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# Liver Histological Patterns among HIV-Infected Individuals and Associated Factors of Fibrosis in Northwestern Tanzania: Is There a Role for Non-Invasive Tests?

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## Abstract

**Background:** There is a paucity of data on HIV related liver pathologies and fibrosis in Tanzania, a lower middle income country, where there is a high burden of HIV and empirical treatment is the norm due to lack of investigative and management guidelines. This study aimed to determine the liver histological patterns among HIV-infected individuals and associated factors of fibrosis, as well as the role of non-invasive tests in the detection of fibrosis. **Patients and Methods:** This was an analytical cross-sectional hospital-based study carried out in Mwanza, Tanzania. HIV-infected individuals with suspected but indeterminate liver disease underwent ultrasound guided liver biopsy to determine histological patterns. Non-invasive tests, APRI and FIB-4, were compared with ultrasound-guided liver biopsy in the detection of fibrosis. Logistic regression analyses were performed to assess the association between outcome and exposure variables with a p-value < 0.05 considered statistically significant. Receiver operating characteristic curve analyses were used to compare APRI and FIB-4 performance against ultrasound-guided liver biopsy for the detection of liver fibrosis. **Results:** A total of 86 participants underwent an ultrasound-guided biopsy and specimen for histopathology. Around 98% of the study participants had histological abnormalities. There was underlying fibrosis in 54.7% of the

participants with the second most common histological pattern being chronic hepatitis (48.9%). Other patterns included malignancy (24.4%) with the majority having hepatocellular carcinoma (HCC). Steatosis or steatohepatitis was present in 17.4%. Granulomatous inflammation was seen in 13.9% with the majority being tuberculous in origin. APRI and FIB-4 reported a lower proportion of fibrosis compared to histology. Advanced age (OR = 4.78, 95% CI =1.64 - 13.91), presence of jaundice (OR = 3.59, 95% CI = 1.27 - 10.16) or hepatomegaly (OR = 3.56, 95% CI = 1.25 - 10.16) were independent predictors of fibrosis. On receiver operating characteristic analyses, FIB-4 performed marginally better than APRI in detecting fibrosis in comparison to histology (AUC = 0.56, 95% CI = 0.46 - 0.67 vs. AUC = 0.51, 95% CI = 0.39 - 0.61). **Conclusions and Recommendation:** Fibrosis is the most prevalent histological pattern among HIV-infected individuals. FIB-4 performed better than APRI as a non-invasive test for detecting fibrosis in comparison to histology. We recommend non-invasive tests for screening underlying fibrosis in HIV-infected individuals.

## Subject Areas

HIV, Hepatology

## Keywords

HIV, Fibrosis, Non-Invasive Tests, Ultrasound-Guided Biopsy

## 1. Introduction

The prevalence of HIV infection among adults in Tanzania is estimated to be 4.4%, corresponding to approximately 1,548,000 adults [1]. Liver pathologies among the HIV-infected have become one of the leading causes of hospitalization and in-patient mortality accounting for 14% - 18% of all deaths and almost 50% of in-patient mortality [2]. There is a paucity of data on the co-existent HIV related liver pathologies and fibrosis in Tanzania, a lower middle income country, where there is a high burden of HIV and empirical treatment is the norm due to lack of investigative and management guidelines. Although liver biopsy as the gold standard of investigation is fraught with risk, incorporation of non-invasive tests for liver fibrosis such as the AST to Platelet Ratio Index (APRI) and Fibrosis-4 index (FIB-4) into the routine clinical care of patients with liver disease is currently being recommended [3] [4]. Judicious investigative confirmation of liver pathologies and fibrosis among the HIV-infected may ultimately guide management. This study aimed to determine the liver histological patterns among HIV-infected individuals and associated factors of fibrosis, as well as the role of non-invasive tests in the detection of fibrosis.

## 2. Patients and Methods

### 2.1. Study Design, Duration and Setting

This was an analytical cross-sectional study carried out over a period of 8 months,

between August 2020 and March 2021, in a zonal and a regional hospital in Mwanza, Northwestern Tanzania at Bugando Medical Centre (BMC) and Sekou-Toure Regional Referral Hospital (SRRH) respectively.

## 2.2. Study Participants

HIV-infected individuals attending the medical outpatient clinics or admitted to inpatient wards with suspected but indeterminate liver disease.

## 2.3. Eligibility Criteria

They were adapted from the American Association for the Study of Liver Disease (AASLD) criteria for liver biopsy [5].

### 2.3.1. Inclusion Criteria

Consenting HIV-infected individual adults (18 years and older) with suspected but indeterminate liver disease based on clinical symptoms and signs (jaundice, hepatomegaly), abnormal liver function tests, and liver parenchymal abnormalities on imaging.

### 2.3.2. Exclusion Criteria

Abnormal bleeding indices (abnormal international normalized ratio (INR) > 1.5), vascular lesions in the liver, ascites and/or unstable patient.

## 2.4. Sample Size and Sampling Technique

A minimum sample size of 86 participants was calculated using a 5% precision, 95% confidence interval and a 94% prevalence of pathologies in HIV-infected individuals, assuming the reported non-diagnostic or indeterminate specimens were pathological [6]. The consecutive sampling technique was applied until the sample size was reached.

## 2.5. Study Variables

Independent variables included age, sex, body mass index (BMI), alcohol intake, ART duration (years), Non-ART drugs use, symptoms and signs (jaundice, hepatomegaly), hepatitis B virus (HBV) surface antigen (HBsAg) positivity, CD4 count, alpha-fetoprotein (AFP), alanine transaminase (ALT), aspartate transferase (AST), platelet count, APRI and FIB-4. Histology was the dependent variable.

## 2.6. Data Collection and Procedures

Data for each patient was entered in a pretested coded questionnaire that included all variables. BMI = body weight/[height]$^2$ in kilogram/metre$^2$ (kg/m$^2$). Blood samples from each patient were taken for serology to determine HBsAg positivity, assay of CD4 count in cells per cubic centimeters (cells/cm$^3$), AFP levels in nanograms per millilitre (ng/ml), ALT and AST levels in international units per litre (IU/L), platelet count in cells × $10^9$ per litre and INR. APRI was calculated using; APRI = [AST level (IU/L)/AST upper limit of normal (IU/L)]/platelet count (cells

$\times 10^9$/L)] × 100. APRI of <0.5 no or minimal fibrosis, 0.5 - 1.5 as significant fibrosis and ≥1.5 as advanced fibrosis or cirrhosis [7]. In this study, a unisex 40 IU/L was used as the ALT and AST upper limit of normal based on our BMC laboratory reference values. FIB-4 was calculated using; FIB-4 = [Age (years) × AST level (IU/L)]/[platelet count ($10^9$cells/L) × square root of ALT level (IU/L)]. FIB-4 of <1.45 no or minimal fibrosis, 1.45 - 3.24 as significant fibrosis and >3.25 as advanced fibrosis or cirrhosis [8]. Ultrasound-guided percutaneous liver biopsy on target lesions was performed by an interventional radiologist using aseptic technique and local anesthesia with a 16-gauge cutting core needle as per AASLD pre- and post-biopsy standard operating procedures [5]. The acquired biopsy specimens were immediately stored in a labeled 10% formalin container and sent to histopathology for processing and interpretation with standard stains. A multidisciplinary team consisting of gastroenterologists, radiologists and pathologists was involved in the diagnosis and management of patients.

### 2.7. Data Management and Statistical Analysis

Data was entered into a spreadsheet, cleaned and analyzed using Stata version 15 (StataCorp LLC, USA). Mean and standard deviation (SD) or median and interquartile range (IQR) were used to describe continuous variables based on distribution while frequencies and proportions were used for categorical variables. Logistic regression analyses were performed to assess the association between outcome and exposure variables with a p-value < 0.05 considered statistically significant. Confounders were adjusted for in multivariate analysis. Receiver operating characteristic curve analyses were used to compare APRI and FIB-4 performance against ultrasound-guided liver biopsy for the detection of liver fibrosis.

### 2.8. Ethical Clearance

This study was conducted in accordance with the Declaration of Helsinki of 1975 (as revised in 2013). The protocol was reviewed and approved by the Catholic University of Health and Allied Sciences Research and Ethics Committee (certificate no. CREC/431/2020, 2020).

## 3. Results

About 138 participants with suspected but indeterminate liver diseases were referred for liver biopsy of which 86 participants were enrolled (**Figure 1**). The majority of the recruited participants were males (M:F = 1.2:1) with an age range of 18 to 84 years. About 45.3% were overweight and obese, 40.7% had a positive history of alcohol intake, 37% were on other drugs other than ART and 26.7% were Hepatitis B positive (**Table 1**). Based on histology, 98% of the study participants had histological abnormalities. There was underlying fibrosis in 54.7% of the participants with the second most common histological pattern being chronic hepatitis (48.9%). Other patterns included malignancy (24.4%) with the majority having hepatocellular carcinoma (HCC). Steatosis or steatohepatitis was present in

17.4%. Granulomatous inflammation was seen in 13.9% with the majority being tuberculous in origin (Table 2). APRI and FIB-4 reported a lower proportion of participants with fibrosis compared to histology (Table 3). Advanced age (OR = 4.78, 95% CI = 1.64 - 13.91, p = 0.004), presence of jaundice (OR = 3.59, 95% CI = 1.27 - 10.16, p = 0.016) or hepatomegaly (OR = 3.56, 95% CI = 1.25 - 10.16, p = 0.017) were independent predictors of fibrosis (Table 4). On receiver operating characteristics curve analysis, FIB-4 performed marginally better than APRI in detecting fibrosis in comparison to histology (AUC = 0.56, 95% CI = 0.46 - 0.67 vs. AUC = 0.51, 95% CI = 0.39 - 0.61) as shown in Figure 2.

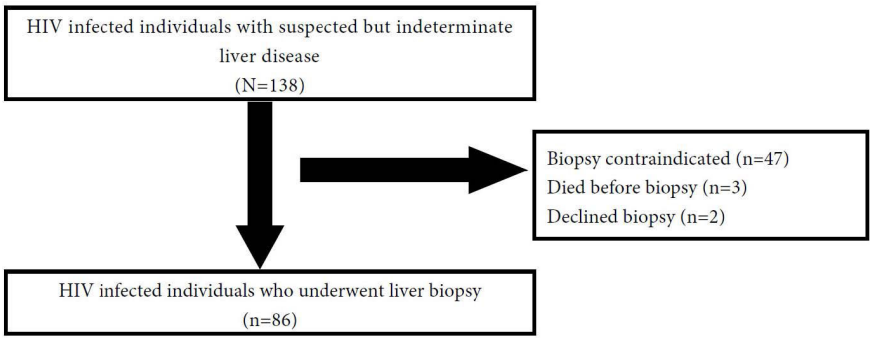


Figure 1. Enrolment flowchart.

Table 1. Participants characteristics (n = 86).

| Variable | n | % |
|---|---|---|
| **Age, years[a]** | 45.6 | (11.9) |
| **Males** | 47 | (54.7) |
| **BMI[a]** | 23.4 | (21.0 - 28.9) |
| Normal (18.5-24.9) | 43 | (50.0) |
| Underweight (<18.5) | 4 | (4.7) |
| Overweight (25-29.9) | 18 | (20.9) |
| Obese (>29.9) | 21 | (24.4) |
| **Alcohol intake** | 35 | (40.7) |
| **ART duration, years[b]** | 3 | (1 - 7) |
| **Non-ART drugs** | 32 | (37.2) |
| **Jaundice** | 57 | (66.3) |
| **Hepatomegaly** | 50 | (58.1) |
| **HBsAg positive** | 23 | (26.7) |
| **CD4 count[b]** | 320 | (172 - 516) |
| **AFP[b]** | 4.2 | (3.2 - 18.0) |
| **ALT[b]** | 43.8 | (25.0 - 53.0) |
| **AST[b]** | 52.0 | (45.0 - 84.4) |
| **Platelet count[b]** | 320 | (246 - 364) |
| **APRI[b]** | 0.45 | (0.37 - 0.80) |
| **FIB-4[b]** | 1.40 | (1.00 - 2.23) |

[a]Mean (SD); [b]Median (IQR).

**Table 2.** Histological patterns.

| Histological patterns | n | (%) |
|---|---|---|
| **Fibrosis** | 47 | (54.7) |
| **Chronic hepatitis** | 42 | (48.9) |
| **Non-specific hepatitis** | 31 | (36.1) |
| **Viral hepatitis** | 11 | (12.8) |
| **Malignancy** | 21 | (24.4) |
| Hepatocellular carcinoma | 15 | (17.4) |
| Lymphoma | 4 | (4.7) |
| Metastatic adenocarcinoma | 2 | (2.3) |
| **Steatosis or Steatohepatitis[a]** | 15 | (17.4) |
| Steatosis | 5 | (5.8) |
| Steatohepatitis | 10 | (11.6) |
| **Granulomatous Inflammation** | 12 | (13.9) |
| Tuberculous | 10 | (11.6) |
| Non-tuberculous | 2 | (2.3) |
| **Cholestasis** | 10 | (11.6) |
| **Nodular Regenerative Hyperplasia** | 1 | (1.2) |
| **Normal** | 2 | (2.3) |

[a]Non-alcoholic fatty liver disease (NAFLD) diagnosed in majority (93%) with Steatosis or Steatohepatitis.

**Table 3.** Grade of fibrosis.

| Grade | APRI | FIB-4 | Histology |
|---|---|---|---|
| | n (%) | n (%) | n (%) |
| **0 (no or minimal)** | 48 (55.8) | 49 (57.0) | 39 (45.4) |
| **1 - 2 (significant)** | 32 (37.2) | 28 (32.6) | 32 (37.2) |
| **3 - 4 (advanced or cirrhosis)** | 6 (7.0) | 9 (10.5) | 15 (17.4) |

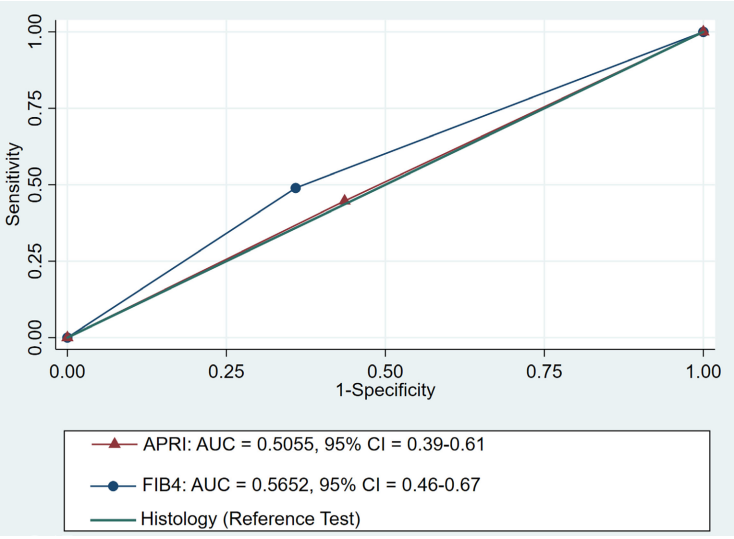


**Figure 2.** Receiver operating characteristics curve.

**Table 4.** Factors associated with histological fibrosis.

| Variable | | Univariate | | Multivariate | |
|---|---|---|---|---|---|
| | n (%) | OR (95% CI) | p | OR (95% CI) | p |
| **Age (≥40)** | 61 (70.9) | 3.77 (1.40 - 10.13) | 0.009 | 4.78 (1.64 - 13.91) | 0.004 |
| **Sex (females)** | 39 (45.4) | 1.67 (0.71 - 3.95) | 0.24 | | |
| **BMI (≥25)** | 39 (45.4) | 2.03 (0.85 - 4.84) | 0.11 | | |
| **Alcohol intake** | 51 (59.3) | 0.69 (0.29 - 1.66) | 0.40 | | |
| **ART duration (≥3)** | 44 (51.2) | 1.75 (0.74 - 4.12) | 0.20 | | |
| **Non-ART drugs** | 32 (37.2) | 1.67 (0.68 - 4.07) | 0.26 | | |
| **Jaundice** | 57 (66.3) | 4.44 (1.70 - 11.61) | 0.002 | 3.59 (1.27 - 10.16) | 0.016 |
| **Hepatomegaly** | 50 (58.1) | 4.67 (1.86 - 11.71) | 0.001 | 3.56 (1.25 - 10.16) | 0.017 |
| **HBsAg positive** | 23 (26.7) | 0.69 (0.26 - 1.79) | 0.44 | | |
| **CD4 count (<200)** | 28 (32.6) | 1.44 (0.58 - 3.61) | 0.43 | | |
| **AFP (>20)** | 15 (17.4) | 0.35 (0.11 - 1.12) | 0.08 | | |
| **Serum ALT (>40)** | 55 (64.0) | 0.81 (0.33 - 1.96) | 0.63 | | |
| **Serum AST (>40)** | 72 (83.7) | 0.89 (0.28 - 2.81) | 0.84 | | |
| **Platelet count (<150)** | 8 (9.3) | 6.65 (0.78 - 56.62) | 0.08 | | |
| **APRI Fibrosis** | 38 (44.2) | 1.05 (0.44 - 2.46) | 0.92 | | |
| **FIB-4 Fibrosis** | 37 (43.2) | 1.71 (0.72 - 4.08) | 0.23 | | |

OR = Odds Ratio, CI = Confidence Interval, p = p-value.

## 4. Discussion

Our study informs on the liver histological patterns in particular fibrosis among the HIV-infected individuals with suspected but indeterminate liver disease in Tanzania. Fibrosis was the most prevalent pathology followed by chronic hepatitis. A high prevalence of HIV-HBsAg co-infection (26.7%) was reported. The global prevalence of HIV-HBsAg co-infection has been documented to be 7.6% with the greatest burden in sub-Saharan Africa (SSA) at 5.3% [9]. A minimal increased risk of fibrosis was seen in the HIV and HBV co-infected participants (OR = 0.69, 95% CI = 0.26 - 1.79), contrary to a previous study done in Tanzania which reported an almost four-fold increased risk of fibrosis in HIV and HBV co-infected participants (OR = 3.78, 95% CI = 1.91 - 7.50) [10]. The previous study utilized APRI as a non-invasive test of fibrosis while in the current study, other than APRI and FIB-4 as non-invasive tests of fibrosis, the histological pattern was also utilized as the gold standard. HIV/HBV coinfection has been shown to accelerate the progression of liver fibrosis, even among patients with successful suppression of viral load [11]. Around 4.2% individuals with chronic HBV infection in SSA have liver cirrhosis and need antiviral therapy as well as liver cancer surveillance [12]. HCC was the prevalent malignancy in our study occurring at a relatively younger age and presenting with an advanced stage of disease, advocating the dire need for screening high risk patients. These findings are similar to other studies [13] [14].

The majority of the study participants with steatosis or steatohepatitis were diagnosed with NAFLD. Features of metabolic syndrome (metS) seen in participants with steatosis and steatohepatitis support how NAFLD is a component of the metS and can be suspected in HIV patients on anti-retroviral therapy with liver disease of unknown etiology and positive metabolic findings. Other studies have also related steatosis and steatohepatitis to metS [15] [16]. Tuberculous granuloma was the commonest cause of granulomatous inflammation among the HIV-infected similar to other studies [17] [18]. Tuberculous granuloma has also been reported to be more common among the HIV-infected compared to non-infected individuals. In a large biopsy study carried out in South Africa involving 262 patients (41% being HIV positive), it was revealed that granulomatous liver disease was more than twice as likely to be found in HIV positive patients compared to HIV negative patients [6].

Fibrosis was the commonest pathology in this study. FIB-4 performed slightly better than APRI as a non-invasive test for fibrosis when compared to histology the gold standard as illustrated in this study. This is analogous to a study carried out in Turkey where FIB-4 was shown to be superior to APRI [19]. The pathophysiology of liver fibrosis in the HIV-infected is a multifactorial process and may include metabolic dysfunction, persistent HIV replication and chronic immune activation as well as microbial translocation and inflammation during HIV infection leading to the production of fibrinogenic mediators and fibrosis [20].

Due to resource constraints, this study was limited by a lack of elastography, a newer sonographic technique for evaluating fibrosis and immunohistochemistry, an antigen specific technique for evaluation of indeterminate cases on histology.

## 5. Conclusion and Recommendation

Fibrosis is the most prevalent histological pattern among HIV-infected individuals. FIB-4 performed better than APRI as a non-invasive test for detecting fibrosis in comparison to histology. We recommend non-invasive tests for screening underlying fibrosis in HIV-infected individuals.

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## Authors' Contributions

RM, PM, SK and PSN were involved in the conceptualization and design. RM, PM, DM, PR and PSN were involved in data collection and interpretation. JK was involved in data analysis. RM and PSN were involved in the initial drafting of the manuscript. All authors critically reviewed and approved the final manuscript.

## Data Availability

The data sets generated and analyzed are available from the corresponding author

upon reasonable request.

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## Conflicts of Interest

All authors declare no conflict of interest.

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