al., 2014; Bartelink et al., 2015). The lack of proper nutrition and/or a diet low in fat are shown to affect ARV absorption, or ARV cellular transport (Lamorde et al., 2012).

We observed the importance of initial ART regimen type and ARV uptake in the past 30 days influencing NVP plasma concentration. Access to ART is only one aspect of an effective HIV management programme (Moosa et al., 2019). Optimal adherence to ART is essential and early studies reported that ≥95% adherence to ART was required to achieve and maintain viral suppression (Paterson et al., 2000). Although recent studies have shown that virologic suppression may still be achieved with <95% adherence levels, this is dependent on the ART regimen, duration of treatment and previous ART exposure (Talam et al., 2008; Ammassari et al., 2016). Furthermore, repeated adherence levels of <100% and treatment interruptions are associated with an increased risk of both NRTI and NNRTI resistance which form the backbone of current first line ART regimens in Kenya and many other developing nations (Haberer et al., 2015; Kimulwo et al., 2017). The important ART regimen type and adherence to treatment cannot be overemphasized in achieving HIV treatment success.

Some of the limitation to this study included: First, the use of an NVP-based ART regimen in Kenya and other developed countries has been reduced if not eliminated in the recent past, limiting the generalization of these results. Secondly, this study did not delve deeper into ethnic profiling of study participants and the actual influence of ethnicity to treatment outcome cannot be ruled out. Lastly the cross-sectional nature of this study only permitted the description of the relationship between NVP plasma concentrations the stated pharmacoecologic factors and not a causal conclusion. Such outcomes can be confirmed in a longitudinal study.

Recognizing these limitations, the following can be concluded, NVP plasma concentration is highly heterogenous with a significant proportion of these patients reporting levels of <3100 ng/mL, correlated with poor viral suppression. The host pharmacoecologic factors, such as gender, age, weight, education level, region of origin (ethnicity), ART regimen type and adherence, are key in influencing NVP plasma concentration. Taking these factors into consideration, HIV treatment may be personalized to achieve optimal treatment success.

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**Declaration of Conflicting Interest**
The author(s) declared no competing interests.
Ethics approval
This study was approved by the Kenya Medical Research Institutes’ (KEMRI) Scientific Review Unit (SERU) (KEMRI/SERU/CVR/002/3214) and NACOSTI (NACOSTI/P/19/11747/28173). Before recruitment in this study, all patients filled in a written informed consent for study participation.

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Informed consent
Written informed consent was obtained from all subjects before the study.

Reference
1. Aizire J, Fowler MG, Wang J, Shetty AK, Stranix-Chibanda L, Kamateeka M, Brown ER, Bolton SG, Musoke PM, Coovadia H. Extended prophylaxis with nevirapine and cotrimoxazole among HIV-exposed uninfected infants is well tolerated. AIDS. 2012 Jan 28; 26(3):325-33.
2. Ammassari A, Trotta MP, Shalev N, Marconi P, Antinori A. Beyond virological suppression: the role of adherence in the late HAART era. Antivir Ther. 2012; 17:785–792.
3. Bartelink IH, Savic RM, Dorsey G, Ruel T, Gingrich D, Scherpier HJ, Capparelli E, Julienn V, Young SL, Achan J, Plenty A, Charlebois E, Kamya M, Havlir D, Aweeka F. The effect of malnutrition on the pharmacokinetics and virologic outcomes of lopinavir, efavirenz and nevirapine in food insecure HIV-infected children in Tororo, Uganda. Pediatr Infect Dis J. 2015 Mar; 34(3):e63-70.
4. Bennett DE, Bertagnolio S, Sutherland D, Gilks CF. The World Health Organization’s global strategy for prevention and assessment of HIV drug resistance. Antivir Ther. 2008; 13 Suppl 2:1–13.
5. Clutter D, Jordan M, Bertagnolio S, Shafer RW. HIV-1 drug resistance and resistance testing. Infection, Genetics and Evolution 46 (2016) 292–307.
6. Čolić A, Alessandrini M, Pepper MS. 2015. Pharmacogenetics of CYP2B6, CYP2A6 and UGT2B7 in HIV treatment in African populations: focus on efavirenz and nevirapine. Drug Metab. Rev. 47, 111–123.
7. Giacomelli A, Rusconi S, Falvella FS, et al.: Clinical and genetic determinants of nevirapine plasma trough concentration. SAGE Open Med. 2018; 6: 2050312118780861.
8. Giacomelli, A., Riva, A., Falvella, F.S. et al. Clinical and genetic factors associated with increased risk of severe liver toxicity in a monocentric cohort of HIV positive patients receiving nevirapine-based antiretroviral therapy. BMC Infect Dis 18, 556 (2018).
9. Gonzalez de Requena D, Bonora S, Garazzino S, et al.: Nevirapine plasma exposure affects both durability of viral suppression and selection of nevirapine primary resistance mutations in a clinical setting. Antimicrob Agents Chemother. 2005; 49(9): 3966–3969.
10. Gopalan BP, Mehta K, D'souza RR, et al. Sub-therapeutic nevirapine concentration during antiretroviral treatment initiation among children living with HIV: Implications for therapeutic drug monitoring. PLoS One. 2017; 12(8): e0183080.
11. Haberer JE, Musinguzi N, Bom Y, Siedner MJ, Mocello AR, Hunt PW, et al., Duration of antiretroviral therapy adherence interruption is associated with risk of virologic rebound as determined by real-time adherence monitoring in rural Uganda. J Acquir Immune Defic Syndr. 2015; 70(4):386–392.
12. Hedrich WD, Hassan HE, Wang H. 2016. Insights into CYP2B6-mediated drug-drug interactions. Acta Pharm. Sin B. 6, 413–425.
13. Hegazi A, Bailey RL, Ahadzie B, Alabi A, Peterson K. Literacy, education and adherence to antiretroviral therapy in The Gambia. AIDS Care. 2010; 22(11):1340-1345.
14. Keny National Bureau of Statistics, 2019. 2019 Kenya Population and Housing Census. Volume III: Distribution of Population by Age and Sex. Pg. 12. December 2019. Accessed from https://www.knbs.or.ke/?wpdmpro=2019-kenya-population-and-housing-census-volume-iii-distribution-of-population-by-age-sex-and-administrative-units.
15. Kimulwo MI, Okendo J, Aman RA, Oguwu BR, Kokwaro G, Ochieng DI, Muigai A, Oloa FA, Ochieng W. 2017. Plasma nevirapine concentrations predict virological and adherence failure in Kenyan HIV-1 infected patients with extensive antiretroviral treatment exposure. PLoS ONE 12(2): e0172960.
16. Lamorde M, Byakika-Kibwika P, Tamale WS, Kiweewa F, Ryan M, Amara A, Tija J, Back D, Khoo S, Boffito M, Kityo C, Merry C. Effect of Food on the Steady-State Pharmacokinetics of Tenofovir and...
Emtricitabine plus Efavirenz in Ugandan Adults. *AIDS Res Treat.* 2012; 2012():105980.

19. Mazanderani AH, Murray TY, Sherman GG, et al.: Non-nucleoside reverse transcriptase inhibitor levels among HIV-exposed uninfected infants at the time of HIV PCR testing - findings from a tertiary healthcare facility in Pretoria, South Africa. *J Int AIDS Soc.* 2019; 22(6): e25284.

20. Moosa A, Gengiah TN, Lewis L, Naidoo K. Long-term adherence to antiretroviral therapy in a South African adult patient cohort: a prospective study. *BMC Infect Dis.* 2019 Sep 5;19(1):775.

21. Ngaimisi E, Habtewold A, Minzi O, Makonnen E, Mugusi S, et al., (2013) Importance of Ethnicity, CYP2B6 and ABCB1 Genotype for Efavirenz Pharmacokinetics and Treatment Outcomes: A Parallel-Group Prospective Cohort Study in Two Sub-Saharan Africa Populations. *PLoS ONE* 8(7): e67946.

22. Oluka MN, Okalebo FA, Guantai AN, McClelland R, Graham SM. Cytochrome P450 2B6 genetic variants are associated with plasma nevirapine levels and clinical response in HIV-1 infected Kenyan women: a prospective cohort study. *AIDS Research and Therapy* (2015) 12:10

23. Paterson DL, Swindells S, Mohr J, Brester M, Vergis EN, Squier C, et al., Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. *Ann Intern Med.* 2000;133(1):21–30

24. Reddy S, Thomas L, Santoshkumar K, Nayak M, Mukhopadhyay A, Thangam S. A LC–MS/MS method with column coupling technique for simultaneous estimation of lamivudine, zidovudine, and nevirapine in human plasma. *Journal of Analytical Science and Technology* (2016) 7:17 DOI 10.1186/s40543-016-0097-2

25. Schipani A, Wyen C, Mahungu T, et al. Integration of population pharmacokinetics and pharmacogenetics: an aid to optimal nevirapine dose selection in HIV-infected individuals. *J Antimicrob Chemother* 2011; 66: 1332–1339.

26. Swaminathan S, Ramachandran G, Agibothu Kupparam HK, Mahalingam V, Soundararajan L, Perumal Kannabiran B, Navaneethapandian PG, Shah I, Karunaianandham R, Sikhamani R. Factors influencing plasma nevirapine levels: a study in HIV-infected children on generic antiretroviral treatment in India. *J Antimicrob Chemother.* 2011 Jun;66(6):1354-9.

27. Talam NC, Gatongi P, Rotich J, et al.: Factors affecting antiretroviral drug adherence among HIV/AIDS adult patients attending HIV/AIDS clinic at Moi Teaching and Referral Hospital, Eldoret, Kenya. *East Afr J Public Health.* 2008; 5(2): 74–8.

28. van Leth F, Phanuphak P, Ruxrungtham K, Baraldi E, Miller S, Gazzard B, Cahn P, Lalloo UG, van der Westhuizen IP, Malan DR, Johnson MA, Santos BR, Mulcahy A, Wood R, Reinhart G, Squires K, Cassetti I, Petit D, Raffi F, Katlama C, Murphy RL, Horban A, Dam JP, Hassink E, van Leeuwen R, Robinson P, Wit FW, Lange JM; 2NN Study team. Comparison of first-line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: a randomised open-label trial, the 2NN Study. *Lancet.* 2004. 363(9417):1253-63.

29. Vreeman R, Nyandiko W, Liechty E, Busakhala N, Bartelink I, Savic R, Scanlon M, Ayaya S, Blaschke T. Impact of Adherence and Pharmacokinetic Characteristics on Nevirapine Pharmacokinetics and Exposure Among HIV-Infected Kenyan Children, *JAIDS Journal of Acquired Immune Deficiency Syndromes:* 2014. 67: 277-286

30. Wang J, Kou H, Fu Q, Han Y, Qiu Z, Zuo L, Li Y, Zhu Z, Ye M, Ma Q, Li T. Nevirapine plasma concentrations are associated with virologic response and hepatotoxicity in Chinese patients with HIV infection. *PLoS One.* 2011;6(10):e26739.

31. World Health Organization. 2016. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. 2nd ed. Geneva: available https://apps.who.int/iris/bitstream/handle/10665/208825/9789241549684_eng.pdf?sequence=1 . Accessed February, 2020.

32. Xu C, Ogburn ET, Guo Y, Desta Z. Effects of the CYP2B6*6 allele on catalytic properties and inhibition of CYP2B6 in vitro: implication for the mechanism of reduced efavirenz metabolism and other CYP2B6 substrates in vivo. *Drug Metab Dispos.* 2012;40(4):717–725.