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Research Article

Suboptimal Antiretroviral Drug Levels and Virologic Failures among PLHIV at A Rural Referral Hospital in South Western Uganda: A Descriptive Cross-Sectional Study

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ABSTRACT

Background: Achieving favorable HIV treatment outcomes is a major challenge, particularly due to non-adherence and consequent sub-therapeutic plasma antiretroviral drug levels. This is often complicated by the development of resistant strains due to mutations. Monitoring antiretroviral drug levels in the blood of patients enrolled on ART can reveal if levels are too high, enough, or too low. High levels may lead to dose-dependent side effects and sub-therapeutic levels could promote treatment failure and resistance. In Uganda, as part of routine HIV care, plasma antiretroviral drug level is estimated indirectly by clinic-based pill counts and patient self-reported adherence, which give no evidence of ingested medication. This study aimed at exploring steady-state nevirapine and efavirenz drug levels in HIV patients accessing ART at a rural referral hospital in South Western Uganda.

Methods: This study was nested into a randomized clinical trial that evaluated the effect of *Artemisia annua L.* and *Moringa oleifera* on immunological response and viral load among persons living with HIV (PLHIV). In the parent study, 250 HIV-infected patients with continued immunologic suppression (CD4 count<350 cells/μL) despite a minimum of one-year on ART were enrolled. Out of 250 clinical trial participants, 95 were randomly selected for steady-state efavirenz and nevirapine plasma concentration sampling having taken the last at bedtime. Additionally, CD4 count, HIV load, liver, and renal function tests were determined. Participants were also interviewed for adherence, and factors that affect blood drug levels.

Results: Of the 95 participants sampled, 67 (71%) and 28 (30%) were on efavirenz or nevirapine based ART respectively. The median viral load for participants on the efavirenz regimen was 490 copies/mL (IQR 116, 1900) while that for the participants on the nevirapine regimen was 500 copies/mL (IQR 137, 1270). The median plasma level for the participants on the nevirapine regimen (6.56 mg/L IQR 4.50, 9.80) was higher than that for the participants on the efavirenz regiment (2.54 mg/L IQR 1.47, 5.12). The prevalence of virologic failure among participants sampled on the efavirenz regimen was higher (37%) compared to that of the participants on the nevirapine regimen (21%). 30% of all plasma samples tested had sub-therapeutic levels of either efavirenz or nevirapine, including 2% in which no drugs were detected.

Conclusion: Periodic therapeutic drug monitoring should be incorporated as one of the components in the monitoring of ART adherence to ensure adequate blood levels among adults accessing ART at MRRH, SW Uganda.

Keywords: Antiretroviral drug levels; Efavirenz; Nevirapine; Virologic failures; Uganda

Abbreviations: ART: Antiretroviral Therapy; EFV: Efavirenz; MRRH: Mbarara Regional Referral Hospital; NEV: Nevirapine; PLHIV: People Living With HIV; TDL: Therapeutic Drug Level; SWU: South Western Uganda

INTRODUCTION

Over the past four decades, there has been an unparalleled effort to provide access to antiretroviral therapy (ART) for people living with HIV (PLWH) in sub-Saharan Africa, the region with the highest HIV burden [1]. Despite the significant reduction in mortality among PLHIV receiving combination ART, 16% of the patients

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fail to achieve a sustained virological [2], and immunological response to therapy [1,3]. This could be a result of many factors including poor adherence leading to sub-optimal drug levels and viral mutations among others.

Multiple approaches exist for measuring adherence to ART [4,5]. Self-report of adherence typically overestimates adherence as it may be influenced by social desirability and recall biases. Clinic-based pill counts may be inaccurate if children or caregivers remove and throw away extra medication to appear more adherent. Moreover, patients may not remember to bring their medication containers to appointments, and bottle openings do not always reflect medication ingestion [3,5]. Achieving favorable HIV treatment outcomes is a major challenge, particularly due to non-adherence and the development of strains harboring resistance-associated mutations. The standard approach for monitoring treatment outcomes in patients on ART depends on the measurement of HIV-load over time [3,6]. According to the WHO guidelines, virologic failure is observed when patients sustain a viral load>1000 copies/mL after 6 to 12 months of ART. The persistent high viral load in most cases is due to non-adherence [3].

Monitoring the levels of antiretroviral drugs in the blood of patients can reveal if levels are too high or too low. High levels may lead to dose-dependent side effects while low levels may not prevent the virus from multiplying. Prevention of viral replication is important for the immune system to recover and to fight opportunistic diseases [7]. There is evidence that monitoring of plasma drug concentrations can be a valuable tool in the treatment of HIV-1 [8,9].

Retrospective data from several cohorts propose an association between nevirapine concentration and a rapid-onset and long-lasting virologic response to ARV therapy. Resistance to nevirapine develops rapidly when the drug is administered in suboptimal regimens, and the emergence of the highly drug-resistant virus has been observed 4 weeks after initiation of monotherapy [6,10]. Efavirenz concentrations have data supporting a correlation with virologic outcomes. In a subgroup analysis, in the 2NN study, not having an efavirenz trough plasma concentration of less than 1.1 mg/L had an 89% negative predictive value. That is, participants achieving plasma EFV concentrations above 1.1 mg/L had an 89% likelihood of not failing virologically [6,11]. This study aimed to assess random ARV plasma levels, viral load; CD4 count, liver, and kidney function among PLHIV enrolled on and using efavirenz and nevirapine containing regimens for at least one year.

MATERIALS AND METHODS

Study design

This sub-study is part of an ongoing randomized clinical trial with three study arms aiming at documenting the effect of Artemisia annua L. and Moringa oleifera on immunological response and viral load in PLWH whose CD4 cell counts remain low despite being on ART for one year. This descriptive cross-sectional sub-study aimed to evaluate random ART drug levels among patients participating in the parent study.

Patient enrolment

From January 2018 to June 2019, the main study had enrolled 250 HIV-infected patients aged 18 years and above with continued immunologic suppression (CD4 count<350 cells/μL) despite a minimum of one-year on efavirenz 600 mg a day in or nevirapine 200 mg twice a day in combination with other antiretroviral agents. The study site was the HIV clinic at Mbarara Regional Referral Hospital (MRRH) located in South Western Uganda (SWU). From the total enrolled participants, 95 participants, 67 on efavirenz, and 28 on nevirapine regimens were randomly selected at enrolment for steady-state efavirenz and nevirapine plasma concentration measurement having taken the last dose at bedtime. Blood samples were also collected from the participants for CD4 count, HIV load, liver, and renal function determination. The participants were asked to state if they were adhering to treatment before enrolment into this study. Participants were also interviewed for factors that affect blood drug levels.

Baseline data collection

Questionnaires were administered to collect socio-demographic data and treatment information including factors that affect blood drug levels. Blood samples for viral-load testing and CD4 count determination, renal, and liver function tests at baseline were drawn from each participant.

We defined virologic failure as a single viral load measure of>1000 copies/mL at enrolment [12]. Liver toxicity was defined as laboratory abnormalities above the normal range. From each study participant single drug concentration blood sample was collected. Samples were labeled for either efavirenz or nevirapine concentration analysis depending on the donor ART history. All samples were centrifuged at 3000 rpm for 10 minutes. Plasma was separated and stored at 800°C. Drug quantification assays were performed at the Department of Pharmacology and Therapeutics, School of Biomedical Sciences, College of Health Sciences Makerere University Kampala-Uganda. Viral load, CD4 count, renal and liver function tests at were done at Devine Mercy Laboratory Mbarara-Uganda.

Drug bioanalysis

Plasma efavirenz and nevirapine were determined by reverse-phase HPLC with ultraviolet (UV) detection as previously described by Mukonzo et al., Minzi et al., [13,14]. The HPLC machine used consisted of a system controller (model SCL-10AVP), a pump (model LC-10ATVP), an auto-injector (model SIL-10ADVP), and a spectrophotometric UV–vis detector (model SPD-10AVP) all supplied by Shimadzu co-operation Kyoto Japan.

For efavirenz determination, the column used: Eclipse 7.5 cm × 4.6 mm 3 μm (Agilent Technologies, USA). The mobile phase consisted of 30% acetonitrile, 30% methanol, 4 mmol l^{-1} potassium hydroxide, and 10 mmol l^{-1} acetic acid (pH 4.3). Plasma proteins were precipitated with acetonitrile. The injection volume was 20 μl. The retention time approximately 7.6 min, run time: 10 min, detected at UV-VIS 1, 210 nm, UV-VIS 2220 nm, and temperature 25° C. This method is linear, with a within-day coefficient of variation of 3.2, 3.3 and 5.1% at concentrations of 0.63 mg/L (n=17), 2.53 mg/L (n=17), and 6.31 mg/L (n=16), respectively, with a between-day coeficient of variation of 4.1% (n=50). The limit of quantification was set at 0.2 mg/L, flow rate: 1.0 mL/min.

The column used for the determination of plasma nevirapine was zorbax eclipse XBD-phenyl 5 μm C18, 4.6 mm × 150 mm (Agilent Technologies, USA). The mobile phase consisted of 75% phosphate buffer and 25% acetonitrile. Plasma

precipitation used 0.55 M perchloric acid. The supernatant 200 µL) was eluted at 50 µL min^{-1} for 15 min. The retention time for nevirapine was 7.5 min as detected at UV-VIS 280 nm, flow rate was 0.6 mL/min. The method was linear, with a within-day coefficient of variation of 3.0, 2.3 and 4.2% at concentrations of 1.0 µg/mL (n=20), 5.0 µg/mL (n=17), and 16.0 µg/mL (n=16), respectively, and a between-day coefficient of variation of 3.7% (n=50). The limit of quantification was set at 0.05 mg/L.

The column used for the determination of plasma nevirapine was zorbax eclipse XBD-phenyl 5 µm C18, 4.6 mm × 150 mm (Agilent Technologies, USA). The mobile phase consisted of 75% phosphate buffer and 25% acetonitrile. Plasma precipitation used 0.55 M perchloric acid. The supernatant 200 µL was eluted at 50 µL min^{-1} for 15 min. The retention time for nevirapine was 7.5 min as detected at UV-VIS 280 nm, flow rate was 0.6 mL/min. The method was linear, with a within-day coefficient of variation of 3.0, 2.3 and 4.2% at concentrations of 1.0 µg/mL (n=20), 5.0 µg/mL (n=17), and 16.0 µg/mL (n=16), respectively, and a between-day coefficient of variation of 3.7% (n=50). The limit of quantification was set at 0.05 mg/L.

Analysis of baseline data

Data collected were entered into an excel sheet version 2016 and was exported to STATA version 13. Descriptive statistics were used to summarise the data. Fisher's exact test was used to determine the association between alcohol use and CD4 count, viral load as well as efavirenz and nevirapine blood levels.

RESULTS

Of the 95 participants whose random nevirapine and efavirenz levels were analyzed, 31 (33%) were male and 64 (67%) were female, with 67 (71%) patients on the efavirenz regimen and 28 (30%) patients on the nevirapine regimen. The age of the participants ranged from 16 to 66 years, with the most representative age group ranging from 16 to 35 years old (Table 1).

Table 1: Participants characteristics at baseline on ART.

Variable	Category	Frequency (%)
Gender	Male	31 (33%)
	Female	64 (67%)
Age (years)	Mean	40
	<35 years	37 (39%)
	35–49 years	36 (38%)
	≥50 years	22 (23%)
ART	NEV	28 (30%)
	EFV	67 (71%)

The median viral load for participants on the efavirenz regimen was 490 copies/mL (IQR 116,1900) while that for the participants on the nevirapine regimen was 500 copies/mL (IQR 137,1270). The median CD4 count for the participants on the efavirenz regimen, 233 (IQR 179,237), was comparable to that of the participants on the nevirapine regimen, 240 (IQR 182,302). The median plasma level for the participants on the nevirapine regimen (6.56 mg/L IQR 4.50, 9.80) was higher than that for the participants on the efavirenz regiment (2.54 mg/L IQR 1.47, 5.12) (Table 2).

Table 2: Participants VL and CD4 count and ARV drug levels at baseline.

Variable (Unit)	Regimen	Median (IQR)	Range
CD4 (cells/µL)	EFV	233 (179, 237)	18 – 341
	NEV	240 (182, 302)	19 – 328
VL (copies/mL)	EFV	490 (116, 1900)	0 – 389000
	NEV	500 (137, 1270)	0 – 942000
Drug level (mg/L)	EFV	2.54 (1.47, 5.12)	0 – 31.81
	NEV	6.56 (4.50, 9.80)	0 – 33.15

Using a threshold of 3 mg/L for optimal therapeutic nevirapine level [15,16] and 2 mg/L for optimal therapeutic efavirenz level [17], 30% (28/95) of all plasma samples tested had sub-therapeutic levels of either efavirenz or nevirapine, including 2% (2/95) in which no drugs were detected. More females than men had drug concentrations above the threshold i.e. for efavirenz plasma levels was 28 (65%) *vs* 15 (35%), while for nevirapine plasma level was 18 (75%) *vs* 6 (25%) respectively (Table 3).

Virologic failure was observed in 37% (16/43) of participants on the efavirenz regimen with concentrations higher than 2 mg/L *vs* 21% (5/24) participants on nevirapine regimen with concentrations higher than 3 mg/L (Table 3).

Liver toxicity (elevated liver enzymes) was observed in 89% (25/28) of the participants on the nevirapine regimen with concentrations higher than 3 mg/L while only 45% (30/67) of the participants on the efavirenz regimen with a concentration above 2 mg/L had liver toxicity (Table 3).

Effect of alcohol consumption on CD4 count, viral load, and ARV drug levels

There were no statistically significant differences in CD4 count (p=0.172), Viral load (p=0.239), efavirenz level (p=0.955) and nevirapine level (p=0.472) between alcohol consumers and non-alcohol consumers although generally, participants who did not drink alcohol had more CD4 counts and lower viral loads than those who consumed alcohol (Table 4).

Table 3: Efavirenz and nevirapine concentration levels.

		67 (71%)	EFV concentrations	NEV concentrations	67 (71%)
		<2 mg/L n=24	>2 mg/L n=43	<3 mg/L n=4	>3 mg/L n=24
Analysis per patient					
Gender	Male (%)	10 (42)	15 (35)	0	6 (25)
	Female (%)	14 (58)	28 (65)	4 (100)	18 (75)
Age (years)	<35 (%)	9 (38)	20 (47)	2 (50)	6 (25)
	35–50 (%)	10 (42)	15 (35)	1 (25)	10 (42)
	>50 (%)	5 (21)	8 (19)	4 (100)	8 (33)

CD4 (<100 cells/μL)	n (%)	1 (4)	5 (12)	0	2 (8)
VL(>1000 copies/mL)	n (%)	6 (25)	16 (37)	2 (50)	5 (21)
ASP (above normal)	n (%)	2 (8)	7 (16)	0	8 (33)
ALT (above normal)	n (%)	2 (8)	12 (28)	1 (25)	10 (42)
Creatinine (above normal)	n (%)	7 (29)	11 (26)	2 (50)	7 (29)

Table 4: Effect of alcohol consumption on CD4 count, Viral load and ARV drug levels

Analysis per patient		Don't drink alcohol (%)	Drink alcohol (%)	P-value
CD4	<100 cells/μL	5.7 (4/70)	16.0 (4/25)	0.172
	>100 cells/μL	94.3 (66/70)	84.0 (21/25)	
VL	<1000 copies/mL	74.3 (52/70)	56.0 (14/25)	0.239
	>1000 copies/mL	25.7 (18/70)	44.0 (11/25)	
EFV	<2 mg/L	29.2 (14/48)	31.6 (6/19)	0.955
	>2 mg/L	70.8 (34/48)	68.4 (13/19)	
NEV	<3 mg/L	50.0 (11/22)	50.0 (3/6)	0.472
	>3 mg/L	50.0 (11/22)	50.0 (3/6)	

DISCUSSION

Clinicians are often confronted with treatment failure or side-effects and need methods to evaluate drug exposure in patients [11]. Adherence to antiretroviral medications prescribed for the treatment of HIV is central to the effective management of the disease and can predict plasma drug levels in HIV patients who are on ART if adherence is high. Efavirenz and nevirapine are extensively used in Uganda as part of the first-line therapy, and viral load monitoring for ART management is not always accessible and affordable. Moreover, drug level and toxicity monitoring are not included in the clinical management of HIV patients. Our findings indicate that 30% of all the patients had sub-therapeutic drug levels of either efavirenz or nevirapine, including those in whom no drugs were detected implying poor adherence to treatments by the patients. These findings are in agreement with a study carried out in Northwestern Tanzania [18] that also reported 28.3% of patients had sub-therapeutic levels of efavirenz and nevirapine.

In a study conducted in China to assess the relationship between mean efavirenz (EFV) plasma concentration and clinical effect, EFV plasma concentrations above 2 mg/L appeared to suppress HIV replication more effectively than concentrations below 2 mg/L [17]. The same study concluded that the emergence of EFV-resistant strains is likely to be facilitated by exposure to sub-therapeutic drug levels, and treatment failure is more frequent in patients with levels lower than 2 mg/L compared with those with higher levels. These findings are in agreement with our study.

We observed that more patients on the efavirenz regimen had a virologic failure (37%) than those on the nevirapine regimen (21%). This is probably because efavirenz is associated with CNS side effects causing fewer adherences to patients on this regimen.

It is well known that virtually all of the antiretroviral drugs available, except tenofovir, lamivudine, and abacavir, can produce a mild elevation in liver function tests. As observed by Ena J et al, the number of patients with elevated ASP who had elevated nevirapine levels (>3 mg/L) was double that of the people on efavirenz (>2 mg/L) [19]. This is in agreement with Bruck S et al., who stated that during therapy with NVP or EFV increases of liver enzymes were not unusual [20]. Our findings are in agreement with the above studies. In our study, more patients on the nevirapine regimen had liver toxicity (89%) than the patients on the efavirenz regimen (45%).

HIV management in Uganda follows the existing national guidelines that include periodic monitoring of viral load and CD4 levels do not include Therapeutic drug level (TDL) monitoring as is often the case in most advanced countries. If ARV drug plasma levels and viral load are used in the routine management of HIV positive patients, treatment failure due to toxicities, concomitant clinical conditions, and development of virologic failure can be detected early leading to early regimen switching preventing the deterioration of the immune system hence giving better treatment outcomes.

It has been reported that individuals with HIV infection may be nonadherent to ART when consuming alcohol due to beliefs regarding adverse interactions between alcohol and ART or because their providers have advised them not to consume alcohol while taking ART [21]. Our findings though not statistically significant show that, generally, patients who did not consume alcohol had slightly lower viral loads (p=0.239) and slightly higher CD4 counts (p=0.172) than those who consumed (Table 4). This is probably because alcohol consumption may reduce adherence to ART, leading to decreased ART effectiveness and, ultimately, increased HIV-related mortality [22]. The difference in efavirenz drug levels among alcohol drug users was not statistically significant (p=0.955). Efavirenz is known to induce the liver enzyme cytochrome P450 (CYP 450) 3A4 that might contribute to the decreased alcohol plasma concentrations.

Since CYP 3A4 is a minor pathway for alcohol metabolism, medications (such as efavirenz) that induce this enzyme function might result in small corresponding changes in the overall metabolism of alcohol [21]. This might explain why alcohol consumption did not significantly affect efavirenz plasma concentration.

CONCLUSION AND RECOMMENDATION

Our study concludes that a large number of patients on nevirapine and efavirenz-based regimen in South Western Uganda have sub-therapeutic drug levels and viral load copies>1000 copies mL. This situation presents a brewing pot

for resistant viral strain selection which in the long run will lead to the two regimens being rendered useless. Regular plasma drug and viral load monitoring are highly recommended in ART care in South Western Uganda.

LIMITATIONS

Our study aimed mainly at measuring efavirenz and nevirapine plasma levels. We took a single blood sample from each patient at the time of enrolment. We, however, did not measure the levels of adherence.

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AUTHORS CONTRIBUTION

STS designed the study, analyzed the data, and drafted the manuscript.

ECA, OPE, and FR designed the study and reviewed the manuscript. JKM conducted the HPLC analysis of the drug levels. All authors read and approved the final manuscript.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request.

ETHICAL APPROVAL

The study was approved by the Mbarara University of Science and Technology Research Ethics Committee with registration number 27/05-17 and Uganda National Council for Science and Technology with approval number NCT03366922. Written informed consent was obtained from each participant and all the procedures used were per the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983. Participants' identities were kept anonymous throughout the study processes.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Ayalew MB, Kumilachew D, Belay A, Getu S, Teju D, Endale D, et al. First-line antiretroviral treatment failure and associated factors in HIV patients at the University of Gondar Teaching Hospital, Gondar, Northwest Ethiopia. HIV AIDS (Auckl). 2016;8:141-146.
2. Rajian M, Gill P, Chaudhary U. Prevalence of virological failure amongst WHO- defined immunological failure HIV patients on first line of highly active antiretroviral therapy in a tertiary care hospital in Haryana, India. Int J Res Med Sci. 2016;4(5):16131619.
3. Chendi BH, Assoumou MC, Jacobs GB, Yekwa EL, Lyonga E, Mesembe M, et al. Rate of viral load change and adherence of HIV adult patients treated with Efavirenz or Nevirapine antiretroviral regimens at 24 and 48 weeks in Yaoundé, Cameroon: a longitudinal cohort study. BMC Infect Dis. 2019;19(1):1-8.
4. Castillo-Mancilla JR, Haberer JE. Adherence measurements in HIV: new advancements in pharmacologic methods and real-time monitoring. Curr HIV/AIDS Rep. 2018;15(1):49-59.
5. Olds PK, Kiwanuka JP, Nansera D, Huang Y, Bacchetti P, Jin C, et al. Assessment of HIV antiretroviral therapy adherence by measuring drug concentrations in hair among children in rural Uganda. AIDS care. 2015;27(3):327-332.
6. Kredo T, Van der Walt JS, Siegfried N, Cohen K. Therapeutic drug monitoring of antiretrovirals for people with HIV. Cochrane Database Syst Rev. 2009(3).
7. Mills EJ, Nachega JB, Buchan I, Orbinski J, Attaran A, Singh S, et al. Adherence to antiretroviral therapy in sub-Saharan Africa and North America: a meta-analysis. Jama. 2006;296(6):679-690.
8. Leth FV, Kappelhoff BS, Johnson D, Losso MH, Boron-Kaczmarska A, Saag MS, et al. Pharmacokinetic parameters of nevirapine and efavirenz in relation to antiretroviral efficacy. AIDS Res Hum Retroviruses. 2006;22(3):232-239.
9. Liu X, Ma Q, Zhang F. Therapeutic drug monitoring in highly active antiretroviral therapy. Expert Opin Drug Saf. 2010;9(5):743-758.
10. Veldkamp AI, Weverling GJ, Lange JM, Montaner JS, Reiss P, Cooper DA, et al. High exposure to nevirapine in plasma is associated with an improved virological response in HIV-1-infected individuals. Aids. 2001;15(9):1089-1095.
11. Marzolini C, Telenti A, Decosterd LA, Greub G, Biollaz J, Buclin T. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. Aids. 2001;15(1):71-75.
12. WHO definitions of clinical, immunological and virological failure for the decision to switch ART regimens. 2013.
13. Mukonzo JK, Röshammar D, Waako P, Andersson M, Fukasawa T, Milani L, et al. A novel polymorphism in ABCB1 gene, CYP2B6* 6 and sex predict single‐dose efavirenz population pharmacokinetics in Ugandans. Br J Clin Pharmacol. 2009;68(5):690-699.
14. Minzi OM, Ngaimisi E. Bioanalytical method for determination of nevirapine in vivo in resource constrained laboratories. J Chem Pharm Res. 2010;2:431-439.
15. Wang J, Kou H, Fu Q, Han Y, Qiu Z, Zuo L, et al. Nevirapine plasma concentrations are associated with virologic response and hepatotoxicity in Chinese patients with HIV infection. PloS one. 2011;6(10):26739.
16. Lamorde M, Byakika-Kibwika P, Okaba-Kayom V, Ryan M, Coakley P, Boffito M, et al. Nevirapine pharmacokinetics when initiated at 200 mg or 400 mg daily in HIV-1 and tuberculosis co-infected Ugandan adults on rifampicin. J Antimicrob Chemother. 2011;66(1):180-183.
17. Sun J, Chen J, Yao Y, Zhang R, Zheng Y. Minimum effective plasma concentration of efavirenz in treatment-naive Chinese HIV-infected patients. Int J STD AIDS. 2010;21(12):810-813.
18. Gunda DW, Kasang C, Kidenya BR, Kabangila R, Mshana SE, Kidola J, et al. Plasma concentrations of efavirenz and nevirapine among HIV-infected patients with immunological failure attending a tertiary hospital in North-western Tanzania. PLoS One. 2013;8(9):75118.
19. Ena J, Amador C, Benito C, Fenoll V, Pasquau F. Risk and determinants of developing severe liver toxicity during therapy with nevirapine-and efavirenz-containing regimens in HIV-infected patients. Int. J. STD AIDS. 2003;14(11):776-781.

20. Brück S, Witte S, Brust J, Schuster D, Mosthaf F, Procaccianti M, et al. Hepatotoxicity in patients prescribed efavirenz or nevirapine. Eur J Med Res. 2008;13(7):343-348.

21. McCance-Katz EF, Gruber VA, Beatty G, Lum PJ, Rainey PM. Interactions between alcohol and the antiretroviral medications ritonavir or efavirenz. J Addict Med. 2013;7(4):264-270.

22. Braithwaite RS, Bryant KJ. Influence of alcohol consumption on adherence to and toxicity of antiretroviral therapy and survival. Alcohol Research & Health. 2010;33(3):280.