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# Association of Abnormal Liver Function Parameters with HIV Serostatus and CD4 Count in Antiretroviral-Naive Rwandan Women

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## Abstract

We determined the associations of HIV infection/CD4 count with markers of hepatocellular damage [elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] and liver synthetic function (decreased albumin) in HIV-infected ($HIV^+$) antiretroviral therapy (ART)-naive and uninfected ($HIV^-$) Rwandan women. In 2005, 710 $HIV^+$ ART-naive and 226 $HIV^-$ women enrolled in the Rwanda Women's Interassociation Study and Assessment. Liver enzymes were measured with abnormality defined as either AST or ALT ≥1.25 times the upper limit of normal. Low serum albumin level was defined as <3.5 g/dl. Multivariable logistic regression analysis identified independent predictors of elevated AST/ALT and low serum albumin. $HIV^-$ women had the lowest prevalence (6.6%) of abnormal AST/ALT, with the highest prevalence (16.4%) in $HIV^+$ women with CD4 <200 cells/$\mu$l ($p = 0.01$). The odds of having serum albumin <3.5 g/dl was 5.7-fold higher in $HIV^+$ than $HIV^-$ women (OR = 5.68, 95% CI: 3.32–9.71). The risk of low albumin decreased from low to high CD4 count, with OR = 2.62, 95% CI: 1.66, 4.14 and OR = 1.57, 95% CI: 1.01, 2.43 in $HIV^+$ women with a CD4 count <200 and 200–350 cells/$\mu$l, respectively vs. $HIV^+$ with CD4 >350 ($p < 0.001$ and $p < 0.05$ for all comparisons). Our findings suggest that HIV-associated liver damage may occur in ART-naive patients. Although liver abnormality prevalences in this cohort of HIV-infected Rwandan women are less than reported in developed countries, caution is needed for risk assessment measures to monitor and screen HIV-infected patients pre- and post-ART initiation in African clinical settings to curtail potential risks associated with HIV infection.

## Introduction

Human immunodeficiency virus (HIV) infection causes morbidity and mortality worldwide, and the number of HIV-infected patients has increased dramatically in the past decade.[1–3] HIV infection causes systemic disease with many complications beyond acquired immunodeficiency syndrome (AIDS) illnesses that may not yet be recognized.[4,5] While liver enzyme elevations are common in HIV-infected patients, their diagnosis or management may be difficult because of the intricacies involved in pathogenic mechanisms of liver functioning.[6,7] In many $HIV^+$ patients with elevated liver enzymes, the elevation is not explained by an identified underlying liver disease or toxin and thus may directly occur either due to antiretroviral drug toxicity or the HIV infection itself.

Additionally, liver enzyme abnormalities in HIV-infected persons may reflect concurrent hepatitis B (HBV) or hepatitis C (HCV) infection, which are more common among $HIV^+$ than $HIV^-$ individuals.[8,9] Other factors that independently contribute to liver damage that may be more common in HIV-infected persons include alcohol-related liver disease, nonalcoholic

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steatohepatitis associated with metabolic syndromes, and medication or illicit drug use.[10,11] Studies from developed countries have reported correlations between HIV viral load and aminotransferase serum levels in HIV-infected antiretroviral (ART)-naive patients.[12] However, a study conducted in Uganda found that the risk of clinically significant hepatotoxicity was low, even in $HIV^+$ patients on ART and among HIV/HBV-coinfected persons.[13] Nevertheless, there is emerging evidence that HIV infection, even in the absence of ART toxicity and other cofactors, may have a direct impact on the liver fibrosis pathogeneses, nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH), and on further progression to liver disease.[14,15]

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are hepatic enzymes whose elevation indicates hepatocellular injury.[12,16] Although reports of elevations in these hepatic enzymes are frequent in HIV-infected patients, direct reports of hepatocellular injury are limited, perhaps because of pitfalls in case definition.[6] In addition to liver enzymes, which indicate hepatocellular damage, serum albumin is a measure of hepatic synthetic function with albumin levels decreased in chronic liver disease, such as cirrhosis,[17] reflecting decreased synthesis. However, albumin levels are dependent on a number of other factors such as catabolism, hormonal factors, and urinary and gastrointestinal losses. These should be taken into account when interpreting low albumin levels. Several studies suggest possible associations of chronically elevated liver enzyme levels and an increased mortality[18,19] in HIV-infected and HIV-uninfected patients irrespective of the causal mechanisms.

We determined the association between HIV infection (and CD4 count among those with HIV infection) and markers of hepatocellular damage (AST and ALT) and liver synthetic function (albumin) in ART-naive HIV-infected Rwandan women compared to an $HIV^-$uninfected group. In particular, hepatotoxicity measured by the levels of ALT or AST elevation was graded according to the AIDS Clinical Trial Group criteria.[20] The goal of the study was to identify predictors of poor liver function in HIV-infected Rwandan women.

## Materials and Methods

### *Study population*

The Rwanda Women's Interassociation Study and Assessment (RWISA) is an observational prospective cohort of 710 ART-naive (at enrollment) HIV-infected and 226 HIV-uninfected Rwandan women enrolled in 2005. Participants were recruited through grassroots women's organizations and clinical care sites for HIV-infected patients. Inclusion criteria were age 25 years or older at study entry, willingness to give informed consent, agreement to be tested for HIV, participation in a baseline visit as an outpatient, and no history of receiving antiretroviral treatment with the allowed exception of single-dose nevirapine to prevent mother-to-child transmission of HIV. Informed consent included a video describing and demonstrating the study, followed by a group question and answer period, and then a private standard written informed consent process.

At study entry, participants provided historical information including sociodemographics, medical history, and symptoms. Anthropometric measurements and fasting blood specimens were taken. The interviews were conducted in the local language Kinyarwanda by trained interviewers with a nursing background. Research staff entered all interview and physical examination data directly into an ACCESS database. The study was funded by the National Institute of Allergy and Infectious Diseases and was approved by the Rwandan National Ethics Committee and the Montefiore Medical Center (Bronx, NY) Institutional Review Board. All RWISA participants for whom AST, ALT, and albumin measurements were performed at the enrollment visit are included in this analysis.

### *Laboratory methods*

The liver enzymes ALT and AST and serum albumin were measured using COBAS Integra Chemistry Analyzer, Roche Diagnostics, Mannheim, Germany at King Faisal Hospital in Kigali. CD4 counts were determined with a FACS counter (Becton and Dickinson, Immunocytometry Systems, San Jose, CA). Diagnosis of HIV infection was determined by having a positive result for HIV-1 antibody ELISA kits (HIV Vironostika, Netherlands, and Murex HIV-1.2, Oxford, UK), which was confirmed by a positive result again with the same test.

### *Exposure variables*

The primary exposure variables were HIV serostatus (positive vs. negative) and in the HIV-positive women CD4 cell count categorized as <200, 200–350, and >350 cells/$\mu$l. Secondary exposure variables included age, income, alcohol consumption, smoking, body mass index (BMI) calculated as weight in kilograms divided by (height in meters)$^2$, serum creatinine, and medication use for prophylaxis of opportunistic infection (sulfamethoxazole/trimethoprim or Dapsone).

### *Outcome variables*

The primary outcomes were elevated transaminases (AST, ALT) and serum albumin. We defined abnormal liver enzymes according to the AIDS Clinical Trial Group Guidelines[20]: liver enzyme elevations of <1.25, 1.25–2.5, 2.6–5, 5.1–10, or more than 10 times the upper limit of normal (ULN) define hepatotoxicity of grades 0, 1, 2, 3, and 4, respectively. For this study an abnormal liver enzyme was defined as an ALT/AST elevation of grade 1 (43 U/liter or 1.25 times the ULN) or higher, using 35 U/liter of ALT or AST as the ULN. Low serum albumin level was defined as an albumin value <3.5 g/dl, the lower limit of normal in the testing laboratory.

### *Statistical methods*

Analyses were done for participants with and without HIV infection combined in order to assess the effect of HIV infection on the liver enzymes and serum albumin. Further analyses were done restricted to $HIV^+$ participants by CD4 category (CD4 >350, 200 $\leq$ CD4 $\leq$350, and CD4 <200 cells/$\mu$l) to assess for the effect of disease progression on the liver enzymes and serum albumin. Descriptive statistics (means, standard deviations) were used to present continuous variables, and numbers and percentages for categorical variables. Analysis of variance compared mean serum albumin levels among groups defined by HIV status and CD4 count while Chi-square tests did so for categorical variables.

To identify predictors of poor liver function in this population we fitted a multivariable logistic regression model with all variables included in the model. Additionally, we did fit

linear regression models using serum albumin as a continuous variable, which gave results similar to those of the categorical models. However, for clinical relevance we believe linear models are not the best option to present and creating categories for levels of serum albumin abnormality is better as previously reported.[13,21]

Abnormal AST/ALT levels were combined both because this is normal clinical practice and due to low rates of abnormality ($\sim$5%) in each. Furthermore, to use ALT and AST as continuous variables they would need to be split up for linear models, which is never done in clinical practice; in addition, each had a skewed distribution (kurtosis $\sim$10) even after log transformation. Variables included in these models were chosen by having a statistical association with aminotransaminase values and serum albumin in univariate analyses. SAS software, version 9.1.3 (Cary, NC), was used for all analysis. $p < 0.05$ was used to indicate a statistically significant association.

## Results

Demographic, clinical, and laboratory parameters of participants are summarized by HIV/CD4$^+$ group (HIV$^-$, HIV$^+$ and for HIV$^+$, CD4 >350, 200 < CD4 <350, and CD4 <200 cells/$\mu$l) (Table 1). Of the 936 participants, 855 (213 HIV$^-$ and 642 HIV$^+$) had data on liver function tests from the enrollment visit. The HIV$^-$ women were older than the HIV$^+$ women (58.2% vs. 21.2% over 40 years old; $p < 0.001$). Among HIV$^-$ women, the prevalence of elevated AST and/or ALT was 6.6% compared to 12.6% in HIV-infected women, with the highest prevalence (16.4%) in HIV-infected women with CD4 <200 cells/$\mu$l. Mean serum albumin (g/dl) was systematically lower with more advanced HIV disease: 3.9 $\pm$ 0.4 in HIV$^-$ women and 3.6 $\pm$ 0.6, 3.5 $\pm$ 0.7, and 3.3 $\pm$ 0.6 g/dl in HIV$^+$ women with CD4 count $\geq$350, 200–350, and <200 cells/$\mu$l, respectively ($p < 0.001$). About 52% of HIV$^+$ and 20% of HIV$^-$ women had serum albumin <3.5 g/dl.

### *Markers of hepatocellular damage (ALT/AST) in HIV-positive subjects*

Table 2 shows factors associated with elevated ALT/AST using univariate (unadjusted) and multivariate (adjusted) logistic regression analysis for all subjects. The risk of having elevated ALT/AST was about 4.4-fold higher in HIV$^+$ women compared to HIV$^-$ women (OR = 4.4, 95% CI: 1.91, 9.97). In analysis restricted to HIV$^+$ subjects, there was no significant difference in liver enzymes among CD4 groups in either univariate or multivariate analysis indicating that the disease progression did not affect liver enzymes (Table 3). However, use of prophylactic drugs (Bactrim and/or Dapsone) was associated with elevated liver enzymes in both univariate and multivariate analysis ($p = 0.045$ and $p = 0.03$, respectively). We did not find any association between alcohol use, age, BMI, and income and elevated liver enzymes in univariate and multivariate analysis.

### *Marker of synthetic liver function (serum albumin) in HIV-positive subjects*

In logistic regression analysis using models including all subjects (Table 4) the odds of having serum albumin <3.5 g/dl was 5.7-fold higher in HIV$^+$ than HIV$^-$ women (OR = 5.68, 95% CI: 3.32, 9.71). In analysis restricted to HIV$^+$ women (Table 5), the odds of having serum albumin <3.5 g/dl was 2.6-fold higher in HIV$^+$ women with CD4 counts <200 and 1.6-fold higher in those with CD4 between 200 and 350 cells/$\mu$l (OR = 2.62, 95% CI: 1.66, 4.14 and OR = 1.57, 95% CI: 1.01, 2.43, respectively) compared to HIV$^+$ women with CD4

Table 1. Baseline Characteristics of Study Population by HIV Status and CD4 Cell Count

| *Participant characteristics* | *HIV negative* (n = *213*) | *HIV positive* | | | p-*value*[a,b] | *All HIV positive* (n = *642*) |
|---|---|---|---|---|---|---|
| | | *CD4 >350* (n = *177*) | *CD4 200–350* (n = *240*) | *CD4 <200* (n = *255*) | | |
| Age (years) | | | | | | |
| <30 | 33 (15.5) | 47 (26.6) | 48 (20.0) | 53 (23.6) | <0.001 | 148 (23.1) |
| 30–40 | 56 (26.3) | 97 (54.8) | 136 (56.7) | 125 (55.6) | | 358 (55.8) |
| 41+ | 124 (58.2) | 33 (18.6) | 56 (23.3) | 47 (20.9) | | 136 (21.2) |
| Income (Rwandan Francs) | | | | | | |
| <10,000 | 86 (44.5) | 57 (32.8) | 87 (36.8) | 82 (37.6) | 0.06 | 226 (35.9) |
| 10,000–35,000 | 74 (38.3) | 96 (55.2) | 119 (50.4) | 101 (46.3) | | 316 (50.3) |
| >35,000 | 33 (17.1) | 21 (12.1) | 30 (12.7) | 35 (16.1) | | 86 (13.7) |
| Alcohol use, *n* (%) | 52 (27.4) | 36 (21.2) | 51 (22.1) | 46 (21.5) | 0.43 | 133 (21.6) |
| Body mass index (kg/m$^2$) Mean $\pm$ SD | 21.32 $\pm$ 3.8 | 21.87 $\pm$ 3.9 | 21.84 $\pm$ 3.8 | 21.07 $\pm$ 3.4 | 0.15 | 21.58 $\pm$ 3.7 |
| Albumin (mean $\pm$ SD), mg/dl | 3.94 $\pm$ 0.4 | 3.58 $\pm$ 0.6 | 3.46 $\pm$ 0.7 | 3.26 $\pm$ 0.6 | <0.001 | 3.43 $\pm$ 0.6 |
| Creatinine, mg/dl (mean $\pm$ SD) | 0.85 $\pm$ 0.19 | 0.91 $\pm$ 0.2 | 0.93 $\pm$ 0.2 | 0.94 $\pm$ 0.2 | <0.001 | 0.93 $\pm$ 0.2 |
| Bactrim and/or Dapsone use in prior year *n* (%) | 38 (18.6) | 127 (72.9) | 219 (91.2) | 202 (91.4) | <0.001 | 548 (86.3) |
| AST > or ALT >43 mg/dl | 14 (6.6) | 21 (11.8) | 23 (9.6) | 37 (16.4) | 0.01 | 81 (12.6) |
| Albumin <3.5 g/dl | 42 (20.1) | 72 (40.9) | 119 (50.0) | 138 (62.7) | <0.001 | 329 (51.9) |

[a]*p* value is from chi-square test comparing all four HIV/CD4 groups (HIV negative, HIV$^+$ CD4 >350, HIV$^+$ CD4 200–350, HIV$^+$ CD4 <200).
[b]Analysis of variance tested for differences among continuous variables among groups defined by HIV/CD4 groups.
*n* (%) for categorical variables or mean $\pm$ standard deviation for continuous variables.
ALT, alanine aminotransferase, AST, aspartate aminotransferase.

Table 2. Factors Associated with Elevated Liver Enzymes (Aspartate Aminotransferase >43 or Alanine Aminotransferase >43 U/liter) in Unadjusted and Multivariate Analyses for All Subjects

| *Parameter* | *Unadjusted OR (95% CI)* | *Overall* p *value* | p-*value* | *Adjusted OR (95% CI)* | *Overall* p-*value* | p-*value* |
|---|---|---|---|---|---|---|
| $HIV^+$ vs. $HIV^-$ | 2.05 (1.14, 3.70) | | 0.02 | 4.36 (1.91, 9.97) | | <0.001 |
| Age (years) | | 0.27 | | | 0.39 | |
| <30 | 1 | | — | 1 | | — |
| 30–40 | 0.66 (0.39, 1.11) | | 0.11 | 0.70 (0.40, 1.21) | | 0.19 |
| >41 | 0.72 (0.41, 1.27) | | 0.25 | 0.79 (0.41, 1.52) | | 0.48 |
| Income (Rwf) | | 0.08 | | | 0.04 | |
| <10,000 | 1 | | — | 1 | | — |
| 10,000–35,000 | 0.59 (0.37, 0.94) | | 0.03 | 0.52 (0.31, 0.87) | | 0.01 |
| >35,000 | 0.76 (0.40, 1.46) | | 0.41 | 0.72 (0.36, 1.46) | | 0.36 |
| Alcohol use (yes vs. no) | 1.41 (0.86, 2.31) | | 0.17 | 1.47 (0.88, 2.46) | | 0.14 |
| Body mass index ($kg/m^2$) | | 0.64 | | | 0.36 | |
| <18.5 | 1 | | — | 1 | | — |
| 18.5–25 | 1.32 (0.74, 2.34) | | 0.34 | 1.44 (0.78, 2.67) | | 0.25 |
| >25 | 1.15 (0.54, 2.46) | | 0.71 | 1.40 (0.61, 3.19) | | 0.43 |
| Bactrim and/or Dapsone use in prior year (yes vs. no) | 1.00 (0.63, 1.60) | | 0.99 | 0.51 (0.28, 0.93) | | 0.02 |
| Serum creatinine >1 mg/dl vs. <1 mg/dl | 1.07 (0.67, 1.73) | | 0.76 | 1.15 (0.69, 1.91) | | 0.58 |

All variables are included in the multivariate model.

counts >350 cells/$\mu$l. Higher monthly income [>35,000 Rwandan francs (FRW) equivalent to 60$ US] was associated with a 40% lower risk of having low serum albumin compared to patients with lower income (<10,000 FRW or 17$) (OR = 0.61, 95% CI: 0.41, 0.99) after excluding the $HIV^-$ group that was confounding the association between income and serum albumin. Higher BMI (>25 $kg/m^2$) was protective against low serum albumin; the risk of having low serum albumin decreased by 70% compared to women with lower BMI (<18.5 $kg/m^2$) (OR = 0.30, 95% CI: 0.16, 0.55). Older age (>40 years) was associated with lower serum albumin compared to younger age (<30 years) (OR = 2.2, 95% CI: 1.28, 3.71).

## Discussion

In this study of ART-naive HIV-infected and HIV-uninfected Rwandan women, HIV serostatus was associated with

Table 3. Factors Associated with Elevated Liver Enzymes (Aspartate Aminotransferase >43 or Alanine Aminotransferase >43 U/liter in Unadjusted and Multivariate Analyses Restricted to HIV Positive Subjects

| *Parameter* | *Unadjusted OR (95% CI)* | *Overall* p-*value* | p-*value* | *Adjusted OR (95% CI)* | *Overall p-value* | p-*value* |
|---|---|---|---|---|---|---|
| CD4 groups | | 0.08 | | | 0.06 | |
| CD4 >350 | 1 | | — | 1 | | — |
| CD4 200–350 | 0.78 (0.42, 1.44) | | 0.43 | 0.86 (0.44, 1.70) | | 0.66 |
| CD4 <200 | 1.44 (0.81, 2.57) | | 0.21 | 1.66 (0.88, 3.14) | | 0.11 |
| Age (years) | | 0.31 | | | 0.30 | |
| <30 | 1 | | — | | | |
| 30–40 | 0.65 (0.38, 1.13) | | 0.12 | 0.74 (0.42, 0.32) | | — |
| >40 | 0.74 (0.38, 1.44) | | 0.37 | 0.70 (0.34, 1.47) | | 0.35 |
| Alcohol use (yes vs. no) | 1.65 (0.97, 2.81) | | 0.06 | 1.65 (0.95, 2.86) | | 0.07 |
| Income (Rwf) | | 0.09 | | | 0.09 | |
| <10,000 | 1 | | — | 1 | | — |
| 10,000–35,000 | 0.58 (0.35, 0.96) | | 0.03 | 0.55 (0.32, 95) | | 0.03 |
| >35,000 | 0.67 (0.32, 1.42) | | 0.29 | 0.56 (0.25, 1.25) | | 0.15 |
| Body mass index ($kg/m^2$) | | 0.83 | | | 0.67 | |
| <18.5 | 1 | | — | 1 | | — |
| 18.5–25 | 1.06 (0.57, 1.95) | | 0.85 | 1.22 (0.64, 2.31) | | 0.55 |
| >25 | 0.86 (0.37, 1.96) | | 0.71 | 1.17 (0.48, 2.82) | | 0.73 |
| | 1.22 (0.78, 1.90) | | 0.30 | 1.10 (0.67, 1.80) | | 0.71 |
| Bactrim and/or Dapsone use in prior year (yes vs. no) | 0.55 (0.30, 0.99) | | 0.045 | 0.50 (0.26, 0.96) | | 0.03 |
| Serum creatinine >1 mg/dl vs. <1 mg/dl | 0.99 (0.60, 1.64) | | 0.96 | 1.20 (0.70, 2.06) | | 0.50 |

All variables are included in the multivariate model.

Table 4. Factors Associated with Albumin (<3.5 vs. ≥3.5 g/dl) in Unadjusted and Multivariate Analysis for All Subjects

| *Parameter* | *Unadjusted OR (95% CI)* | *Overall* p-*value* | p-*value* | *Adjusted OR (95% CI)* | *Overall* p-*value* | p-*value* |
|---|---|---|---|---|---|---|
| $HIV^+$ vs $HIV^-$ | 4.29 (2.96, 6.22) | | <0.001 | 5.68 (3.32, 9.71) | | <0.001 |
| Age (years) | | 0.51 | | | 0.003 | |
| <30 | 1 | | — | 1 | | — |
| 30–40 | 1.22 (0.85, 1.74) | | 0.27 | 1.20 (0.81, 1.77) | | 0.36 |
| >40 | 1.21 (0.82, 1.79) | | 0.33 | 2.08 (1.31, 3.30) | | 0.002 |
| Income (Rwf) | | 0.054 | | | 0.28 | |
| <10,000 | 1 | | — | 1 | | — |
| 10,000–35,000 | 0.94 (0.70, 1.26) | | 0.68 | 0.88 (0.63, 1.24) | | 0.46 |
| >35,000 | 0.61 (0.39, 0.94) | | 0.02 | 0.68 (0.41, 1.11) | | 0.12 |
| Body mass index ($kg/m^2$) | | <0.001 | | | <0.001 | |
| <18.5 | 1 | | — | 1 | | — |
| 18.5–25 | 0.56 (0.40, 0.80) | | <0.001 | 0.53 (0.36, 0.78) | | <0.001 |
| >25 | 0.39 (0.24, 0.62) | | <0.001 | 0.39 (0.23, 0.66) | | <0.001 |
| Alcohol use (yes vs. no) | 0.88 (0.63, 1.23) | | 0.44 | 1.02 (0.71, 1.46) | | 0.92 |
| Bactrim and/or Dapsone use (yes vs. no) | 2.25 (1.65, 3.09) | | <0.001 | 0.97 (0.63, 1.51) | | 0.89 |
| Serum creatinine ≤1 vs. >1 | 1.11 (0.82, 1.50) | | 0.51 | 0.97 (0.69, 1.36) | | 0.86 |

All variables are included in the multivariate model.

markers of liver damage. The prevalence of liver transaminase abnormalities and impaired synthetic function was higher in $HIV^+$ than $HIV^-$ women. Among the HIV-infected women there was no significant difference in liver enzymes across CD4 categories. Even though the prevalence of liver transaminase abnormalities was higher in HIV-infected women with CD4 <200 compared to CD4 >350, this difference was not statistically significant, suggesting that elevated liver enzymes are mainly due to HIV infection rather than HIV disease progression as a further contributing factor in this process.

Studies from resource-rich countries suggest that HIV infection is associated with a higher prevalence of elevated liver enzymes.[16,22] A study of prevalence and factors associated with liver test abnormalities among HIV-infected persons in San Diego, California found that 27% of $HIV^+$ patients had abnormal liver test results during a 6-month study period after ART initiation.[22] However, the excess

Table 5. Factors Associated with Albumin (<3.5 vs. ≥3.5 g/dl) in Unadjusted and Multivariate Analysis Restricted to HIV-Positive Subjects

| *Parameter* | *Unadjusted OR (95% CI)* | *Overall* p-*value* | p-*value* | *Adjusted OR (95% CI)* | *Overall* p-*value* | p-*value* |
|---|---|---|---|---|---|---|
| CD4 groups | | <0.001 | | | <0.001 | |
| CD4 >350 | 1 | | — | | | — |
| CD4 200–350 | 1.45 (0.98, 2.15) | | <0.06 | 1.57 (1.01, 2.43) | | <0.04 |
| CD4 <200 | 2.44 (1.62, 3.67) | | <0.001 | 2.62 (1.66, 4.14) | | <0.001 |
| Age (years) | | 0.004 | | | | |
| <30 | 1 | | — | | 0.005 | — |
| 30–40 | 1.11 (0.76, 1.64) | | 0.58 | 1.12 (0.74, 1.72) | | 0.58 |
| >40 | 2.08 (1.29, 3.36) | | 0.003 | 2.18 (1.28, 3.71) | | 0.004 |
| Income (Rwf) | | 0.08 | | | 0.49 | |
| <10,000 | 1 | | — | 1 | | — |
| 10,000–35,000 | 0.70 (0.50, 0.99) | | 0.04 | 0.84 (0.57, 1.23) | | 0.36 |
| >35,000 | 0.62 (0.37, 1.02) | | 0.05 | 0.61 (0.41, 0.99) | | 0.04 |
| Body mass index ($kg/m^2$) | | <0.001 | | | <0.001 | |
| <18.5 | 1 | | — | 1 | | — |
| 18.5–25 | 0.37 (0.24, 0.57) | | <0.001 | 0.38 (0.24, 0.61) | | <0.001 |
| >25 | 0.26 (0.15, 0.45) | | <0.001 | 0.30 (0.16, 0.55) | | <0.001 |
| Alcohol use (yes vs. no) | 0.95 (0.65, 1.40) | | 0.81 | 1.11 (0.73, 1.68) | | 0.63 |
| Bactrim and/or Dapsone use (yes vs. no) | 0.85 (0.54, 1.35) | | 0.49 | 0.67 (0.39, 1.15) | | 0.14 |
| Serum creatinine ≤1 vs. >1 | 0.91 (0.65, 1.27) | | 0.57 | 0.97 (0.66, 1.41) | | 0.85 |

All variables are included in the multivariate model.

elevated liver enzymes observed in the latter study might be due to ART intake in contrast to our study in which all $HIV^+$ women were ART naive. Contrary to studies conducted in developed countries, a prospective study of outpatients in Uganda found that the risk of clinically significant hepatotoxicity was low, even in patients on ART.[13] Similarly, the prevalence of liver abnormalities observed in our study is lower than that reported in studies from developed countries among HIV-infected persons.[16,22] However, other factors need be considered when comparing studies from sub-Saharan Africa and those from developed countries as HIV-infected persons in the resource-rich countries are more likely to have chronic HCV infection, which itself is associated with grade III–IV liver enzyme elevation,[23] and to be obese with nonalcoholic fatty liver disease.

We observed a significant association of low serum albumin with HIV infection. This trend remains in multivariate models that adjust for other factors, which suggests the possibility that the liver injury could be partially due to the effect of HIV infection itself. Some studies suggest that HIV can alter the permeability of the gastrointestinal tract, leading to increased levels of circulating lipopolysaccharide that may affect liver function parameters,[24] and in advanced HIV infection, there is an elevated rate of protein turnover, which persists irrespective of sufficient intake of calories and protein.[25] Much of this is thought to be driven by an increase in circulating inflammatory factors. Elevated circulating concentrations of cytokines such as tumor necrosis factor (TNF), interleukin (IL)-6, and interferon (IFN)-$\alpha$ have been demonstrated in subjects with HIV infection and have significant effects on protein metabolism.[25,26] A possible mechanism of the pathogenesis of liver damage in our study is that when HIV infection progresses and the CD4 count falls the whole-body protein turnover is increased. The whole-body protein turnover is markedly increased in stage IV HIV infection despite normal protein balance.[25]

Contrary to other studies we found no association between alcohol consumption and serum liver enzyme activities.[27,28] The possible reason is that HIV-infected persons were educated on risk factors for HIV disease progression (that drug and alcohol use can interfere with their treatment or impair judgment and lead to risky behaviors) and thus avoided taking alcohol. Another possible reason is that having our study subjects consisting only of women could have decreased the effect of alcohol consumption as a recent study found that male gender was associated with liver enzyme elevations.[13]

Our study did not find any association between BMI and liver enzymes. The fact that BMI was not associated with elevated liver enzymes in our study population, contrary to studies from developed countries,[29,30] may be explained by the fact that the large majority of women in our study population were lighter, with a mean BMI around 21 $kg/m^2$, and thus did not have a predisposition to hepatic steatosis. We did find, however, that a higher BMI was associated with a lower risk of low serum albumin; serum albumin <3.5 mg/dl was 70% and 62% lower in women with BMI >25 and 18.5–25 $kg/m^2$, respectively, compared to women with BMI <18.5 ($p < 0.001$). Previously published data from this population showed that $HIV^-$ subjects with low BMI tended to have serum albumin levels in the normal range, whereas the mean serum albumin among $HIV^+$ women with low BMI was well below the lower limit of normal.[31] This suggests a specific effect of advanced HIV disease in lowering serum albumin rather than a direct association of low albumin with low BMI. Our findings are similar to other studies among HIV-infected persons, which showed that albumin <3.5 mg/dl was associated with faster HIV disease progression and mortality in HIV-infected women,[31,32] and suggests that impaired albumin synthesis is probably a consequence of HIV infection. Furthermore, the association between higher income and lower odds of serum albumin suggests that micronutrient intake and diet quality are affected by socioeconomic status (SES). In a study assessing whether social class predicts diet quality, Darmon and colleagues found that SES or income inequality variables may have a causal influence on diet quality.[33]

This study is limited by generalizability to men as the study population included only women. Second, we lacked data on hepatitis B and C viral infections and therefore we could not take into account coexisting liver infections that may have caused increases in liver enzymes. However, a recent study in Rwanda indicated no statistical differences in median ALT and AST levels at baseline and 12 months post-ART between HBV- and HCV-exposed and unexposed groups.[34] Additionally, this population essentially had no exposure to illicit drug use (IDU), which may suggest that a low prevalence of HCV and HIV/HCV coinfections is likely to be less common in East Africa due to the differences in transmission routes.[35] We utilized self-reported alcohol use, which is subject to underreporting due to the cultural stigma associated with alcohol consumption among women in Rwanda, but alcohol use in our setting appears to be very low.

In summary, our findings suggest that liver injury in ART-naive HIV-infected African women could partially be due to the effect of HIV infection itself, and that liver abnormalities were less common than previously reported in developed countries. Impaired liver synthesis is probably a consequence of advanced HIV disease. Future studies of the association between HIV infection and liver function in low-income settings, using direct or indirect liver damage measures (e.g., imaging) to improve recognition, diagnosis, and management, should be considered. In addition, prospective research on HIV infection, liver disease progression, and ART effects on reducing aminotransferase levels in these patients should be developed in order to determine the incidence of liver abnormalities and to establish causal relationships. Finally, given the critical importance of lipopolysaccharide and Kupffer cells in the pathogenesis of many forms of liver injury, the measurement of soluble CD14 might provide some indication of whether increased Kupffer cell activation might be driving the transaminase increase.

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### Author Disclosure Statement

The authors declare no competing financial interests.

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