Hepatic Function and its Association with Clinical Outcomes in Non-Adherent HIV-1 Adults

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Background and study aim: Hepatic derangements are emerging as prominent causes mortality and morbidity among HIV patients on Highly Active Antiretroviral therapy (HAART). Among this population, high HIV viremia, low CD4+ count and HAART have been established as risk factors for hepatic injury. Although HAART mitigates these risks, it is unknown whether non-adherence to it contributes to the development of hepatic derangements. The current study aimed at exploring whether non-adherence to HAART affects hepatic function of HIV-1 infected adults and to associate hepatic function makers with Viral loads, Body mass index and CD4+ counts of the same population.

Materials and Methods: This cross-sectional analytical study targeted HIV-1 infected adults on first-line HAART comprising of Tenofovir Disoproxil Fumarate, Lamivudine and Efavirenz. A total of 163 adult participants were enrolled. Adherence to HAART was calculated using Pharmacy refill records. Hepatic enzyme levels were measured using a Mindray BS-200 automated clinical chemistry analyzer. Descriptive statistics, Kruskal Wallis test and Bonferroni post hoc statistical tests were performed using SPSS version 25.

Results: Levels of total protein and globulin were elevated among HAART non-adherent participants relative to HAART adherent participants. Furthermore, Albumin to total protein and albumin to globulin ratios were lower in the HAART non-adherent participants relative to the HAART adherent group. CD4+ counts positively correlated with globulin levels. However, levels of Aspartate transaminase, Alanine transaminase, Alkaline phosphatase and Gamma glutamyl transferase were similar between HAART non-adherent and HAART adherent participants.

Conclusion: Non-adherence to HAART dysregulates hepatic globulin synthesis without significant hepatic damage .

INTRODUCTION

The Human immunodeficiency virus (HIV) remains a threat to global public health. Approximately 84.2 million HIV infections and 40.1 million HIV-related mortalities have been reported globally since the onset of HIV **[1].** Currently, the global HIV incidence stands at 1.5 million HIV infections totaling to 38.4 million people living with HIV(PLHIV). Despite tremendous decline of HIV incidence in Eastern and Southern Africa region, the region remains disproportionately affected and threatened by a slow HIV response **[2].** HIV is also a public health concern in Kenya which bears a heterogenous HIV burden largely clustered around the Lake Victoria basin **[3].** Siaya county, located on the Lake Victoria basin bears a HIV prevalence of .

12.3% , ranking it among the top five counties with highest HIV burden in Kenya **[4]**.

HAART remains the global standard for HIV therapy and with optimal adherence it effectively suppresses viral replication to undetectable levels **[5].** By 2021, approximately 28.7 million PLHIV worldwide received HAART of which 16.2 million were in the Eastern and Southern Africa region **[6].** Approximately 83% of all PLHIV in Kenya have access to HAART **[7].** Scale-up of HAART globally has led to a tremendous reduction in new HIV infections and decline in HIV related mortality over the past decade. Consequently, HIV has gradually evolved to a chronic manageable disease and great improvement in the quality of life among PLHIV realized.

Contrary to earlier guidelines requiring initiation of HAART at CD4+ cell count <200cells/mm^3, HAART is currently indicated upon all patients who test positive for HIV regardless of their CD4+ count **[8]**. Consequently, HIV related mortalities have decreased dramatically and lifespans of all PLHIV on HAART prolonged. However, the burden of Non-HIV related morbidity and mortality has drastically increased in the same population **[9]**. This is largely attributed to the development of systemic derangements such as Immune reconstitution inflammatory syndrome, metabolic dysregulation, nephrotoxicity and hepatotoxicity subsequent to cumulative exposure to HAART **[10]**.

Emerging evidence shows that liver related derangements, common among PLHIV are among the leading determinants of non-HIV mortality **[11]**. Such derangements arise from liver damage caused by multiple stimuli, eventually translating into acute or chronic liver disease **[12]**. Among the HAART naive patients, high HIV viraemia and low CD4+ counts mediate liver damage by promoting fibrogenesis and hepatocyte apoptosis **[13]**. HAART also advances structural liver damage via mitochondrial dysfunction, insulin resistance, hepatic steatosis and fibrosis **[14]**. Although current antiretroviral agents used in HAART are effective in protecting against hepatic fibrosis, their efficiency is largely dependent on optimal adherence **[15]**. As such, non-adherence to HAART may predispose PLHIV to higher rates of liver derangements.

Liver injury among HIV infected patients can be detected using liver enzyme levels. For instance, among the HAART naive HIV patients, elevation of gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) without underlying liver disease indicates HIV mediated cholestatic liver damage (16). Likewise, elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serve as indicators of hepatocellular injury among the same population. Hypoalbuminemia, consistent with HIV progression also occurs among HAART naive patients **[17]**. Among the HAART experienced, mild and self-limiting to severe transaminitis as well as hyperbilirubinemia is common **[18]**. Therefore, HAART non-adherent patients are at risk of hepatic damage from both HIV and HAART. Despite this, monitoring of liver damage among PLHIV at baseline and during follow-up remains inadequate especially in resource limited settings. WHO guidelines and Kenyan national guidelines on antiretroviral therapy only indicate non-mandatory determination of AST and ALT levels **[19]**. This hampers early detection, diagnosis and management liver injury among PLHIV.

Previously, associations between higher HIV viral loads with elevated AST and ALT have been reported among HIV patients with poorly controlled viremia, reflecting HIV mediated liver injury **[20]**. In addition, low CD4+ counts are associated with increased incidence of transaminitis and rapid progression of fibrosis among the same population. However, effective HAART counters hepatocyte fibrosis and inflammation, restoring levels of AST and ALT within normal ranges **[21]**. Furthermore, higher body mass index (BMI) is associated with decreased risk of hypoalbuminemia among HIV infected patients is **[22]**. While these associations have been reported among HIV patients naïve and adherent to HAART, it remains largely unknown whether they hold true among HIV patient's non-adherent to HAART. As such, the current study sought to evaluate liver function of HAART non-adherent participants and associate them viral load, BMI and CD4+ counts.

MATERIALS AND METHODS

Study design: This was a cross-sectional analytical study.

Study site and population

This study was carried out among consenting HIV-1 adults attending Siaya county referral hospital, a public facility in Siaya county, Nyanza region of Kenya. Siaya has the third highest HIV burden of 15.3% after Kisumu (19.6%) and Homabay (17.5%) counties. It also bears the lowest levels of viral suppression amongst the three counties **[4].** Study participants were enrolled through systematic random sampling.

Inclusion and exclusion criteria

Only individuals aged 18-60 years on first line HAART regime comprising of Tenofovir, Lamivudine and Efavirenz (TDF+3TC+EFV) for a period not less than 6 months were enrolled. The study excluded individuals with a history of hepatic, renal or cardiovascular disease, tuberculosis, hepatitis B virus, hepatitis C virus, diabetes, hypertension, cancer, substance abuse and pregnant women. Individuals on any other long-term medication other than HAART were also excluded. A total of 163 adults comprising of HIV-1 negative (n=51), HAART naïve (n=23), HAART adherent (n=47) and HAART non-adherent (n=42) were recruited into the study. Sample size was determined using the Daniel (1999) formula and previously reported HIV prevalence **[4]**.

Demographic and anthropometric measures

An open-ended questionnaire was used to collect demographic data of study participants. A Seca 786 (Hamburg, Germany) stadiometer was used to measure body weight and height with participants wearing light clothes and standing in an upright posture. Weight measurement was made to the nearest gram and height to the nearest centimeter. Body mass index (BMI, kg/m^2) was calculated by dividing weight (kg) with height (m) and categorized into underweight (<18.5 kg/m^2) normal (18.5 – 24.9 kg/m^2) and overweight (>25.0 kg/m^2) BMI groups based on World Health Organization (WHO) adult nutrition status.

Sample collection and processing.

An experienced phlebotomist collected 5ml of venous blood from participants' antecubital vein into a syringe. 3ml of the blood was transferred to into an Ethylenediamine tetraacetic acid vacutainer (EDTA) and 2ml into plain BD vacutainer® tubes (Becton Dickinson, Franklin Lakes, USA). The tubes were then labelled with the participants code, date and sample collection time. Blood samples in EDTA tubes were used for CD4+ T cell enumeration immediately after collection. Blood in plain vacutainer tubes was allowed to clot for five minutes prior to serum extraction by centrifugation at 3000 r.p.m for 5 minutes. Obtained serum was used for hepatic function tests.

HIV-1 diagnosis.

Confirmation of HIV-1 was done using rapid immunochromatographic test kit, Determine™ (Abbott Laboratories, Tokyo, Japan) and first response™ (Trinity Biotech Plc, Bray, Ireland). In accordance with the Kenyan national HIV testing algorithm, Participants were considered HIV-1 infected if they had HIV positive results for Determine and HIV-1 positive results using first response kits.

Clinical chemistry measurements

Liver function tests were ran using an automated Mindray™ BS-200 Clinical Chemistry analyzer (Mindray Medical Intl, Shenzhen, China) as per manufacturer's instructions and standard operation procedures. Quality controls for selected liver function tests were done and parameters which failed were recalibrated. Briefly, 1000ul of Serum was pitted into new, clean, unused Mindray™ BS-200 cuvettes and loaded inside the sample chamber of the machine. Subsequently, measurement of serum Aspartate Aminotransferase, Alanine aminotransferase, Albumin, Total Protein, Gamma Glutamyl transpeptidase and C-reactive protein levels was done using the same machine.

Calculation of adherence to HAART

Adherence to HAART was assessed using comprehensive care clinic pharmacy refill records and percentage adherence calculated using the following formula.

$$\text{Percentage adherence} = \frac{\text{No. of doses patient should have taken} - \text{No. of doses missed}}{\text{No. of doses the patient should have taken}} \times 100$$

CD4+ T cell counts

The levels of CD4+ T cells were determined using a Pima™ point of care CD4 cytometer (Inverness medical™, Morges, Switzerland). 25.0 µl of well mixed whole blood sample collected in EDTA BD vacutainer® tube was placed in the Pima CD4 test cartridge sample

collector, capped and inserted into the Pima™ CD4 cytometer. Enumeration of CD4+ T cells was based on quantification of fluorescent signals emitted by fluorescent-labelled anti CD3 and CD4 specific monoclonal antibodies interacting with the sample in the cartridge. The total signals collected were converted into a CD4+ count displayed as cells/µl within 20 minutes. CD4+ counts were then classified into various HIV immunological stages of HIV as follows; ≥ 500 CD4+ cells/ µl as Stage I, 350 – 499 cells/ µl as Stage II, 200 – 349 cells/ µl as Stage III and < 200 cells/ µl as stage IV in accordance with WHO guidelines.

HIV viral load determination

Viral load was determined using an automated Abbott m2000 System (Abbott Molecular Inc., Illinois, USA). RNA was extracted from 200µl of serum sample. Reverse transcription of the RNA to cDNA was done followed by amplification of the cDNA using HIV-specific primers. The extract was fed into the analyzer which converted intensity of HIV probes to viral loads. WHO guidelines were used to categorize viral loads with viral loads <1000 HIV-RNA copies/ml indicating viral suppression.

Statistical analysis

The data was analyzed using IBM SPSS version 25.0 (SPSS Inc. Chicago, USA). Categorical variables such as gender and duration of HAART were summarized into proportions and tabulated. Continuous variables (laboratory measures, age, height, weight, BMI) were described using medians (IQR). Chi-square test was used to determine differences in proportions. Kruskal wallis test was used to compare continuous data across the study groups followed by Bonferroni post hoc correction for multiple comparisons. Spearman correlation test was used to determine the association of each liver function marker with the viral load, CD4+ count and BMI.

RESULTS

Demographic and laboratory characteristics of study participants

A total of 163 adult participants comprising of males, (n = 73) and females (n = 90) were enrolled to the study. Of these, there were HIV-1 negative healthy controls (n= 51), HIV-1 positive HAART naive, (n=23), HIV-1 positive HAART adherent (n = 47) and HIV-1 positive HAART non-adherent (n = 42). A summary of demographic characteristics of study participants is presented in **Table I**. Age (P = 0.838) and height (P = 0.465) of study participants were similar across study groups but body weight differed across the study groups (P = <0.0001). HAART non-adherent participants (median, 59.1 kg; IQR, 11.8; P<0.0001) weighed less than the HAART adherent group (median, 66.6 kg; IQR, 14.7). However, the HAART adherent group (median, 66.6 kg; IQR, 14.7; P<0.0001) weighed more than the HAART naive group (median,56.9 kg; IQR, 11.0). Likewise, body mass index (BMI) varied across groups (P = <0.0001) with the HAART non-adherent group (median, 21.1 kgm^2; IQR, 3.72; P = 0.002) having lower BMI than the HIV-1 HAART adherent group (median, 23.5 kgm^2; IQR, 5.49). In addition, HAART non-adherent group (median, 21.1 kgm^2; IQR, 3.72; P<0.0001) and HIV-1 HAART naive (median, 20.4 kgm^2; IQR, 5.11; P= 0.003) had lower BMI compared to HIV-1 negative healthy control group (median, 24.5 kgm^2; IQR, 5.27).

Distribution of participants into underweight, normal and overweight BMI varied significantly across the groups (P = <0.0001). Majority of underweight participants were found in the HAART non-adherent while overweight participants were most frequent in the healthy control group. Varied distribution of participants amongst various CD4+ T cell categories was observed (P = <0.0001). The HAART non-adherent group accounted for majority of participants in WHO stage III. Similarly, distribution of participants between viral load categories differed significantly across the groups (P <0.0001). Proportion participants with low viral loads was highest in the HAART adherent group while the HAART non-adherent group had the highest proportion of participants with high viral loads.

Liver function parameters of study participants

Albumin levels varied significantly across groups (P < 0.0001) and were higher in the HAART non- adherent group (median, 3.6 g/dl; IQR, 1.7; P = 0.008) and HAART adherent group (median, 4.0 g/dl; IQR, 1.1; P < 0.0001) compared to the naive group (median, 3.2 g/dl; IQR, 1.2). **Table II** summarizes the liver function parameters of study participants. Likewise, globulin levels differed across the

groups (*P*< 0.0001) with the HAART non-adherent group having higher globulin levels (median, 3.4 g/dl; IQR, 1.9; *P* < 0.0001) relative to the HAART adherent group (median, 2.1 g/dl; IQR, 1.2). In addition, both the HAART adherent and HAART non-adherent groups had lower globulin compared to the HAART naive group (median, 3.4 g/dl; IQR, 1.9; *P* = 0.006 and median, 2.1 g/dl; IQR, 2.1; *P* < 0.0001 versus median, 4.1 g/dl; IQR, 1.0) respectively.

Total protein levels differed significantly among the groups (*P*< 0.0001) with between group analysis showing elevated levels of total proteins in the HAART non-adherent group (median, 7.3 g/dl; IQR, 0.8; *P* < 0.0001) compared to the HAART adherent group (median, 6.5 g/dl; IQR, 0.6). In contrast, the HAART adherent group (median, 6.5 g/dl; IQR, 0.6; *P* <0.0001) had lower total protein levels in relative to HAART naive (median, 7.5 g/dl; IQR, 1.0;) and healthy control group (median, 7.1 g/dl; IQR, 1.1). Levels of AST differed significantly across groups (*P*< 0.0001) and were higher in the HAART non-adherent (median, 31.6 IU/L; IQR, 8.0; *P* <0.0001), HAART adherent (median, 32.6 IU/L; IQR, 12.4; *P* <0.0001) and HAART naive (median, 33. 3 IU/L; IQR, 9.8; *P* <0.0001) relative to the healthy control group (median, 23.9 IU/L; IQR, 13.4). Likewise, levels of alanine aminotransferase (ALT) differed significantly across the groups (*P* = 0.003) with the HAART non-adherent group (median, 29.0 IU/L; IQR, 14.6; *P*<0.0001) having higher ALT levels relative to the healthy control group (median, 25.0 IU/L; IQR, 9.9).

The activity of GGT and ALP varied significantly in a similar fashion across groups at the same level of statistical significance (*P* < 0.0001). GGT activity was significantly higher in HAART non-adherent group (median 42.4 IU/L; IQR, 34.3; *P*<0.0001), HAART adherent group (median 51.7 IU/L; IQR, 32.0; *P*<0.0001) and in HAART naive group (median 46.3 IU/L; IQR, 31.0; *P*<0.0001) in comparison to healthy control group (median 28.0 IU/L; IQR, 25.3). Similarly, ALP activity was higher in the HAART non-adherent group (median 100.8 IU/L; IQR, 27.0; *P*<0.0001), HAART adherent group (median 108.7 IU/L; IQR, 27.6; *P*<0.0001) and in HAART naive group (median 102.5 IU/L; IQR, 31.0; *P*<0.0001) in comparison to HIV negative healthy controls (median 28.0 IU/L; IQR, 25.3).

Albumin, total protein, globulin, ALT and AST ratios of study participants.

Across group analysis revealed significant variations in albumin to total protein ratio (*P* <0.0001) as shown in **Table II**. Subsequent between group analysis revealed lower albumin to total protein values in HAART non-adherent group (median, 0.5; IQR, 0.2; *P* <0.0001) compared to the HAART adherent group (median, 0.6; IQR, 0.1). In contrast, albumin to total protein ratio was significantly higher in the HAART non-adherent (median, 0.5; IQR, 0.2; *P* = 0.009) and HAART adherent group (median, 0.6; IQR, 0.1; *P* <0.0001) in comparison to the HAART naive group (median, 0.4; IQR, 0.1). Similarly, values of albumin to globulin ratio were significantly higher in the HAART adherent group (median, 0.6; IQR, 0.1; *P* = 0.003) relative to the healthy control group (median, 0.5; IQR, 0.1).

Correlation of liver function with Clinical outcomes HIV-1 HAART non-adherent

There was a weak significant positive correlation between globulin levels with CD4+ counts (ρ = 0.308, *P* = 0.048) among HIV-1 HAART non-adherent participants as shown in **Table III.**

Table I: Demographic and laboratory characteristics of study participants

Characteristic	**HIV-1 [-] Control** (n = 51)	**HIV-1 [+] HAART naive** (n = 23)	**HIV-1 [+] HAART adherent,** (n = 47)	**HIV-1 [+] HAART non-adherent,** (n = 42)	***P*-value**
Gender:					
Female n (%)	27 (52.9)	14 (60.9)	32 (68.1)	17 (40.5)	
Male, n (%)	24 (47.1)	9 (39.1)	15 (31.9)	25 (59.5)	0.065
Age, years	37.0 (6.0)	38.0 (7.0)	37.0 (7.0)	36.5 (9.3)	0.838
Height, m	1.7 (0.1)	1.7 (0.1)	1.7 (0.1)	1.7 (0.1)	0.465
Weight, Kg	67.8 (18.0)	56.9 (11.0) [a]	66.6 (14.7) [b]	59.1 (11.8) [a, c]	**<0.0001**
BMI, Kgm^2	24.5 (5.27)	20.4 (5.11) [a]	23.5 (5.49)	21.1 (3.72) [a, c]	**<0.0001**
BMI category, n (%)					
Underweight (<18.5kg/m^2)	4 (7.8)	3 (13.0)	3 (6.4)	9 (21.4)	
Normal (18.5 – 24.9 kg/m^2	22 (43.1)	16 (69.6)	26 (55.3)	30 (71.4)	**<0.0001**
Overweight (>25.0 kg/m^2**)**	25 (49.0)	4 (17.4)	18 (38.3)	3 (7.1)	
WHO HIV immunological staging, n (%)					
Stage III	0 (0.0)	10 (43.5)	5 (10.6)	18 (42.8)	
Stage II	3 (5.9)	7 (30.4)	13 (27.7)	12 (28.6)	**<0.0001**
Stage I	48 (94.1)	6 (26.1)	29 (61.7)	12 (28.6)	
Log_{10} HIV-1 RNA, Copies/ml	0 (0.0)	4.5 (1.6)	3.4 (1.9) [a]	4.6 (1.1) [b, c]	**<0.0001**
Viral Load Categories, n (%)					
Low	0 (0.0)	4 (17.4)	20 (42.6)	3 (7.3)	**<0.0001**
High	0 (0.0)	19 (82.6)	27 (57.4)	38 (92.7)	

Data shown are numbers (n) and proportion (%) of study subjects for categorical variables and median, interquartile range (IQR) for continuous variables. HIV-1 [-]; Human immunodeficiency virus type 1 negative. HIV-1 [+]; Human immunodeficiency virus type 1 positive. HAART; Highly active antiretroviral therapy, BMI; Body mass index. Data analysis was conducted using chi-square tests for proportions and Kruskal wallis test for continuous data. Thereafter, Bonferroni post-hoc test for was used for comparisons between study groups. Bonferroni correction was set at P <0.0125 and significant groups denoted as; [a] P< 0.0125 vs HIV-1[-] control, [b] P<0.0125 vs HIV-1 [-] naive, [c] P<0.0125 vs HIV-1 [+] HAART adherent. P values in bold are significant.

Table II: Liver function parameters of study participants

Parameter	HIV-1 [-] healthy control n = 51	HIV-1 [+] HAART naive n = 23	HIV-1 [+] HAART adherent, n = 47	HIV-1 [+] HAART non-adherent, n = 42	*P*
Liver function parameters					
Albumin, g/dl	4.0 (3.0-5.4)	3.2 (2.1-4.5) [a]	4.3 (2.7-5.8) [b]	3.6 (2.6-5.6) [b]	**<0.0001**
Globulin, g/dl	3.2 (3.0-5.4)	4.1 ((2.3-6.1) [a]	2.1 (0.5-3.7) [a, b]	3.4 (1.2-5.1) [b, c]	**<0.0001**
Total protein, g/dl	7.1 (6.0-8.4)	7.5 (6.8-8.2)	6.5 (5.6-7.3) [a, b]	7.3 (6.6-8.0) [c]	**<0.0001**
ALB/T.PROT ratio	0.5 (0.4-0.8)	0.4 (0.3-0.6) [a]	0.6 (0.4-0.9) [a, b]	0.5 (0.3-0.8) [b, c]	**<0.0001**
ALB/GLB ratio	1.2 (0.6-4.7)	0.8 (0.3-1.8)	2.1 (0.7-11.2) [a, b]	1.1 (0.5-4.8) [c]	**<0.0001**
AST, IU/L	23.9 (9.1-36.9)	33.3 (19.1- 42.3) [a]	32.6 (18.8-45.0) [a]	31.6 (21.6-42.7) [a]	**<0.0001**
ALT, IU/L	25.0 (11.4-37.4)	25.4 (15.6-36.4)	29 (14.8-43.9) [a]	28.8 (15.7-35.3)	**0.003**
AST/ALT ratio	1.0 (0.3-2.6)	1.2 (0.7-2.1)	1.1 (0.5-2.2)	1.2 (0.6-2.1)	0.025
GGT, IU/L	28.0 (9.4-48.0)	46.3 (22.6-73.5) [a]	51.7 (28.2-84.7) [a]	42.4 (23.4-73.6) [a]	**<0.0001**
ALP, IU/L	74.4 (43.1-115.6)	102.5 (68.1-130.2) [a]	108.7 (64.1-133.9) [a]	100.8 (72.2-132.8) [a]	**<0.0001**

Data shown are median, and interquartile range (IQR) for continuous variables of study subjects. HIV-1 [-]; Human immunodeficiency virus type 1 negative. HIV-1 [+]; HIV-1 positive. HAART; Highly active antiretroviral therapy. AST; Aspartate aminotransferase. ALT; Alanine aminotransferase. GGT; Gamma glutamyl transpeptidase. ALP; Alkaline phosphatase. IU/L; international units per liter. Data analysis was conducted using Kruskal wallis test. Thereafter, Bonferroni post-hoc test was used for comparisons between study groups. Bonferroni correction was set at P <0.0125 and significant groups denoted as [a] P< 0.0125 vs HIV-1[-] control, [b] P<0.0125 vs HIV-1 [-] naive, [c] P<0.0125 vs HIV-1 [+] HAART adherent. P values in bold are significant.

Table III. Correlation of liver function with Clinical outcome measures HIV-1 HAART non-adherent

Parameter	BMI, kg		Viral loads		CD4+ count	
	ρ	*P*	*ρ*	*P*	*ρ*	*P*
Total protein, g/dl	-0.272	0.081	-0.183	0.246	0.064	0.688
Albumin	0.113	0.476	0.158	0.318	-0.214	0.173
Globulin	-0.214	0.173	-0.275	0.078	**0.308**	**0.048**
ALB: GLB ratio	0.157	0.322	0.209	0.185	-0.249	0.112
ALB: T.PRT ratio	0.157	0.322	0.209	0.185	-0.249	0.112
AST, IU/L	0.201	0.203	-0.111	0.485	0.131	0.408
ALT, IU/L	-0.054	0.735	0.164	0.298	-0.211	0.179
AST:ALT ratio	0.150	0.342	-0.194	0.218	0.278	0.074
ALP, IU/L	-0.036	0.819	0.195	0.216	-0.169	0.285
GGT, IU/L	0.129	0.414	0.202	0.199	-0.229	0.145

Data presented are correlation coefficient (rho, *ρ*) with associated p-values. Statistical analysis was performed using Spearman's rank correlation test. BMI; body mass index, ALB/GLB ratio; albumin-to-globulin ratio, ALB/T.PRT ratio; albumin-to-total protein ratio, AST; Aspartate aminotransferase, ALT; Alanine aminotransferase, ALP; alkaline phosphatase, GGT; gamma glutamyl transferase.

DISCUSSION

Non-adherence to HAART is a global public health concern associated with HIV progression and transmission **[23]**. In the current era of widespread HAART use, non-AIDS related conditions are emerging as the leading causes of mortality among PLHIV with liver diseases being amongst the most profound determinants of mortality **[11]**. While both HAART and HIV are known to contribute to liver injury, the role of non-adherence to HAART in emergence of liver injury among PLHIV remains poorly characterized. Therefore, the current study determined liver function markers among HIV-1 HAART non-adherent and adherent adults from western Kenya.

Significantly lower weight and BMI as well as higher rates of underweight BMI among HAART non-adherent participants relative to the HAART adherent counterparts were indicative of HIV-induced malnutrition. Furthermore, higher viral loads and higher rates of participants with HIV immunological stage III in HAART non-adherent group indicated poor viral suppression and HIV progression. The findings resonate with previous studies done in Uganda and Ethiopia **[24]**. Non-adherence to HAART results in suboptimal suppression and promotes HIV progression, ultimately predisposing HAART non-adherent patients to HIV associated malnutrition, poor nutrient absorption and utilization as well as metabolic disturbances resulting in weight loss **[25]**. Plausibly, malnutrition may have also resulted from food insecurity is identified as a predictor of non-adherence to HAART.

Elevated albumin levels in the HAART non-adherent and HAART adherent group relative to the HAART naive group suggests improved hepatic albumin synthesis. This finding corroborates previous studies indicating a significant increase in albumin levels upon initiation to HAART **[26]**. HAART suppresses HIV viral replication and promotes immune restoration, ultimately improving hepatic synthetic function **[27]**. Although albumin is a negative acute phase protein whose levels decline with HIV progression, no significant difference was revealed in albumin levels between HAART adherent and non-adherent groups. It is possible that the competing effects of HIV and HAART as well as albumin's long half-life prevented sharp declines of albumin levels among the HAART non-adherent group **[28].** As such, our findings suggest that use of HAART, regardless of adherence to it, among PLHIV promotes the liver's synthetic function.

Elevation of globulin and total protein levels as well as viral load levels among the HAART non-adherent participants was indicative of disease progression. Increases in globulins are attributable to dysregulated globulin synthesis

resulting from poor viral suppression, common among HAART non-adherent patients **[29]**. As such, high viral loads impair hepatic and B-cell functions and promote hepatocyte inflammation leading to overproduction of α, β and gamma globulins, subsequently elevating globulin levels **[30]**. Consequently, albumin to globulin ratio is reduced among HAART non-adherent patients as the case with the present study. Our findings are consistent with previous studies which observed elevation of globulin levels among HAART naive individuals, suggesting that hyperglobulinemia observed among HAART non-adherent participants was HIV- induced **[31]**. As such, increased globulins levels and reduced albumin to globulin ratio present possible surrogate markers for prediction of hepatic damage among HAART non-adherent patients.

In contrast with our findings, earlier studies associate HAART with a reduction of total protein levels **[32]**. This is explained by HAART's ability to dysregulate protein metabolism and induce adverse gastrointestinal disturbances which result in malnutrition. However, this effect may vary depending on adherence to HAART. Notably, effective HAART decreases globulin levels via immune reconstitution and rectification of dysregulated immune function with subsequent restoration immunoglobulin levels **[33]**. In addition, indirect evidence showing lower levels of IgG and IgA among virally suppressed HIV infected patients but not among viremic patients, suggests that adherence to HAART may play a role in dysregulating globulin levels **[34]**. Since poor viral suppression and progressive liver damage results in sustained globulin overproduction, our findings indicate that adherence to HAART modulates total protein levels by promoting elevation of globulin levels.

Elevation of AST levels among HIV infected participants naive, adherent and non-adherent to HAART relative to healthy controls suggests synergistic HIV and HAART induced liver injury. This is supported in part by significantly higher ALT levels in the HAART adherent group relative to the healthy control group. Together, our findings imply that HIV progression and use of HAART can cause transaminitis. This corroborates previous studies conducted elsewhere (35). HIV directly injures hepatocytes via induction of apoptosis while antiretroviral agents such as Efavirenz induce mild to moderate hepatotoxicity **[36]**. Elevation of ALT in the HAART adherent group alone suggested that HAART is a significant determinant to liver damage among PLHIV. Similar findings have been reported in Ethiopia **[37]**. Our findings differ from previous studies demonstrating marked elevation of AST and ALT among HIV patients on HAART **[38]**, probably due to exclusion of participants coinfected with viral hepatitis, tuberculosis as well as those with history of alcohol consumption.

Concurrent elevation of GGT and ALP among the HAART non-adherent, adherent and naive groups relative to HIV-1 negative healthy controls was indicative of progression to cholestasis and ongoing liver damage. Similar findings have been reported by earlier studies **[39]**. We attribute elevations of GGT and ALP to HIV infection and HAART. For instance, HIV infected Kupffer cells, while mitigating gut microbial translocation in HIV, inadvertently trigger hepatic inflammatory and fibrogenic processes, ultimately causing hepatic damage **[40]**. In addition, HAART induces mitochondrial dysfunction and mitochondrial toxicity by promoting unregulated production of reactive oxygen species and impairment of intracellular drug metabolism which causes hepatic cholestasis.

Significant positive correlation of globulin levels with CD4+ counts as well as higher total protein and viral loads among HAART non-adherent participants was indicative of deranged B cell function and HIV progression. This finding partially resonates with a Nigerian study which reported positive correlation between CD4+ count and α-1 macroglobulin **[41]**. Poor viral suppression among the HAART non-adherent patients is associated with partial immune reconstitution, progressive liver damage and sustained globulin overproduction by B cells **[42]**. It's also possible that CD4+ auto antibody production previously reported among virologically suppressed HIV infected patients, may have been heightened among HAART non-adherent patients, ultimately resulting in hypergammaglobulinemia and dysregulated B-cell activation.

CONCLUSION

Non-adherence to HAART is associated with alterations of globulin, total protein, albumin to globulin and albumin to total protein ratios

possibly indicating dysregulation of hepatic globulin synthesis but without significant hepatic damage. Further prospective studies on the value globulin, total protein and albumin to globulin indices as surrogate markers for non-adherence to HAART is warranted.

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Ethical consideration

Scientific and ethical approval for this study was obtained from Masinde Muliro University of science and Technology Ethical Review Board (MMUST IERC), approval number **MMUST/IERC012/2022** and the National commission for science, technology and innovation (NACOSTI), license number **NACOSTI/P/22/16221**. Written informed consent was obtained prior to enrollment in the study and confidentiality maintained via coding and restricting access to study participants' information. Random selection of participants ensured equal chances of benefit for all. Free health education on importance of proper adherence to HAART and clinical consequences of non-adherence to HAART were given to all participants.

HIGHLIGHTS

1. Hepatic derangements are emerging as the leading causes of mortality among HIV patients on HAART.
2. High HIV viremia, low CD4+ count and HAART are associated with development of hepatic derangements among HIV patients.
3. Non-adherence to HAART was found to dysregulate hepatic globulin synthesis without causing significant hepatic damage.

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