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ORIGINAL ARTICLE

Sub therapeutic drug levels among HIV/TB co-infected patients receiving Rifampicin in northwestern Tanzania: A cross sectional clinic based study

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Plasma ARV drug levels;
Therapeutic drug monitoring

Abstract *Background:* Tuberculosis/Human Immunodeficiency Virus (TB/HIV) is a very common co-infection which carries a high mortality rate. Though World Health Organization recommends co-treatment of TB/HIV to improve its outcome, Rifampicin potentially induces metabolism and sub-therapeutic antiretroviral plasma levels of non nucleoside reverse transcriptase inhibitors and protease inhibitors which may cause inadequate virological suppression if corrections are not timely done. In Tanzania Therapeutic drug monitoring is not done; so the proportion of sub-therapeutic ARV plasma levels among TB/HIV patients co-treated with anti-tuberculous drugs is not known. The aim of this study was therefore to determine the magnitude and risk factors of sub-therapeutic ARV plasma levels among adult HIV patients co-treated with anti tuberculous Medications.
Materials and methods: A cross sectional hospital based study was conducted among adult HIV patients on ARV and TB co-treatment for at least one month. Patients were serially enrolled through routine HIV care and treatment services until the sample size was reached. The information about demographic, clinical and adherence level, Anti-TB duration, viral load, baseline and enrollment CD4 counts, Hepatitis B co-infection and ARV plasma levels was collected and analyzed using STATA 12 software.

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Results: In total 118 patients were included in this study; of whom 26 (22%) had sub-therapeutic ARV plasma levels. The sub-therapeutic ARV levels were independently associated with adherence <95% (OR = 6.8, $p = 0.001$), female gender (OR = 3.4, $p = 0.028$) and virological failure (OR = 3.8, $p = 0.016$). NVP based regimen was associated with sub-therapeutic drug levels on univariate model (OR = 2.1, $p = 0.010$).

Conclusion: The magnitude of sub-therapeutic ARV plasma levels is high among adult HIV/TB co-infected patients on anti-TB co-treatment in Tanzania. These patients stand a high risk of inadequate virological suppression with a potential resistance development and a long term poor clinical outcome. Identifying at risk patients and adherence enhancement could potentially improve the overall outcome of this subgroup of patients in resource restricted setting like ours where TDM is not available.

1. Introduction

Tuberculosis/Human immunodeficiency virus (TB/HIV) co-infection has been a common phenomenon for decades, causing a substantially high morbidity and mortality with Tuberculosis ranking as the most common opportunistic infection and the most common cause of mortality among people living with HIV/AIDS (PLWHA) especially in resource restricted countries.[1,2] In the year 2013 alone about 1.1 million new cases of TB were reported in HIV positive patients globally where Majority of them (up to 78%) occurred in Africa.[3] Tuberculosis occurs as the first manifestation of HIV/AIDS in more than 50% of HIV positive patients [4] and deaths that are linked to TB are significantly high especially in sub-Saharan Africa where in some countries this rate is reported to be in excess of 50%.[5]

Early initiation of Antiretroviral therapy (ART) in the course of TB treatment has been shown to have a mortality benefit [6,7] and WHO strongly recommends on co-treatment of HIV/TB co-infection,[8] with a rapid scaling up of Antiretroviral therapy programs especially in resource restricted countries, where tuberculosis is for the most part the widespread opportunistic disease.[9,10] In these areas thus ART is regularly initiated when patients are being treated for tuberculosis,[11,12] with a goal line being to provide an effective and safe Antiretroviral therapy and anti-tuberculosis management which is efficient enough to cure and prevent recurrence and resistance.[2,13]

Despite this overall success, HIV and TB co-treatment faces a number of important challenges including induction of sub-therapeutic levels of both Non Nucleotide Reverse Transcriptase Inhibitors (NNRTIs) and Protease Inhibitors (PIs). Rifampicin which is the most important component of anti tuberculous medications is remarkable for its induction effect on CYT P450 iso-enzymes which may adversely increase the metabolism and disposition of both NNRTIs and PIs which can potentially cause inadequate plasma levels of these drugs and severely limiting the treatment options for optimal Highly Active Antiretroviral Therapy (HAART) regimens [14–19] especially in resource limited settings. Whereas it has been established from prior studies that Rifampicin may be a cause of significant suboptimal levels of both NNRTIs and PIs,[20,21] sub-therapeutic ARV plasma levels as a consequence have been demonstrated to be associated with inadequate virological suppression which may subsequently lead into selection of resistant strains and a long term inadequate immune recovery and overall poor clinical outcome.[22,23]

In developed countries this challenge is overcome using therapeutic drug monitoring (TDM) that is readily available for routine practical use where the patients' NNRTIs and PIs plasma levels are monitored for any adverse drug levels, and corrections of dosages are timely done to improve the therapeutic outcomes.[24,25] TDM has been usefulness in a number of clinical settings including monitoring of ARV plasma levels in TB/HIV co-treatment. In this regard a better treatment outcome has been documented among patients whose treatment was TDM guided than those whose ARV plasma levels were not monitored.[25,26] Even though TDM is not done in most of the resource limited countries, the available studies from these settings demonstrate that a significant proportion of HIV patients co-treated with anti Tuberculous drugs (Rifampicin) have sub-therapeutic NNRTIs and PIs plasma levels and some of the locations have reported even higher rates of sub-therapeutic ARV (NNRTIs and PIs) plasma levels than most of resource rich countries.[21,27–29]

In Tanzania no study has ever reported ARV plasma levels in HIV positive patients who are co-treated with Rifampicin. The current study was therefore designed to determine the magnitude and the associated risk factors of sub-therapeutic NNRTIs (Efavirenz and Nevirapine) and PIs (Lopinavir) plasma levels among adult HIV positive patients who were co-treated with anti-TB in northwestern Tanzania. The results from this study will be useful to assist the overall optimization of management of patients on ARV/anti-TB co-treatment especially in resource limited settings. Also the results from this study will provide a base for further studies on the subject and add to the existing body of knowledge regarding ARV plasma levels especially in resource limited countries.

2. Materials and methods

2.1. Study design and setting

This was a cross-sectional hospital based study which was done between April 2012 and July 2013 at Bugando Medical Centre (BMC) at Care and Treatment Center (CTC) in Mwanza, Tanzania. BMC is a tertiary and teaching hospital for the North Western part of Tanzania. It has a capacity of 1000 beds, and it serves around 13 million people. At Bugando,

CTC services started a way back 2004, and are routinely done as part and parcel of outpatient activities. Currently the center serves a total of more than 10,000 patients, whereby more than 4000 of them are active on ART. Tuberculosis screening is routinely done on daily bases and as of now about 300 patients are on TB/HIV co-treatment.

2.2. Study population

This study involved adult HIV and TB co-infected patients diagnosed to have either Pulmonary TB (smear positive and smear negative) or Extra Pulmonary Tuberculosis (EPTB) according to WHO TB diagnosis guideline 2010 [30] and put on anti-TB Treatment. The HIV diagnosis was as per WHO guidelines and all patients were treated with standard dose of Nevirapine 200 mg twice daily or Efavirenz 600 mg once daily or Lopinavir/r 400/100 mg twice daily and Rifampicin 600 mg or 450 mg once daily for patients weighing more than 45 kg and less than 45 kg respectively. All patients aging over 18 years and co-treated with ARV and anti-tuberculosis medications for at least one month were included in this study.

2.3. Sample size, patients' enrollment and data collection

A minimum sample of 100 patients was estimated from cross sectional studies' formula by Leslie Kish, assuming 30% of adult HIV positive patients co-treated with Rifampicin had subtherapeutic ARV levels [21,28] at an allowable error of 0.09. After a written informed consent a structured questionnaire was used to collect information about demographic data, body mass index (BMI), date of HIV diagnosis, date of ART initiation, the ART regime, ART adherence level, baseline CD4 and on study CD4 count, Hepatitis B status, type of tuberculosis, time on anti tuberculous medication, viral load and plasma NNRTIs and PIs levels.

The ART adherence level in the last 30 days was assessed using pill counts.[31] The pill counts were performed by the study pharmacist, who counted the number of remaining pills at each drug refill visit. Pill count-based adherence was assessed using the formula [Adherence = (Number of pills dispensed -Number of pills returned 100)/(Number of pills prescribed daily $\times$ Number of days between pharmacy visits)]. Adequate adherence level was defined as a value $\geqslant$95% pills whereas poor adherence was defined as a value $\leqslant$95%. The patients were instructed to take their medication at night as prescribed and come the following morning for blood sample collection before their next ART dose. Two blood samples were drawn, one for viral load which was done at BMC main laboratory and the other sample was sent to Germany for TDM to determine the plasma concentrations of Efavirenz, Nevirapine and Lopinavir.

2.4. Sample collection, processing and analysis

For each patient, 5 ml of whole blood was collected in plasma EDTA bottles for TDM, approximately 8–12 h after the last dose of antiviral drugs and just before the next dose was done. The samples were immediately centrifuged at 3000 rpm for 3 min to obtain plasma that was transferred into cryovials. The cryovials were stored at -20 °C before shipment. The samples were packed and shipped to Germany in cold boxes with cooling packs maintaining a temperature of -30 °C. The plasma concentrations of NVP, EFV, and LPV were determined by a sensitive validated simultaneous assay using reverse-phase (Zorbax XD8-CI8; Agilent Technology) high performance liquid chromatography (HP 1100; Agilent Technology), coupled with tandem mass spectrometry (MS-MS) (API 2000; Applied Biosystems) as described previously.[32] An additional 5 ml of whole blood was collected in a tube supplemented with EDTA (BD Biosciences) for plasma preparation and sent to BMC main laboratory for viral load analysis using COBAS AmpliPrep/COBAS TaqMan (Roche Molecular Systems, USA) according to manufacturer's guidelines as described previously.[33]

2.5. Data management and analysis

Data were managed using Epi Data 3.1 (CDC Atlanta, USA) and analysis was done using STATA version 12 (College Station, Texas, USA). ARV drug concentrations were recorded as continuous variables. The therapeutic ranges in ng/ml were defined as 1000–4000 for EFV, and 3400–8000 and 3000–7000 for NVP and LPV respectively; thus three categories of ARV plasma drug concentrations were defined as used in other studies,[24] and any levels below the lower limit of the respective drug were coded as being sub-therapeutic ARV level for that drug and drug levels that were within therapeutic range were coded as therapeutic ARV levels, while those that were above the upper limit of therapeutic range were coded as supra-therapeutic ARV levels. Categorical variables were summarized as proportion and the significance of the difference in distribution within the categories of ARV plasma drug concentrations was assessed using Pearson's Chi-square test or Fisher's exact test where appropriate. We used probability plots and Shapiro-Wilk normality test to assess the normality of continuous variables. Parametric continuous data were summarized as mean with standard deviation and the significance of difference in means within categories of ARV plasma drug concentrations was assessed using Student's *t*-test. Non-parametric continuous data were summarized as median with interquartile range and the difference in medians within the categories of ARV plasma drug concentrations was compared using Wilcoxon rank-sum test. The odds ratios (ORs) and 95% confidence intervals (CIs) of risk factors associated with sub-therapeutic ARV plasma levels were calculated using univariate logistic regression model followed by multivariate logistic regression model. All factors associated with sub-therapeutic ARV plasma levels in the univariate model with p-values less than 0.05 were considered for inclusion in the multivariate model. A stepwise approach was used to derive a Parsimonious model, and all associated factors in the final model were considered significant if the P-value was less than 0.05.

3. Ethical consideration

The permission to conduct this study and publish the results was found from The Catholic University of Health and Allied Sciences and Bugando Medical Centre (CUHAS/BMC) joint ethics review board. Written consent was obtained from all study participants. Patients' identifiers were not included to maintain confidentiality.

4. Results

4.1. Baseline demographic, clinical and laboratory characteristics of 118 adult HIV positive patients cotreated with tuberculosis drugs at Bugando CTC

A total of 118 patients were included in this study, where more than 58% of these patients were females with a median age of 38 [32–43] years. The median time on anti-TB was 5 [2–6] months, and on ARV 10 [3–22] months with an adherence rate of >95% in more than 79% of the studied patients (Table 1). The most common regimen used was TDF + FTC + EFV 80 (67.8%), followed by AZT + 3TC + EFV 24 (20.3%) which shows that most patients 104 (88%) were on EFV based regimen as summarized in Fig. 1.

4.2. Subtherapeutic ARV plasma levels and associated factors among 118 adult HIV positive patients co-treated with anti-tuberculosis drugs

In total 26 (22%) of the studied patients had sub-therapeutic ARV plasma levels (Table 1), and by regimen 19 (28.3%), 57 (54.8%), and 28 (26.9%) of those who were on EFV based regimen had sub-therapeutic, therapeutic and supra-therapeutic EFV plasma levels respectively, whereas 4 (40%), 5 (50%), and 10 (10%) had sub-therapeutic, therapeutic and supra-therapeutic LPV plasma levels respectively. Furthermore Sub-therapeutic and therapeutic NVP plasma levels were found in 3 (75%) and 1 (25%) respectively and there was no study participant who had supra-therapeutic NVP plasma levels (Fig. 2). Fig. 3 summarizes the distribution of variables by plasma ARV levels. Several factors were tested for association with sub-therapeutic ARV plasma levels. On a univariate model the sub-therapeutic ARV plasma levels were strongly associated with a female gender (OR = 2.9, $p = 0.031$), adherence level of less than 95% (OR = 4.5, $p < 0.002$), virological failure (OR = 2.9, $p = 0.027$), and NVP based regimen (OR = 2.1, $p = 0.010$), whereas on a multivariate model only Female gender (OR = 3.4, $p = 0.028$) and adherence <95% (OR = 6.8, $p = 0.001$) remained independently associated with sub-therapeutic ARV plasma levels. Moreover the patients with sub-therapeutic ARV plasma levels were consequently more likely to have virological failure (OR = 3.8, $p = 0.016$) (Table 2). However there was no significant statistical association found between sub-therapeutic ARV plasma levels and age, geographical location, BMI, WHO clinical stage, CD4 levels, Hepatitis B co-infection, time on ART, time on anti-TB, and viral load of more than 400 copies/μl and TB category.

Table 1 The baseline demographic, clinical and laboratory characteristics of 118 adult HIV Positive patients attending CTC at Bugando with ARV and anti-TB Co-treatment.

Factor	Number (%) or median [IQR]
Gender	
Female	69 (58.5)
Males	49 (41.5)
Age (years)	38 [32–43]
Geographical location	
Urban	52 (44.1)
Rural	66 (55.9)
WHO stage	
Stage 3	75 (63.6)
Stage 4	43 (36.4)
BMI index(kg/M^2)	
Under weight	22 (18.6)
Normal weight	79 (67.0)
Overweight	10 (8.5)
Obese	07 (5.9)
Hepatitis B	
Positive	09 (07.6)
Negative	109 (92.4)
ARV regimes	
EFV based	104 (88.1)
LPV based	10 (08.5)
NVP based	4 (03.4)
Subtherapeutic ARV	26 [22.0]
Subtherapeutic regimen	
EFV	19 (18.3)
LPV	4 (40.0)
NVP#	3 (75.0)
Duration on ART (months)	10 [3–22]
Adherence level	
>95%	92 (79.6)
<95%	24 (20.4)
Duration on anti-TB (months)	5 [2–6]
TB category	
PTB Sputum Positive	71 (60.2)
PTB Sputum Negative	24 (20.3)
EPTB	23 (19.5)

*ART: antiretroviral therapy, CD4: cluster of differentiation 4, CTC: Care and Treatment center; EFV: Efavirenz, EPTB: extra pulmonary TB, LPV: Lopinavir, NVP: Nevirapine, PTB: Pulmonary Tuberculosis.

5. Discussion

The objective of this study was to determine the proportion and risk factors of sub-therapeutic ARV plasma levels among adult HIV positive patients co-treated with anti-TB. In this study, 26 (22%) of the patients co-treated with anti-TB had subtherapeutic ARV plasma levels, and by regimen 19/104 (18.3%) had subtherapeutic EFV levels, while 3/4 (75%) and 4/10 (40%) had subtherapeutic NVP and LPV levels respectively. The sub-therapeutic ARV plasma levels were independently associated with female gender and adherence level of less than 95% on a multivariate analysis; otherwise, NVP based regimen was only strongly in association with sub-therapeutic ARV plasma levels on univariate model alone.

Comparable ranges of sub-therapeutic ARV levels have been reported in different studies across the world assessing plasma concentration of NNRTIs or PIs. In previous studies sub-therapeutic EFV plasma levels were reported with a prevalence varying between 20% and 32%. For example in London Sathia et al. reported EFV sub-therapeutic plasma levels in 20% of patients who were co-treated with Rifampicin,[21] while in Kenya [34] this was reported in 32% of patients who were co-treated with Rifampicin. The magnitude of sub-therapeutic

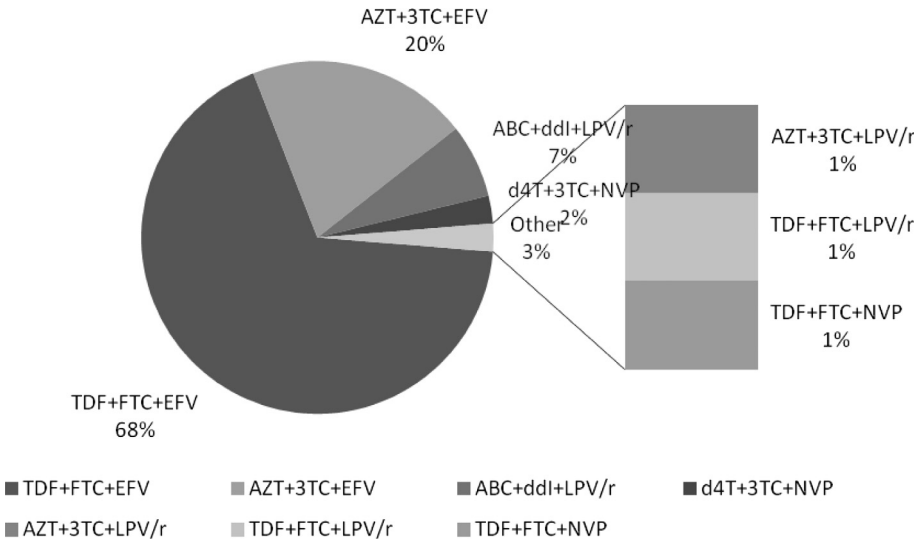

Figure 1 The combined Anti retroviral therapy regimens. *__ABC__: Abacavir; **AZT:** Zidovudine; **d4T:** Stavudine; **ddI:** Didanosine; **EFV**: Efavirenz; **FTC:** Emtricitabine; **LPV:** Lopinavir; **NPV:** Nevirapine, **r:** ritonavir; TDF: Tenofovir; **3TC:** Lamivudine.

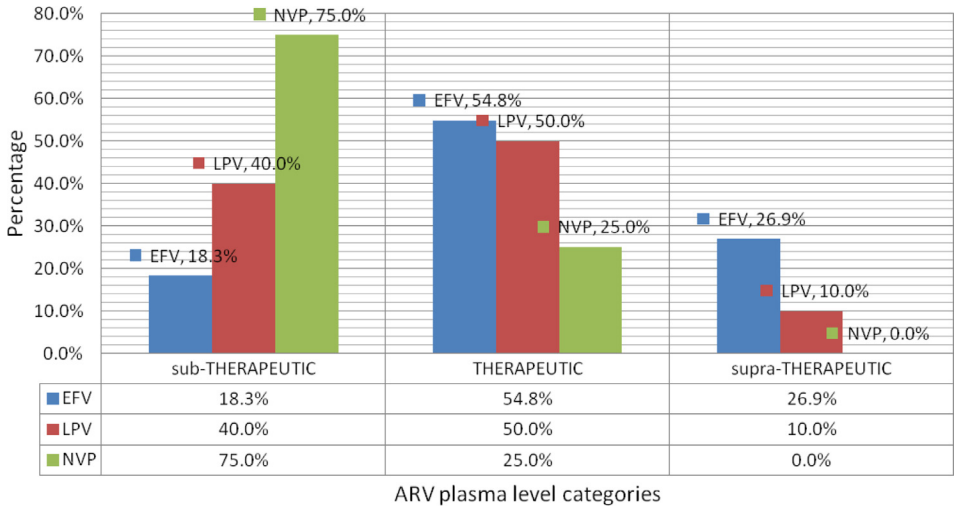

Figure 2 Distribution of ARV plasma levels as sub-therapeutic, Therapeutic or supra-therapeutic among 118 adult HIV patients cotreated with TB drugs. *__ARV:__ Antiretroviral; **EFV:** Efavirenz; **HIV**: Human immunodeficiency virus; **LPV:** Lopinavir; **NVP:** Nevirapine; **TB:** Tuberculosis.

EFV levels reported from Kenya is comparatively higher than our finding, probably because this study was done among patients with a number of other co-morbidities requiring several other co-medications which could have considerably contributed to this higher report of sub-therapeutic Efavirenz plasma levels.

Sub-therapeutic NVP level is also reported in a range of 34–39% among patients co-treated with Rifampicin in various other studies. In Kenya 34.5% of patients studied for clinically significant drug interaction had sub-therapeutic NVP.[34] In studies from UK [21] and South Africa,[28] the sub-therapeutic NVP plasma levels were reported in 36% and 39% of patients co-treated with Rifampicin respectively. These rates are on the other hand lower than our findings, but of note all these studies suggest that TB/HIV co-treated patients on NVP-based regimen were the most likely to have subtherapeutic ARV drug levels, as compared to EFV based regimen even though the number of patients who were on NVP based regimen in our study was much smaller.

Rifampicin is remarkable for its CYP45O induction effect, which subsequently increases the metabolism of NNRTIs and PIs.[17,35,36] This phenomenon affects NVP more extensively than EFV, and it is essentially because of the differences in biotransformation pathways which are largely influenced by CYP2B6 and NAT2 genetic polymorphism.[37–39] Nevirapine metabolism occurs through a number of CYP450 isoenzymes inclusive of CYP2B6, CYP3A and CYP2C which are Rifampicin ''inducible'',[40] an event that has also been suggested to affect the PIs substantially.[10,36,41] As a consequence this increases the clearance of NVP and PIs among those individuals who are co-treated with Rifampicin containing tuberculosis drugs. A number of studies have demonstrated the conse-

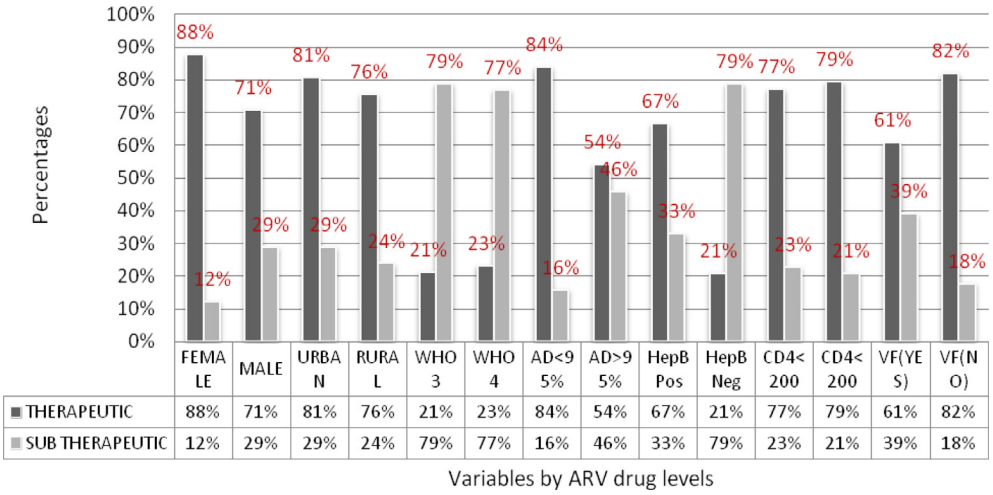

Figure 3 Distribution of variables by plasma ARV drug levels as sub-therapeutic or therapeutic. *__AD < 95%:__ Adherence level of less than 95%; **AD > 95%:** Adherence level of more than 95%; **ARV:** Antiretroviral; **HepB pos**: Hepatitis B positive; **HepB neg:** Hepatitis B positive; **VF**: virological failure; **WHO**: world health organization; **WHO 3:** WHO clinical stage 3; **WHO 4**: WHO clinical stage 4.

quence of this interaction between NVP and Rifampicin including a recent study from Mozambique which indicated that NVP plasma concentrations were much lower while on anti tuberculosis drugs than after discontinuation of tuberculosis drugs[42] with a large proportion of NVP concentration being below therapeutic range for the period of cotreatment with tuberculosis drugs than thereafter.

Even though protease inhibitors including Lopinavir have a short half life requiring multiple dosages a day, LPV is still the most preferred PI when used in its co formulated form with ritonavir as a pharmacological booster (LPV400 mg/r100 mg), since it is well tolerated and has a better virological efficacy[43,44] as compared to other PIs. However at this strength of LPV/r co formulation Rifampicin significantly increases the clearance of both drugs leading into failure of virological control. Based on this a number of studies have demonstrated a better virological control with super boosted LPV/r including combined strengths of 400/400 mg or 800/200 mg regimens,[45,46] however with increased risk of toxicity which could negatively affect the patients' adherence to medications.

On the contrary Efavirenz is mainly metabolized by CYP2B6.[47,48] Though this enzyme can also be induced by Rifampicin it has been shown that Isoniazid is one of important components of the TB fixed dose combination, inhibits CYP2B6 especially among individuals who are slow metabolizers (with CYP2B6 loss of function), and decreases the EFV clearance with attendant increase in plasma EFV concentration.[49,50] This phenomenon is reportedly more frequent among African than European descendants,[51] and it has been observed in about 40% of African individuals in some study settings.[52] Among patients who were on TB treatment in Mozambique their plasma EFV concentrations were higher with anti tuberculosis than after discontinuation of anti tuberculosis drugs.[42] In this study it was further shown that only a small proportion of patients had sub-therapeutic EFV plasma levels (9%) as compared to more than 37% who had supra-therapeutic levels of EFV while on anti tuberculosis.

These observations are similar to our findings that of the 104 patients who were on EFV based regimen, 28 (26.9%) had supra-therapeutic (>4000 ng/mL) EFV plasma concentrations (Fig. 2) whereas only 1 (10%) participant on LPV and none of those who were on NVP based regimen had supra-therapeutic plasma levels. These findings suggest that patient on HIV/TB co-treatment should as well be monitored for potential ART drug toxicities.

One aim of this study was to determine the factors that will give clinical prediction of subtherapeutic ARV levels in adult patients who are co-treated with anti-TB. These factors which have also been reported by other studies, include adherence of <95%,[53,54] NVP based regime[55] and female gender.[34,53] In the face of this association our results suggest that the occurrence of any of these risk factors independently augments the likelihood of sub-therapeutic ARV levels plasma among patients co-treated with Rifampicin.

Many of the findings of this study were consistent with previous studies and logical but one surprising finding is that of the female gender being a predictor of sub-therapeutic plasma ARV levels among adult HIV patients co-treated with anti-TB. The female gender is documented as one of the inter-individual differences which have been shown to alter the apparent oral clearance of ARVs.[56] This may be one of the explanations that the female sex stands as one of the predictors of sub-therapeutic ARV levels among patients co-treated with anti-TB. Additional factors which may alter oral clearance of ARV are inclusive of geographical location and Hepatitis B Virus (HBV) co-infection.[56,57] However in our study these two additional factors did not show any significant statistical association to sub-therapeutic ARV levels.

Apart from the fact that both NNRTIs and PIs undergo their disposition through CYP450 iso enzymes,[10] on the other hand the plasma levels of these drugs are determined by adherence levels to medications especially among patients on EFV based regimens.[42] Prior studies had indicated that the sub-therapeutic EFV plasma levels on TB co-treatment are less frequent and are more common among those patients with inadequate adherence level.[42] Since isoniazid reduces clearance of EFV, improvement of adherence to ART medications may positively alter the plasma levels of EFV and possibly give

Table 2 Univariate and multivariate analysis for factors associated with sub-therapeutic ARV plasma levels among 118 adult HIV positive patients on TB co-treatment at Bugando.

Variables	Sub-therapeutic ARV levels		Unadjusted		Adjusted	
	Yes (n) = 26	No (n) = 82	OR (95% CI)	p-Val	OR (95% CI)	p-Val
Gender						
Male	06 (12.2)	43 (87.8)				
Female	20 (29.0)	49 (71.0)	2.9 (1.1–7.9)	**0.031**	3.5 (1.1–10.6)	**0.028**
Age in years	33 [27–44]	38 [32–43]	1.0 (0.9–1.1)	0.513		
Geog location						
Urban	10 (19.2)	42 (80.8)				
Rural	16 (24.2)	50 (75.8)	0.7 (0.3–1.8)	0.515		
BMI index						
Under WT	04 (18.2)	18 (81.8)				
Normal WT	19 (24.1)	60 (75.9)				
Over WT	03 (17.7)	14 (82.3)	1.0 (0.9–1.1)	0.842		
WHO stage						
Stage 3	16 (21.3)	59 (78.7)				
Stage 4	10 (23.3)	33 (76.7)	1.1(0.5–2.7)	0.808		
Initial CD4						
< 200 cells/μl	18 (22.8)	61 (77.2)				
> 200 cells/μl	08 (20.5)	31 (79.5)	0.9 (0.3–2.2)	0.779		
Enroll CD4						
< 200 cells/μl	09 (20.0)	36 (80.0)				
> 200 cells/μl	17 (23.3)	56 (76.7)	1.2 (0.5–3.0)	0.676		
HBV co-infection						
Yes	03 (33.3)	06 (66.7)				
No	23 (21.1)	86 (78.9)	0.4 (0.0–3.5)	0.424		
Adherence						
> 95%	15 (16.0)	79 (84.0)				
< 95%	11 (45.8)	13 (54.2)	4.5 (1.7–11.8)	**0.002**	6.8 (2.2–20)	**0.001**
ART months	9.5 [2–20]	11 [3–22]	1.1 (0.9–1.4)	0.999		
VL (copies/μl)						
> 400	11 (23.9)	35 (76.1)				
< 400	15 (20.8)	57 (79.2)	1.2(0.5–2.9)	0.694		
VF (VL ⩾ 10^4/μl)						
Yes	09 (39.1)	14 (60.9)				
No	17 (17.9)	78 (82.1)	2.9(1.1–7.9)	**0.027**	3.8 (1.2–11.4)	**0.016**
Anti-TB months	5 [3–6]	4.5 [2–6]	1.1(0.9–1.4)	0.261		
TB category						
PTB SP	14 (19.7)	57 (80.2)				
PTB SN	09 (37.5)	15 (62.5)				
EPTB	03 (13.0)	20 (87.0)	1.0(0.6–1.7)	0.112		
ARV regimen						
EFV	19 (18.3)	85 (81.7)				
LPV	04 (40.0)	06 (60.0)				
NVP#	03 (75.0)	01 (25.0)	2.1(1.2–2.8)	**0.010**	-	-

*__ART__: antiretroviral therapy, **CD4:** cluster of differentiation 4, **CI:** confidence interval, **EFV**: Efavirenz, **EPTB**: extra pulmonary TB, **Geog:** Geographical, **HBV**: Hepatitis B, **IQR:** Interquartile range, **LPV:** Lopinavir, **NVP**: Nevirapine, **PTB:** Pulmonary Tuberculosis, **SN:** Smear Negative, **SP:** Smear positive, **VF:** Virological failure, **VL:** viral load.

patients a much positive virological outcome as compared to NVP and Lopinavir based regimens.

Therapeutic drug levels are a key to successful ART,[57,58] and any low drug levels observed in patients on ART have been extrapolative of a failure to achieve an immediate virological containment and a longer term immunological failure.[53,59] Findings of sub-therapeutic drug levels among patients co-treated with anti-TB in the current study are accompanied by a co-occurrence of both high levels of virological failure and substantially low CD4 counts on enrollment as a consequent. In this study virological failure was found in 39.1% of the patients with sub-therapeutic ARV levels and

on enrollment CD4 count of < 200 cells/μl was found in more than one-third, 9/26 (34.6%) of these patients with sub-therapeutic NNRTIs and PIs plasma levels.

On clinical grounds these findings suggest that these patients stand a high risk of a subsequent potential of developing and accumulating resistant viral strains,[23,60,61] when these drug levels are not corrected quickly. However this is a great challenge in Tanzania and other resource limited settings where TDM is not done. Our findings that female gender, poor adherence, and certain regimens are predictive of sub-therapeutic drug levels are helpful to clinicians in these settings who need to maintain a high index of suspicion for sub-therapeutic drug levels in such patients and to counsel patients on the importance of adherence. This study had a number of limitations including a small sample size especially of patients on NVP and LPV based regimen. Also this being a cross sectional and a single clinic based study, the results from this study may not necessarily be generalizable to the general population; therefore, a longitudinal study with a larger sample size is recommended.

6. Conclusion

The magnitude of sub-therapeutic ARV plasma levels is significantly high among adult HIV positive patients on ARV and anti-TB co-treatment attending HIV care and treatment centers in Tanzania. These patients are at a high risk of immediate inadequate virological suppression with a potential resistance development and a long term poor clinical outcome. Since TDM is not available in most resource limited settings to assist in identifying these patients early and making timely correction to improve their overall outcome on ARVS, Clinician in resource limited settings such as Tanzania should maintain a high index of suspicion and identify potential patients for a closer clinical follow-up and adherence augmentation.

Competing interest

Authors declare that they had no competing interest and all the authors approved the final manuscript before submission.

Authors' contribution

CK, SEK, HK and DWG conceived the idea and designed the study; DWG and CK performed the experiments and acquired the data; DWG and BRK analyzed and interpreted the data; DWG, CK, and

BCM did literature search; DWG did draft the manuscript; DWG, BCM, CK, BRK, HK, GWK and

SEK critically reviewed and edited the manuscript for its intellectual content.

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References

1. Manosuthi W et al. Standard-dose efavirenz vs. standard-dose nevirapine in antiretroviral regimens among HIV-1 and tuberculosis co-infected patients who received rifampicin. *HIV Med* 2008;**9**(5):294–9.
2. Corbett EL et al. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med* 2003;**163**(9):1009–21.
3. WHO, Global Tuberculosis Report; 2014.
4. WHO, Interim policy on collaborative TB/HIV activities; 2004.
5. WHO, Global tuberculosis control 2011, WHO., Editor; 2011.
6. Torok ME, Farrar JJ. When to start antiretroviral therapy in HIV-associated tuberculosis. *The New Engl J Med* 2011;**365**(16):1538–40.
7. Lawn SD, Torok ME, Wood R. Optimum time to start antiretroviral therapy during HIV-associated opportunistic infections. *Current Opin Infect Diseases* 2011;**24**(1):34–42.
8. Friedland G et al. Administration of efavirenz (600 mg/day) with rifampicin results in highly variable levels but excellent clinical outcomes in patients treated for tuberculosis and HIV. *J Antimicrob Chemother* 2006;**58**(6):1299–302.
9. Brinkhof MW et al. Tuberculosis after initiation of antiretroviral therapy in low-income and high-income countries. *Clin Infect Dis* 2007;**45**(11):1518–21.
10. Boulle A et al. Outcomes of nevirapine- and efavirenz-based antiretroviral therapy when coadministered with rifampicin-based antitubercular therapy. *JAMA* 2008;**300**(5):530–9.
11. Lawn SD et al. Burden of tuberculosis in an antiretroviral treatment programme in sub-Saharan Africa: impact on treatment outcomes and implications for tuberculosis control. *AIDS* 2006;**20**(12):1605–12.
12. Lawn SD, Wood R. Tuberculosis control in South Africa–will HAART help? *S Afr Med J* 2006;**96**(6):502–4.
13. Collins KR et al. Impact of tuberculosis on HIV-1 replication, diversity, and disease progression. *AIDS Rev* 2002;**4**(3):165–76.
14. Fletcher CV et al. Concentration-controlled compared with conventional antiretroviral therapy for HIV infection. *AIDS* 2002;**16**(4):551–60.
15. McIlleron H et al. Complications of antiretroviral therapy in patients with tuberculosis: drug interactions, toxicity, and immune reconstitution inflammatory syndrome. *J Infect Dis* 2007;**196**(Suppl 1):S63–75.
16. Ramachandran G et al. Increasing nevirapine dose can overcome reduced bioavailability due to rifampicin coadministration. *J Acquir Immune Defic Syndr* 2006;**42**(1):36–41.
17. Rae JM et al. Rifampin is a selective, pleiotropic inducer of drug metabolism genes in human hepatocytes: studies with cDNA and oligonucleotide expression arrays. *J Pharmacol Exp Ther* 2001;**299**(3):849–57.
18. Pepper DJ et al. Combined therapy for tuberculosis and HIV-1: the challenge for drug discovery. *Drug Discov Today* 2007;**12**(21–22):980–9.
19. Ribera E et al. Rifampin reduces concentrations of trimethoprim and sulfamethoxazole in serum in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother* 2001;**45**(11):3238–41.
20. Pedral-Sampaio DB et al. Efficacy and safety of Efavirenz in HIV patients on Rifampin for tuberculosis. *Braz J Infect Dis* 2004;**8**(3):211–6.
21. Sathia L et al. Concomitant use of nonnucleoside analogue reverse transcriptase inhibitors and rifampicin in TB/HIV type 1-coinfected patients. *AIDS Res Hum Retroviruses* 2008;**24**(7):897–901.
22. Clevenbergh P et al. Improving HIV infection management using antiretroviral plasma drug levels monitoring: a clinician's point of view. *Curr HIV Res* 2004;**2**(4):309–21.

23. Gunda DW 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):e75118.
24. Duong M et al. Usefulness of therapeutic drug monitoring of antiretrovirals in routine clinical practice. *HIV Clin Trials* 2004;**5**(4):216–23.
25. Khoo SH et al. Pharmacologic optimization of protease inhibitors and nonnucleoside reverse transcriptase inhibitors (POPIN)–a randomized controlled trial of therapeutic drug monitoring and adherence support. *J Acquir Immune Defic Syndr* 2006;**41**(4):461–7.
26. Leth FV et al. Pharmacokinetic parameters of nevirapine and efavirenz in relation to antiretroviral efficacy. *AIDS Res Hum Retroviruses* 2006;**22**(3):232–9.
27. Kigen G et al., Prevalence of potential drug-drug interactions involving antiretroviral drugs in a large Kenyan cohort. PLoS One. 6(2): p. e16800.
28. Elsherbiny D et al. Population pharmacokinetics of nevirapine in combination with rifampicin-based short course chemotherapy in HIV- and tuberculosis-infected South African patients. *Eur J Clin Pharmacol* 2009;**65**(1):71–80.
29. van Oosterhout JJ et al. Nevirapine-based antiretroviral therapy started early in the course of tuberculosis treatment in adult Malawians. *Antivir Ther* 2007;**12**(4):515–21.
30. WHO, Treatment of tuberculosis Guidelines. 2010(WHO/HTM/TB/2009.420): p. 147.
31. Grossberg R, Gross R. Use of pharmacy refill data as a measure of antiretroviral adherence. *Curr HIV/AIDS Rep* 2007;**4**(4):187–91.
32. Langmann P et al. High-performance liquid chromatographic method for the determination of HIV-1 non-nucleoside reverse transcriptase inhibitor efavirenz in plasma of patients during highly active antiretroviral therapy. *J Chromatogr B Biomed Sci Appl* 2001;**755**(1–2):151–6.
33. COBAS®, COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version2.0. Doc Rev. 5.0 ed., R.m. systems, Editor. 2010, Roche molecular systems USA. p. 1–30.
34. Kigen G et al. Prevalence of potential drug-drug interactions involving antiretroviral drugs in a large Kenyan cohort. *PLoS One* 2011;**6**(2):e16800.
35. De Requena DG, Jimenez-Nacher I, Soriano V. Changes in nevirapine plasma concentrations over time and its relationship with liver enzyme elevations. *AIDS Res Hum Retroviruses* 2005;**21**(6):555–9.
36. Piscitelli SC, Gallicano KD. Interactions among drugs for HIV and opportunistic infections. *N Engl J Med* 2001;**344**(13):984–96.
37. Bertrand J et al. Dependence of efavirenz- and rifampicin-isoniazid-based antituberculosis treatment drug-drug interaction on CYP2B6 and NAT2 genetic polymorphisms: ANRS 12154 study in Cambodia. *J Infect Dis* 2014;**209**(3):399–408.
38. Cohen K et al. Effect of rifampicin-based antitubercular therapy and the cytochrome P450 2B6 516G > T polymorphism on efavirenz concentrations in adults in South Africa. *Antivir Ther* 2009;**14**(5):687–95.
39. Bertrand J et al. Multiple genetic variants predict steady-state nevirapine clearance in HIV-infected Cambodians. *Pharmacogenet Genomics* 2012;**22**(12):868–76.
40. Riska PS et al. Biotransformation of nevirapine, a non-nucleoside HIV-1 reverse transcriptase inhibitor, in mice, rats, rabbits, dogs, monkeys, and chimpanzees. *Drug Metab Dispos* 1999;**27**(12):1434–47.
41. Patel A et al. Safety and antiretroviral effectiveness of concomitant use of rifampicin and efavirenz for antiretroviral-naive patients in India who are coinfected with tuberculosis and HIV-1. *J Acquir Immune Defic Syndr* 2004;**37**(1):1166–9.
42. Bhatt NB et al. Nevirapine or efavirenz for tuberculosis and HIV coinfected patients: exposure and virological failure relationship. *J Antimicrob Chemother* 2015;**70**(1):225–32.
43. Cvetkovic RS, Goa KL. Lopinavir/ritonavir: a review of its use in the management of HIV infection. *Drugs* 2003;**63**(8):769–802.
44. Oldfield V, Plosker GL. Lopinavir/ritonavir: a review of its use in the management of HIV infection. *Drugs* 2006;**66**(9):1275–99.
45. Murphy RA et al. Coadministration of lopinavir/ritonavir and rifampicin in HIV and tuberculosis co-infected adults in South Africa. *PLoS One* 2012;**7**(9):e44793.
46. Sunpath H et al. Double-dose lopinavir-ritonavir in combination with rifampicin-based anti-tuberculosis treatment in South Africa. *Int J Tuberc Lung Dis* 2014;**18**(6):689–93.
47. Ogburn ET et al. Efavirenz primary and secondary metabolism in vitro and in vivo: identification of novel metabolic pathways and cytochrome P450 2A6 as the principal catalyst of efavirenz 7-hydroxylation. *Drug Metab Dispos* 2010;**38**(7):1218–29.
48. Riska P et al. Disposition and biotransformation of the antiretroviral drug nevirapine in humans. *Drug Metab Dispos* 1999;**27**(8):895–901.
49. Gengiah TN et al. The influence of tuberculosis treatment on efavirenz clearance in patients co-infected with HIV and tuberculosis. *Eur J Clin Pharmacol* 2012;**68**(5):689–95.
50. Luetkemeyer AF et al. Relationship between weight, efavirenz exposure, and virologic suppression in HIV-infected patients on rifampin-based tuberculosis treatment in the AIDS Clinical Trials Group A5221 STRIDE Study. *Clin Infect Dis* 2013;**57**(4):586–93.
51. Uttayamakul S et al. Effects of CYP2B6 G516T polymorphisms on plasma efavirenz and nevirapine levels when co-administered with rifampicin in HIV/TB co-infected Thai adults. *AIDS Res Ther* 2010;**7**:8.
52. Arnaldo P et al. Frequencies of cytochrome P450 2B6 and 2C8 allelic variants in the mozambican population. *Malays J Med Sci* 2013;**20**(4):13–23.
53. Alexander CS et al. Antiretroviral concentrations in untimed plasma samples predict therapy outcome in a population with advanced disease. *J Infect Dis* 2003;**188**(4):541–8.
54. Ahoua L et al. Risk factors for virological failure and subtherapeutic antiretroviral drug concentrations in HIV-positive adults treated in rural northwestern Uganda. *BMC Infect Dis* 2009;**9**:81.
55. Manosuthi W et al. A randomized trial comparing plasma drug concentrations and efficacies between 2 nonnucleoside reverse-transcriptase inhibitor-based regimens in HIV-infected patients receiving rifampicin: the N2R Study. *Clin Infect Dis* 2009;**48**(12):1752–9.
56. Kappelhoff BS et al. Nevirapine and efavirenz pharmacokinetics and covariate analysis in the 2NN study. *Antivir Ther* 2005;**10**(1):145–55.
57. Veldkamp AI et al. High exposure to nevirapine in plasma is associated with an improved virological response in HIV-1-infected individuals. *AIDS* 2001;**15**(9):1089–95.
58. Gieschke R et al. Relationships between exposure to saquinavir monotherapy and antiviral response in HIV-positive patients. *Clin Pharmacokinet* 1999;**37**(1):75–86.
59. Burger DM et al. Low plasma concentrations of indinavir are related to virological treatment failure in HIV-1-infected patients on indinavir-containing triple therapy. *Antivir Ther* 1998;**3**(4):215–20.
60. Sigaloff KC et al. Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa. *J Acquir Immune Defic Syndr* 2011;**58**(1):23–31.
61. Johannessen A et al. Virological efficacy and emergence of drug resistance in adults on antiretroviral treatment in rural Tanzania. *BMC Infect Dis* 2009;**9**:108.