

## **Fatty Liver Disease and its Correlates among People Living with HIV/AIDS Attending Care and Treatment Clinic at Temeke Regional Referral Hospital in Dar es Salaam**

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**Abstract****Background**

Fatty Liver Disease (FLD) is projected to be the leading cause of chronic liver disease among People living with HIV (PLHIV).

**Broad objective**

This study aimed at determining the magnitude and the associated factors for Fatty Liver Disease among People living with HIV attending Care and Treatment Clinic (CTC) at Temeke Regional Referral Hospital in Dar es Salaam, Tanzania.

**Methodology**

A hospital-based descriptive cross-sectional study was conducted between September and November 2020. Consenting adults aged  $\geq 18$  years and living with HIV, were enrolled in the study. A questionnaire with structured questions was used to collect socio-demographic, anthropometric measurements and clinical characteristics. Patients were fasted for a minimum of 8 hours before sonography and taking samples for lipid profile. Abdominal Ultra sonography (USS) was performed using B-mode and 3.5 MHz convex probe transducer (Dawei-DW 580, China, 2020) by a single trained investigator and confirmed by an experienced Radiologist; discrepancies were discussed by revisiting the images. FLD was defined as an increase in liver echogenicity compared to the right kidney. Independent predictors of FLD were analyzed using multivariate logistic regression; p value of  $< 0.05$  was considered to be statistically significant.

**Results**

A total of 454 patients were enrolled into the study. FLD was visible from 118 (25.9%) (95% CI 22.0%-30.3%) patients. Factors significantly associated with FLD at multivariate analysis were; age group 40-60 years (aOR 1.74; 95% CI: 1.02 – 2.96  $p=0.043$ ), overweight (aOR 1.92; 95%CI: 1.05-3.51:  $p=0.034$ ), obesity (aOR 3.46; 95% CI: 1.80 – 6.65:  $p < 0.001$ ) and dyslipidemia (a OR: 2.63 95%CI: 1.58-4.39;  $p < 0.001$ ). HIV viral load status and duration on combination antiretroviral therapy were not significantly associated with FLD.

**Conclusion and Recommendations**

One out of four PLHIV at Temeke Regional Referral Hospital CTC had Fatty liver Disease. Factors associated with FLD were age 40-60 years, overweight, obesity and dyslipidemia. We recommend weight reduction and regular screening for FLD among PLHIV with above risk factors.

**Keywords:** *Fatty liver, HIV, PLHIV, “People living with HIV”, Tanzania, Liver, Steatohepatitis, Non-Communicable Diseases, NCD, Overweight, Obesity, Dyslipidemia.*

**Introduction**

Fatty Liver Disease (FLD) is the pathological accumulation of fat in the hepatocytes specifically more than 5% of total liver weight (1). It is recognized as a spectrum of diseases comprising of simple steatosis, steatohepatitis, and fibrosis(1). It is estimated that about 4.7% of adult Tanzanians have HIV infection, and 75% of these have been enrolled on Care and Treatment Clinic services and are on antiretroviral therapy (ART) (2). As the survival of People living with HIV (PLHIV) increases morbidity due to liver disease has been increasing, with FLD being the leading cause of HIV unrelated morbidity and mortality (1). The global prevalence of FLD among PLHIV ranges between 30-100%, the wider variation is reportedly due to different methods of diagnosis (3). PLHIV are at an increased risk of developing FLD compared to the general population due to insulin resistance caused by HIV infection, unwanted effect of combination antiretroviral therapy on lipid metabolism and, shared mode of transmission with hepatitis B and C infections(4,5). Moreover, traditional risk factors such as alcohol consumption, obesity and dyslipidemia do play a role on pathogenesis of FLD among PLHIV(6).

The gold standard tool in the diagnosis of FLD is liver biopsy, but being an invasive procedure it involves the risk of bleeding(4,5). Abdominal USS is one of the validated non-invasive tools used in screening and diagnosing FLD(4,5). We conducted a study determine the magnitude and the associated factors for Fatty Liver Disease among People living with HIV attending Care and Treatment Clinic (CTC) at Temeke Regional Referral Hospital (TRRH) in Dar es Salaam, Tanzania.

**Methods****Study Setting**

This was a hospital-based cross-sectional descriptive study, conducted at Temeke Regional Referral Hospital (TRRH) from September 2020 to November 2020.

**Study population**

The study included people living with HIV/AIDS on combination antiretroviral therapy for at least one year attending CTC at TRRH, aged 18 years and above. The sample size was 454 participants, calculated using the following formula for a prevalence study:  $n = z^2 \times p(1-p) / \epsilon^2$  whereby  $n$  = minimum sample size,  $z$  = standard normal deviation (1.96),  $p$  = 7% which was the prevalence of FLD among patient with HIV diagnosed by Abdominal USS in a study done by Lesi A. O et al in Nigeria (18) and  $\epsilon$  = maximum likely error taken as 3%. All patients provided written informed consent. The MUHAS Research and Publications Committee approved the study.

**Data collection**

The investigator and two assistants who were qualified nurses who had been working at the Care and Treatment Clinic (CTC) for more than 3 years prospectively collected the data using a questionnaire with structured questions. The questionnaire comprised of 4 sections; socio-demographic, clinical, laboratory and radiology sections for data collection. It was pilot tested among researchers and a small sample of patients. Improvement was made in the questions accordingly and the final version of the questionnaire was used for data collection. HIV related data included duration on combination antiretroviral therapy (c-ART), current c-ART regimen (within six months) and current HIV/RNA viral load (copies/ml) as obtained from a CTC card and/or electronic data.

Data on clinical parameters included hypertension which was defined as blood pressure  $\geq 140/90$  mmHg and/or treatment with antihypertensive (7). Body Mass Index was calculated as weight (kg) /height ( $m^2$ ), and central obesity was defined as a waist circumference  $\geq 90$  centimetres in males and  $\geq 84$  centimetres in females(8).

Body fat percent was calculated using the following anthropometric measurements; neck circumference, waist circumference, hip circumference, and height (both in inches) using the following formula. The equation for women was: % body fat=  $[163.205 \times \text{Log}_{10} (\text{waist} + \text{hip} - \text{neck})] - [97.684 \times \text{Log}_{10} (\text{height})] - 78.387$ . The equation for men was: % body fat=  $[86.010 \times \text{Log}_{10} (\text{waist} - \text{neck})] - [70.041 \times \text{Log}_{10} (\text{height})] + 36.76$  (9)

Diabetes mellitus was defined as FBG  $\geq 7$  mmol/l OR RBG  $\geq 11$  mmol/l plus hyperglycemic symptoms and/or using antidiabetic medication(10).

Dyslipidemia was defined as fasting low density lipid cholesterol  $\geq 130$  mg/dl and/or total cholesterol  $\geq 200$  mg/dl(11).

**Fatty Liver Disease assessment**

To assess for FLD an Abdominal USS was performed by a trained investigator using a 3.5 MHz convex probe transducer (Dawei-DW 580, China, 2020) using a B mode under fasting condition (minimum of 8 hours). Study participants' particulars (age, sex, CTC card number) were entered in the USS machine and the machine was set on abdominal examination mode. Investigator asked the participant to lie on left lateral position, with the right arm raised above his/her head to widen the intercostal acoustic window. Ultrasound gel was applied over the right subcostal margin along mid axillary line. The transducer was placed over the gel in parasagittal plane for visualization of the cross-section of the right liver and right kidney. A minimum of two images upon breath holding after deep inspiration for few seconds were frozen and saved. Stored images were interpreted by the trained investigator and a senior

radiologist. Upon discordance, the interpretation of the senior radiologist was taken as final. Fatty Liver Disease (FLD) as a categorical variable in this study was defined as presence of hepatic steatosis presented as increased hepatic echogenicity compared to the right kidney and was graded according to the previous established criteria(12).

### Statistical analysis

The prevalence of Fatty Liver Disease (FLD) was calculated as the total number of study participants with FLD divided by the total number of people living with HIV attending the CTC at Temeke Regional Referral Hospital enrolled in the study.

Categorical variables were summarized as proportions. Univariate logistic regression analysis was used to identify factors associated with FLD. All variables with p-values <0.2 at univariate analysis were entered into multivariate logistic regression models. In addition, sex was added into the multivariable model despite being non-significant at univariate analysis as it is a known confounder a priori. The odds ratio (OR) and 95% confidence intervals (95%CI) were used to estimate the association of each variable with FLD. All statistical analyses were performed with the Statistical Package for Social Sciences (SPSS) version. 26, p<0.05 was considered significant.

### Results

A total of 454 People living with HIV (PLHIV) at Temeke Regional Referral Hospital (TRRH) HIV Care and Treatment Clinic (CTC) were enrolled in this study. Socio-demographic characteristics of study participants are presented in Table 1. The median age of the study participants was 44 years (IQR 38, 49). Majority (57.7%) of them were in the age category of 40-60 years. Most of them were female 330 (72.7%). Current alcohol consumption was reported in 82 (18.1%), and 14 (17.1%) among the alcohol consumers took hazardous amounts of alcohol. Majority 73(89.0%) reported to be consuming regular beer, while 9 (11.0%) were taking local brews and spirits. Seventy-three (16.1%) of our study participants were current cigarette smokers.

A summary of the clinical characteristics of study participants is presented in Table 2. Hypertension was found among 161(35.5%) participants, and diabetes mellitus was found in 31(6.8%) participants. Fasting serum LDL cholesterol  $\geq 130$  mg/dl and fasting total serum cholesterol  $\geq 200$  mg/dl were found among 68 (15.0%) and 84 (18.5%) participants respectively. Some study participants presented with both elevated LDL and total cholesterol, and were thus counted once. A total of 101(22.0%) participants were found to have

dyslipidemia. Nearly half of study participants 226 (49.7%) were overweight and/or obese. A total of 301(66.3%) participants had high body fat percent.

The median (Interquartile Range) duration on combination ART use was 7 (4, 11) years. Majority of the recruited PLHIV 422 (93.0%) had attained HIV viral suppression. Most of study participants 439 (96.7%) were on Dolutegravir based antiretroviral therapy (Tenofovir + Lamivudine + Dolutegravir (TLD) at the time of the study.

It was found that the HIV associated factors such as the type of current combination ART, HIV viral load status, and median duration on combination ART were not associated with FLD.

Among the 454 study participants who underwent Abdominal USS, FLD was present in 118 (25.9 %) participants with a 95%CI of 22% - 30.3 %, Figure 1.

**Table 1: Socio-demographic characteristics of People Living with HIV at TRRH HIV Care and Treatment Clinic n=454**

Variable	Category	Frequency (Percentage)
Age(years)	<40	165 (36.3%)
	40-60	262 (57.7%)
	>60	27 (6.0%)
Sex	Female	330 (72.7%)
Marital status	Single	132 (29.1%)
	Married	183 (40.3%)
	Divorced	79 (17.4%)
	Widow/widower	60 (13.2%)
Level of education	Informal	27 (6.0%)
	Primary	292 (64.3%)
	Secondary	122 (26.9%)
	College/university	13 (2.9%)
Current alcohol consumption	Yes	82 (18.1%)
	No	372 (81.9%)
Hazardous alcohol consumption	Yes	14 (17.1%)
	No	68 (82.9%)
Cigarette smoking	Yes	73 (16.0%)
	No	381 (84.0%)

*Hazardous alcohol consumption; drinking >3 and >4 units of alcohol per occasion in males and females respectively (4)*

**Table 2: Clinical characteristics of People living with HIV at Temeke Regional Referral Hospital HIV Care and Treatment Clinic, n=454**

Variable	Category	Frequency (Percentage)
Diabetes mellitus	Yes	31 (6.8%)
	No	423 (93.2%)
Hypertension	Yes	161 (35.4%)
	No	293 (64.6%)
LDL cholesterol (mg/dl)	≥130 mg/dl	68 (15.0%)
Total cholesterol(mg/dl)	≥200 mg/dl	84 (18.5%)
Dyslipidemia	Yes	101 (22.2%)
	No	353 (77.8%)
Central obesity	Yes	197 (43.4%)
	No	257 (56.6%)
BMI categories	Underweight	30 (6.6%)
	Normal	198 (43.6%)
	Overweight	125 (27.5%)
	Obese	101 (22.2%)
Body fat percent	Low	40 (8.8%)
	Normal	113 (24.9%)
	High	301 (66.3%)
Median duration of ART (IQR) years 7 (4,11)		
Current ART regime	DTG based	439(96.7%)
	PI based	15(3.3%)
HIV viral load status	Suppressed	422(92.9%)
	Not suppressed	32(7.1%)

Central obesity was defined as waist circumference  $\geq 94$  cm for men and  $\geq 80$  cm for women, Normal ranges for body fat percent as per age category and sex; 20-39 yrs (21%-32% F, 8%19% M), 40-59yrs (23%-33%F, 11%-21%M), 60-79yrs (24%-35%F, 13-24%M)

BMI=Body Mass Index; ART= Antiretroviral Therapy; PI=Protease Inhibitor;

IQR=Interquartile range; LDL=Low Density Lipoprotein: HIV=Human Immunodeficiency Virus; DTG based=Tenofovir Disoproxil Fumarate+ Lamivudine+ Dolutegravir;

PI based =Abacavir+lamivudine+Lopinavir/r, Tenofovir+Lamivudine+Atazanavir/r ;

Abacavir+lamivudine+ Atazanavir/r

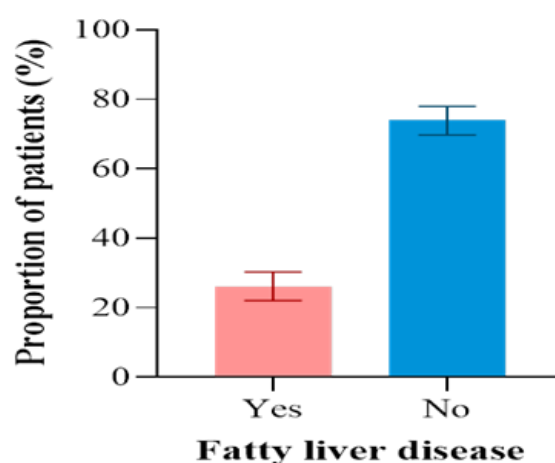
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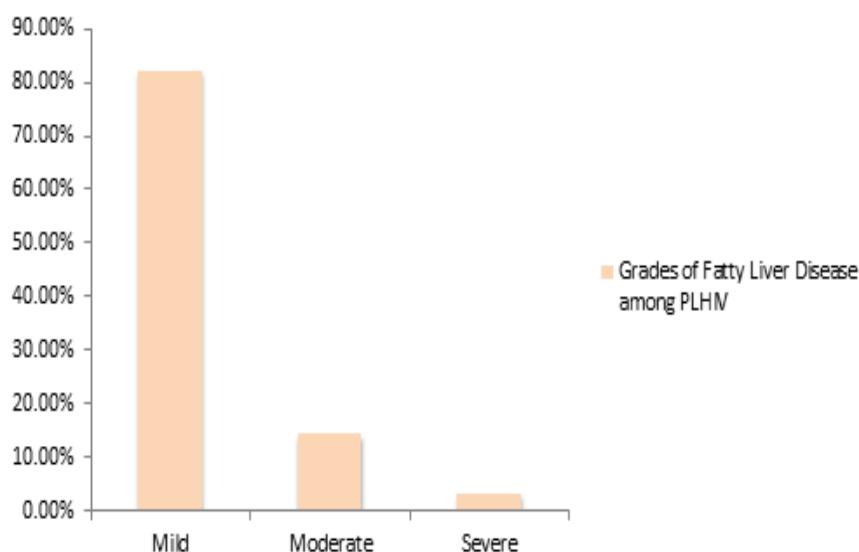
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Among the 454 study participants who underwent Abdominal USS, FLD was present in 118 (25.9 %) participants with a 95%CI of 22% - 30.3 %, Figure 1.



**Figure 1. Prevalence of Fatty Liver Disease among People living with HIV at Temeke Regional Referral Hospital HIV Care and Treatment Clinic**

Majority of our study participants had grade 1(mild) form of FLD, Figure 2.



**Figure 2. Distribution of severity of FLD among PLHIV at TRRH HIV CTC (n = 454)**



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Factors associated with FLD are presented in univariate and multivariate analysis table (Table 3).

**Table 3: Univariate and multivariate analysis of the factors associated with FLD among PLHIV At Temeke Regional Referral Hospital HIV Care and Treatment Clinic n=454**

Variable	Category	Univariate analysis			Multivariate analysis		
		cOR	95% CI	p - value	aOR	95% CI	p – value
Age	>60	2.54	1.06 – 6.09	0.036	2.32	0.86 – 6.27	0.097
	40- 60	1.80	1.12 – 2.89	0.015	1.74	1.02 – 2.96	0.043
	< 40	Ref	-	-	-	-	-
Sex	Female	1.14	0.71 – 1.84	0.593	0.68	0.36-1.27	0.224
	Male	Ref	-	-	-	-	-
Marital status	Widow/widower	2.11	1.09 – 4.09	0.026	1.67	0.77-3.60	0.19
	Divorced	1.15	0.60 – 2.21	0.669	1.08	0.53-2.191	0.84
	Married	1.11	0.65 – 1.88	0.702	0.97	0.58-1.74	0.91
	Single	Ref	-	-	-	-	-
Current alcohol consumption	Yes	1.74	1.04 – 2.90	0.034	1.69	0.95-2.98	0.07
	No	Ref	-	-	-	-	-
BMI (kg/m <sup>2</sup> )	Obesity	3.58	2.08 – 6.14	< 0.001	3.46	1.80-6.65	< 0.001
	Overweight	1.95	1.14 – 3.33	0.014	1.92	1.05 – 3.51	0.034
	Underweight	0.97	0.35 – 2.70	0.945	1.03	0.35-3.03	0.95
	Normal	Ref	-	-	-	-	-
Body fat percent	High	1.57	0.93 – 2.64	0.092	0.57	0.26-1.23	0.16
	Low	1.14	0.48 – 2.72	0.774	0.73	0.28-1.87	0.51
	Normal	Ref	-	-	-	-	-
Central obesity	Yes	1.89	1.21-2.94	0.005	1.73	0.78-3.89	0.18
	No	Ref	-	-	-	-	-
Dyslipidemia	Yes	3.21	1.77-5.82	<0.001	2.63	1.58-4.39	<0.001
	No	Ref	-	-	-	-	-
Diabetes mellitus	Ye	2.19	1.04 – 4.62	0.040	1.14	0.49-2.62	0.75
	No	Ref	-	-	-	-	-

Key: cOR =Crude Odds Ratio; aOR= Adjusted Odds Ratio; Ref= Reference group

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In univariate logistic regression analysis, Diabetes mellitus (cOR 2.19; 95%CI: 1.04 – 4.62 p value=0.040), central obesity (c OR 1.89; 95%: 1.21-2.94; p value =0.005) and current history of alcohol consumption (cOR 1.74; 95% CI: 1.04 – 2.90; p-value =0.034) demonstrated a positive association with FLD.

Factors associated with FLD in multivariate logistic regression analysis were; age between 40-60 years (aOR 1.74; 95% CI: 1.02 – 2.96; p value= 0.043), overweight (aOR 1.92; 95% CI: 1.05-3.51; p value =0.034), obesity (aOR 3.46; 95% CI: 1.80-6.65; p value <0.001) and dyslipidemia (aOR 2.63; 95% CI 1.58-4.39; p value<0.001).

**Discussion**

In this study we aimed at determining the magnitude and the associated factors for Fatty Liver Disease among People living with HIV attending Care and Treatment Clinic (CTC) at Temeke Regional Referral Hospital (TRRH) in Dar es Salaam, Tanzania. We found that one out of four PLHIV had FLD. The predictors of FLD were age category of between 40-60 years, overweight, obesity and dyslipidemia.

The prevalence of FLD among PLHIV in our study was 25.9%. This prevalence is higher compared to prevalence in the general population (13.9%) as it has been revealed in a recent study by Simalene N (13). This could be attributed to presumably higher rates of HCV/HBV co-infection among PLHIV as revealed in previous studies (14,15) which compound the pathogenesis of FLD. Neither our study nor that by Simalene, determined the serological status of HBV and HCV infection in study participants. A study by Lesi O et al among Nigerians on combination ART, showed the prevalence of FLD was 13.3%(16).The lower prevalence found by Lesi O et al could be due to a smaller sample size and exclusion of those with prior history of heavy alcohol consumption. On the other hand, the high prevalence found in our study is in keeping with Pezzini and colleagues who found that the prevalence of FLD by using Abdominal USS was 31.6% among Brazilians(17). Like Tanzania, the prevalence of co-infections with HBV/HCV and HIV is as high as 4.4% among PLHIV in Brazil (18). Moreover, FLD was found on 45 (73%) of 62 study participant by Morse CG et al using histopathological results from liver biopsies taken from HIV mono-infected individuals (19). It is known that a diagnostic tool and race greatly influence the presence of FLD and histopathology is the gold standard test for FLD (4,5). So, in general terms it fair to conclude that the prevalence of FLD is high among PLHIV but it varies from place to place and based on the methodological differences.

Our study found that dyslipidemia was independently associated with FLD. Similar to our findings, previous studies have shown the association between serum levels of low density lipoprotein (LDL) and cholesterol  $\geq 130$  mg/dl and FLD (16,17). Elevated levels of LDL cholesterol have been shown to impair lipid metabolism, increasing insulin resistance leading to excessive delivery to and storage of fatty acids in the liver (20). This study once again underscores the effect of dyslipidemias in the pathogenesis of FLD supporting the evidence gathered in the previous studies.

Obesity is a well-documented risk factor for development of FLD. Several previous studies have shown the association between obesity and FLD among PLHIV (16,17). In the current study, high body mass index was correlated with central obesity and strongly associated with FLD. Obesity causes insulin resistance which impairs hormone sensitive lipase, and leads to increased accumulation of fatty acids in the liver (4). Lipodystrophy, an adverse effect of combination ART, is blamed for high BMIs and central obesity in this cohort due to peripheral adipocyte death, and hence abnormal fat content (4,5). In our study abnormal fat distribution (lipodystrophy) was not assessed in its entirety and we acknowledge it as our limitation. However, truncal obesity was significantly associated with FLD, while overall high body fat percent was not significantly associated with FLD at multivariate analysis. The absence of statistical significance should be taken with caution as it could result from the absence of validated tools for assessing body fat percent to African population. Overall, obesity is one of the consistent factors associated with FLD and its control should be one of the measures to take in the management of FLD.

Alcohol consumption is not uncommon among PLHIV in our setting (21) and has been linked to poor ART adherence (6). In our study, current alcohol consumption was significantly associated with FLD in univariate analysis. Our results are in agreement with a study by Lesi O et al (16), although the association was not statistically significant. The association is brought about by alcohol's pleotropic effects of inducing mitochondrial toxicity and gut bacterial translocation due to increased intestinal permeability leading to chronic inflammatory state; leading to insulin resistance (4,5). On the contrary, in our study hazardous alcohol consumption was not statistically significantly associated with FLD. This finding may have arisen from measurement error of the amount consumed due to self-reporting bias and fear of disappointing the health care workers. Nevertheless, alcohol consumption is known cause for alcohol related fatty disease and can compound the frequency of FLD among the PLHIV. Cessation of alcohol consumption part of the treatment plan of FLD.

In our study contrary to what is previously reported a high HIV viral load was not significantly associated with FLD ( $p=0.582$ ). It is known that high HIV viral load induces chronic

inflammatory state, the release of cytokines leads to insulin resistance; a culprit of FLD (4, 5). In the recent years, due to global policy change in HIV treatment and preventing strategy, starting ART as soon as the diagnosis is made and early combination ART as well as effective viral suppression may be the reason why this association between combination ART and FLD has not been observed. In our study only 3.3% of study participants had detectable viremia. Similarly, Lesi O et al did not find association between FLD and median copies of HIV RNA load.

In the current study we could not establish association between sex and FLD. Previous studies have reported varying results regarding the effect of sex on FLD. A study done by Pezzini MF et al (17) in Brazil, showed that male sex was independently associated with FLD. Similar to our study, by Lesi O et al (16) showed no association between FLD and male sex. Over representation of female (72.7%) in our study could be the reason for this.

Noteworthy, this study revealed an age range between 40 and 60 years is an independent risk factor for FLD. Different from previous studies (16,17), we found an association between this age group and FLD. The relationship between increasing age and FLD could be a result of either; impaired mitochondrial function and increase of oxidative stress as a result of aging or a reflection of accumulated effect of viral inflammation over time (22). With more advanced age, it is expected to have advanced FLD (steatohepatitis), which is associated with higher levels of adiponectin that reduces hepatic fat content (23). Also, the lack of association of FLD for those who aged more than 60 years in our study could be due to their low proportion (5.9%).

This study used Abdominal USS in assessing the presence of Fatty Liver Disease (FLD) among People living with HIV (PLHIV) on combination antiretroviral therapy. To our knowledge this is the first local study which determined the magnitude and factors associated with FLD among PLHIV. The prevalence of FLD among PLHIV is high in our set-up, which has never been documented previously. At this point, we therefore have local data showing high prevalence of a potentially fatal but reversible condition. Factors that may contribute to FLD were clearly ironed out providing a platform for intervention studies or/and programmatic approach to reducing the burden in our setting. Our relatively larger sample size allowed for exploration of many factors.

This study was cross-sectional in nature done in a single centre, Temeke Regional Referral Hospital (TRRH), hence its results cannot be generalized. The use of B mode Abdominal USS in assessment of FLD, has the limitation of being less sensitive (60.9-65%) in detecting mild hepatic steatosis (> 5% of hepatocyte infiltrated with fat) (60). This could lead to underestimation of FLD. Also, abdominal USS is associated with inter-observer variability.

**Conclusion**

In conclusion, Fatty Liver Disease (FLD) occurs in one out of four PLHIV on combination ART in Temeke Regional Referral Hospital. Factors associated with FLD in our study include, age between 40- 60 years, obesity, overweight and dyslipidemia. We recommend weight reduction and regular screening for FLD among PLHIV with above risk factors.

**Abbreviations**

FLD	Fatty Liver Disease
USS	Abdominal Ultrasonography
PLHIV	People Living With HIV
NCD	Non communicable diseases
ART	Antiretroviral Therapy
CTC	Care and Treatment Clinic
TRRH	Temeke Regional Referral Hospital
HIV/AIDS	Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome.
cART	Combined Antiretroviral Therapy

**Conflict of interest**

The authors declare that they have no conflict of interest in this study.

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**Authors contributions**

FM participated in protocol writing, was the major contributor in data collection and analysis and participated in manuscript writing. EVK played a major role in conceptualization and manuscript writing but also participated in protocol writing, data collection and analysis. TJN played a major role in conceptualization and manuscript writing but also participated in protocol writing, data collection and analysis. LF participated in analysis and took part in manuscript writing. JR participated in analysis and took part in manuscript writing. All authors read and approved the final manuscript.

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