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Second-line virologic failure and elevated bilirubin as a potential surrogate marker of ART adherence among people living with HIV in Eastern Uganda

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Abstract

Second-line antiretroviral therapy (ART) failure remains a challenge in HIV Programs. We conducted a cross-sectional study among people living with HIV on second-line ART in Eastern Uganda to determine the prevalence and associated factors of virological failure and to assess elevated serum bilirubin as a surrogate marker of adherence. The prevalence of virological failure was 7.5%. Elevated bilirubin showed poor sensitivity and specificity for predicting adherence or virological failure. The findings highlight the need for routine viral load monitoring, as bilirubin is not a reliable surrogate marker of treatment adherence or virological failure.

Keywords Second-line virological failure, Elevated bilirubin, And adherence

Background

The rapid scale-up of the use of antiretroviral therapy has led to a global decline in the prevalence of HIV and new infections in the past ten years [1, 2]. Despite these improved outcomes in many people living with HIV (PLWHIV) on ART, some eventually fail on first-line and second-line treatment, resulting in increased morbidity and mortality [3].

Elevated bilirubin has been proposed as a proxy for adherence, which in turn influences virological outcome than pill count adherence in clients receiving atazanavir/ritonavir-based second-line antiretroviral therapy [4]. The use of elevated serum bilirubin as a surrogate marker to monitor adherence and virological treatment failure of the ATV/r-based second-line regimen is not a current practice in Uganda. Additionally, there is a paucity of data concerning second-line ART virological failure in Uganda. Addressing concerns of treatment failure is key to achieving the 95-95-95 targets by 2030, particularly viral load suppression. If treatment failure increases unchecked, viral load suppression may not be reached due to limited therapeutic options and the higher costs of expensive second and third-line treatments [5]. With the US funding cuts On HIV programs, alternative marker of

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adherence such as bilirubin could be viable [6].This study sought to determine the prevalence and factors associated with second-line treatment virological failure and evaluate elevated serum bilirubin as a potential surrogate marker of adherence among people living with HIV in eastern Uganda.

Materials and methods

Study design and setting

A cross-sectional study was carried out from 20/10/2022 to 12/03/2023. The study was conducted in the ART clinics of Tororo General Hospital and Mbale Regional Referral Hospital (MRRH). Tororo General Hospital HIV has a total of 2335 clients on ART therapy of which 162 clients were on second-line regimen. The second line regimen that is used in the facility is ATV/r, LPV/r-based regimen, and dolutegravir (DTG). Similarly, MRRH runs an ART clinic daily with a total number of clients on ART 5420 of which 522 (%) are on ATZ/r-based regimen and LPV/r-based regimen-based second-line therapy.

Study population

PLWHIV on a second-line regimen for more than six months were eligible for the study as per the Uganda Ministry of Health Clinical guidelines, while clients who declined consent were not included. The 294 study participants were selected using a sequential sampling method.

Data collection methods

Data was collected by reviewing the antiretroviral treatment (ART) records, interviewing participants, and performing laboratory tests for total bilirubin. Liver function tests, especially bilirubin levels, were performed to assess adherence. Standard operating procedures were followed to obtain and process the blood samples.

Data analysis and presentation

STATA (version 15.0) was used for analysis. Descriptive statistics were used to summarize the study population characteristics and the overall prevalence of second-line virological failure. Continuous variables were summarized using mean and standard deviation (SD), while categorical variables were summarized using frequency and percentages. We used bivariate analysis to identify factors associated with virological failure. Statistically significant variables were then subjected to multivariate analysis to determine the predictors of virological failure. We then used a multivariate logistic regression model fitted through the backward selection of variables to assess the adjusted effect of predictor variables on the primary outcome. Second-line antiretroviral therapy (ART) virological failure is defined as PLWHIV who are on a second-line regimen and have a high viral load level (> 1000 copies/mL) in two consecutive measurements after 6 months of treatment and on repeat tests after 3 months [7]. Sensitivity and specificity of bilirubin was computed using the two-by-two tables.

Results

Characteristics of the participants

Overall, 294 participants were enrolled in the study, of which 271 (92.2%) were aged 20 years and above with a mean age of 45 years (SD 13.9 years). The majority, 116 (39.5%), had been on a second-line ART regimen for about 24–36 months with a mean duration on ART of 38.5 months (SD 16.0 months), of which 44 (15.0%) had elevated bilirubin levels, and 22 (7.5%) had second-line virological failure. The prevalence of virological failure was 7.5% (Table 1).

The age of the participant, drug frequency, alcohol consumption, duration on second line ART, most recent CD4 count, adherence to Art and the most recent WHO-clinical stage were associated with virological failure.

Discussion

Prevalence of virological failure

The prevalence of second-line virological failure was found to be 7.5% using the WHO definition of virological failure of > 1000 viral copies/ml after six or more treatment cycles. This was higher compared to that found by a similar study done in Malawi [4] and much lower than reported in the earlier systematic meta-analysis about treatment outcomes of clients on second-line ART treatment in resource-limited countries [8]. The higher prevalence of virological failure in our context could be because a large proportion consumed alcohol and had a CD4 count < 200 cells/mm^3. Despite the lower prevalence values illustrated by this finding, the second-line virological failure is of health and financial importance [9], and this undermines the efforts to achieve the 95-95-95 strategy by 2030.

Factors associated with increased risk of developing second-line virological failure

This study found two factors posing an increased risk of developing virological failure: taking alcohol and having a the most recent CD4 count < 200 cells/mm^3 (Table 2).

CD4 count cells have long been known to be determinants of the body's immunity, thus enabling the body to clear viruses; however, the findings of our study have shown that having the most recent CD4 count of < 200 cells/mm^3 increased the risk of second-line virological failure. This contradicts several studies done elsewhere [3, 10, 11].

Taking alcohol has been found in previously conducted studies to be a risk factor for virological failure [12]. This might be related to increased factors that were not within

Table 1 Bivariate analysis of factors associated with second-line virological failure

Variable	Suppressed n (%), *N* = 272	Persistent non-suppression n (%), *N* = 22	COR (95% CI)	*p*-value
Age (completed years)				
< 10	3 (1.1)	2 (9.1)	Ref	0.417
10–19	14 (5.2)	4 (18.2)	0.4(0.035–3.999)	0.016
≥ 20	255 (93.8)	16 (72.7)	0.1 (0.016–0.659)	
Drug frequency				
Once	269 (98.9)	17 (77.3)	Ref	0.001
Twice	3 (1.1)	5 (22.7)	26.4 (5.809 –119.735)	
Alcohol consumption				
Yes	48 (17.7)	8 (36.4)	8.2 (3.245–20.552)	0.001
	224 (82.4)	14 (63.6)	Ref	
Disclosure				
No	30 (11.0)	6 (27.3)	0.3 (0.120–0.910)	0.032
	242 (88.8)	16 (72.7)	Ref	
Backbone				
ABC	13 (4.8)	6 (27.3)	Ref	0.109
AZT	23 (8.5)	3 (13.6)	0.3 (0.060–1.323)	0.001
TDF	236 (86.8)	13 (59.1)	0.1 (0.039–0.365)	
Protease/integrase				
ATV/r	66 (24.3)	11 (50.0)	Ref.	0.05
LPV/r	1 (0.4)	2 (9.1)	12.2 (1.016 –146.005)	
DTG	205 (75.4)	9 (40.8)	0.1 (0.107–0.676)	0.01
Duration on 2nd line ART (months)				
12–24	34 (12.5)	0 (0.0)	1 empty	**0.044**
24<–36	105 (38.6)	11 (50.0)	0.4 (0.131–0.975)	**0.001**
36<–48	107 (39.3)	3 (13.6)	0.1 (0.023–0.377)	
> 48	26 (9.6)	8 (36.4)	Omitted	
Most recent CD4 count (cells/mm3)				
< 200	39 (14.3)	10 (45.5)	0.2 (0.071–0.441)	0.001
> 200	233 (85.7)	12 (54.6)	**Ref**	
Adherence to ART				
Good	272 (100)	0 (0.0)	Perfectly predict the outcome	
Poor	0 (0.0)	22 (100.0)		
Most recent WHO-clinical stage				
One	152 (55.9)	4 (18.2)	**Ref**	**0.035**
Two	81 (29.8)	8(36.4)	3.8 (1.097–12.842)	**0.001**
Three	34 (12.5)	9 (40.9)	10.1 (2.925–34.589)	0.093
Four	5 (1.8)	1 (4.6)	7.6 (0.714–80.932)	
Bilirubin level (mg/dl)				
Elevated	38 (14.0)	6 (27.3)	0.4 (0.159–1.176)	0.101
Normal	234 (86.0)	16 (72.7)	**Ref**	

Empty refers to those categories falling on the side of the dependent outcome, ref-reference, COR- crude odds ratio, ABC- abacavir, TDF-tenofovir, DTG- dolutegravir, GOVT- government employee, NGO- non-government organization employee, ART-Antiretroviral therapy

the scope of this study, like the risk of depressive symptoms, hepatitis C co-infection, and alcohol-associated low CD4 count, as was revealed in an earlier study in 2007 [13].

Bilirubin as a surrogate marker of adherence to the ATV/r second-line ART regimen

Adherence has long been known as a determinant of virological suppression; however, the finding of this study reveals no difference in the adherence levels between virologically failing and virologically suppressing HIV-infected clients. This leaves us with two explanations: either the method of assessing adherence in place is ineffective or the emergence of resistance.

Objective biologic measures of ART adherence are more reliable in predicting poor adherence and VF than pill count or the client's self-report, which often overestimate adherence levels. The study done earlier in Malawi revealed that normal bilirubin values in the setting of ATV/r-based therapy better predicted treatment failure than pill count. However, the findings of this study have shown that the sensitivity was found to be at 50%, with a low specificity of 31.8%. More studies are needed

Table 2 Multivariate analysis of factors associated with second-line virological failure

Variable	COR (95% CI)	*p*-value	AOR (95% CI)	*P*-value
Age (years)				
< 10	Ref	0.417	0.7	0.881
10–19	0.4 (0.035–3.999)	**0.016**	(0.013–40.476)	0.985
≥ 20	0.1 (0.016–0.659)		1.0 (0.023–41.343)	
Drug frequency				
Once	Ref	0.001	Ref	0.550
Twice	26.4 (5.809–119.735.809.735)		2.4 (0.137–41.659)	
Alcohol consumption				
Yes	8.2 (3.245–20.552)	0.001	5.2 (1.224–21.764)	0.025
	Ref		Ref	
Disclosure				
No	0.3 (0.120–0.910)	0.032	0.5 (0.054–5.319)	0.593
	Ref		Ref	
Backbone				
ABC	Ref	0.109	0.3	0.325
AZT	0.3 (0.060–1.323)	0.001	(0.019–3.757)	**0.030**
TDF	0.1 (0.039–0.365)		0.1 (0.014–0.807)	
Protease/integrase				
ATV/r	Ref	**0.05**	5.5 (0.040–63.938.040.938)	0.498
LPV/r	12.2(1.016–146.005.016.005)	**0.01**	0.7 (0.138–4.547)	0.793
DTG	0.1 (0.107–0.676)			
Duration on 2nd line ART (months)				
12–24	1 empty	**0.044**	1 empty	0.406
24<–36	0.4 (0.131–0.975)	**0.001**	0.5 (0.097–2.570)	**0.007**
36<–48	0.1 (0.023–0.377)		0.08 (0.012–0.501)	
> 48	Omitted		Omitted	
Most recent CD4 count (cells/mm3)				
< 200	0.2 (0.071–0.441)	0.001	0.07 (0.019–0.253)	0.000
> 200	**Ref**		Ref	
Baseline WHO-clinical stage				
One	**Ref**	**0.035**	Ref	0.423
Two	3.8	**0.001**	1.9	0.298
Three	(1.097–12.842)	0.093	(0.403–8.756)	0.798
Four	10.1 (2.925–34.589)		2.5 (0.438–4.802)	
	7.6 (0.714–80.932		1.5 (0.080–6.495)	

The variable was considered significant when its p-value is less than 0.05 at a 95% confidence interval

to support the use of bilirubin as a surrogate marker of adherence to ART.

Conclusions

The prevalence of virological failure among people living with HIV on second-line ART regimens in two ART clinics was high. Alcohol consumption and a baseline CD4 count were independent factors associated with second-line virological failure among people living with HIV at clinics in eastern Uganda. Elevated bilirubin is not a surrogate marker for virological failure in people living with HIV on ATV/r based second-line ART regimen.

Recommendations

We recommend continued adherence support to clients after switching to second-line treatment and continuous viral load monitoring in line with the national clinical guidelines.

A qualitative inquiry into the barriers and facilitators to understand why some PLWHIV on second-line treatment remain non adherent to contextualize interventions. Community sensitization regarding the dangers of alcohol consumption needs to be implemented, focusing more on HIV-infected populations.

Health workers need to actively identify potential advanced HIV disease and the common markers of treatment failure.

Limitations

Participants on TB treatment, known NCDs or on other medications that may elevate bilirubin levels were not excluded from this study.

Abbreviations

HIV	Human immunodeficiency virus
ARV	Anti-retroviral
CD4	Cluster of differentiation
WHO	World Health Organization
ART	Anti-retroviral therapy
ATV/r	Atazanavir/ritonavir
UGT	Uridine glucuronide transferase
LPV/r	Lopinavir/ritonavir
MRRH-REC	Mbale regional referral hospital research Ethics Committee
NGO	Non-Governmental Organization
DTG	Dolutegravir
MRRH	Mbale region referral hospital

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Author contributions
This work was carried out in collaboration among all authors. Author SML conceived and designed the study, and participated in data collection, analysis, and manuscript writing. Author PO supported data collection, analysis, and manuscript writing. Authors RN, MAO, JE, PO, HI, LVNS, and JN performed the statistical analysis, wrote the protocol and managed the literature searches, wrote the first draft of the manuscript, and managed the study analyses. Authors JSI and David Okia served as research mentors and supervisors who participated in the study conception, design, preparation for approval, and proofreading of the final results and manuscript. All authors read and approved the final manuscript.

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Data availability
The datasets generated and/or analyzed during the current study are available in the OSF repository [osf.io/mqf3b](https:/osf.io/mqf3b). Also, the corresponding author can provide the data if required.

Declarations

Ethics approval and consent to participate
Ethical clearance was obtained from the Mbale Regional Referral Hospital Research Ethics Committee (MRRH-2022-204). Permission to collect data was sought from the administration of MRRH and Tororo Hospital. Written informed consent was obtained from all participants who completed the questionnaires individually to ensure privacy, confidentiality, and anonymity. Participation was voluntary, and participants could withdraw from the study at any time without penalty.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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