Original Research Article

Title: Prevalence and Predictors of Liver Fibrosis Using Fib 4 and Association between Liver and Immunological Functions in HIV Positive Patients on antiretroviral therapy (ART)

Abstract

Introduction

Liver disease remains a severe co-morbid complication in HIV patients despite advances in treatment with anti-retroviral drugs.

The aim of this study is to evaluate and report the prevalence of liver disease as evident by the presence and extent of fibrosis in association with variables such as age, CD4,TNF –alpha and liver enzyme biomarkers using ALT,AST,ALP and blood proteins of HIV positive patients on ARV in university of port Harcourt teaching hospital.

Materials and methods

A hospital-based study was conducted with a sample of 210 patients who were tested using randomized selection. Patients' age and gender were considered, and blood samples were obtained for CD4, TNF, and LFT profiles. Liver fibrosis was determined using the FIB-4 index as a non-invasive measuring index to determine the presence and extent.

FIB-4=[age×AST (IU/liter)/platelet count (10^9/liter)×ALT (IU/liter)$^{1/2}$].

Results.

The prevalence of liver fibrosis reported was 21% was a slight male dominance in the prevalence ratio. Liver fibrosis correlated negatively with lower CD4 counts and elevated liver function biomarkers and TNF-alpha.

Conclusion

The prevalence of liver fibrosis is high from this study. Increasing in age with elevated liver function biomarkers, TNF-alpha and reduced CD4 counts can be considered as predictors for liver fibrosis. Males are more likely to be affected than females.

Key Words: TNF, FIB 4, CD4 ALT, AST, ALP, Liver fibrosis

Introduction

The menace of the Human Immune Deficiency Virus and Acquired Immune Deficiency Syndrome (HIV/AIDS) which came into limelight in the early 1980s as sexually transmitted diseases continue to cause devastating effects worldwide as it causes the untimely death of many, and rendering many more incapacitated particularly in some developing countries. HIV/AIDS has defiled the global trend in epidemiologic transition from infectious to non-communicable disease as it continues to pose a challenge with a rising incidences in morbidity and mortality [1], up until 2004 when mortality worldwide seem to have plateaued [2] as a direct benefit of antiretroviral therapy (ART).

In recent times, combination antiretroviral therapy (cART) has dramatically reduced Acquired Immunodeficiency Syndrome (AIDS) – related morbidities and mortality, but deaths from co-morbidities have been as a result of non- AIDS-related causes[3]. The liver disease appears to be the most common cause of death in adults infected with HIV, 14 – 18 % of deaths recorded in HIV infected adults in America and Europe has attributed to liver disease. In HIV- infected patients liver disease may result directly from the virus itself or antiretroviral toxicity[5]. While reports have shown that HIV- infection causes elevated enzymes, the exact mechanism is still not precise, a possible mechanism is that HIV- related liver injury may result from interactions between liver cells and HIV, HIV glycoproteins interact with hepatic stellate cells which stimulates collagen production[6] resulting in liver damage.

Liver fibrosis is the primary predictor of end-stage liver disease and preceded by inflammation; its evaluation is very crucial in the clinical management of liver diseases including HIV- related liver diseases [7]. Although suppressed immunity, gender, age, and alcohol have been recognized as significant contributors to faster liver fibrosis progression, the underlying mechanism is still poorly understood[8]. Adaptive immunity is critical in HIV infection, production of pro-inflammatory cytokines like tumor necrosis factor alpha (TNF alpha), interferon gamma (IFN gamma) and interleukin IL6 promote inflammation and cell-mediated immunity, as well as macrophage and stellate cell activation to regulate infection[10], but overproduction of these cytokines as seen in chronic inflammation results to other damages.

Observations have shown that antiretroviral agents related to hepatotoxicity have decreased in recent times. Integrase inhibitors seem to have little or no intrinsic hepatotoxicity. However, patients treated previously with specific agents may continue to experience issues related to prior exposures. Data has shown significant association with severe liver outcomes in patients with the greatest increasing exposure to stavudine (Zerit), didanosine, or tenofovirdisoproxilfumarate (TDF)[11]. These outcomes include noncirrhotic portal hypertension, which is significantly associated with the use of didanosine[12].

The Gold standard for evaluating the occurrence and degree of liver inflammation and fibrosis is liver biopsy [13], however, it's invasive nature makes it less desirable. Therefore, for this study, Fibrosis -4 (FIB – 4), a non-invasive method to accurately measurement liver fibrosis was employed. This analytical tool utilizes the level of other serum biomarkers and has been validated to detect liver fibrosis[14].

The need to better describe the deleterious consequences of liver fibrosis in HIV-infected patients in Nigeria has led to this study. Thus, the aim of this study was to analyze and report the prevalence and predictors of liver fibrosis in Nigerian HIV-infected adults on antiretroviral therapy and whether levels of TNF-α and CD4 count are associated with liver fibrosis as measured by FIB-4 (a noninvasive index that includes platelets, age, and liver enzyme levels) using gender and age of patients. Secondly, we also try to explore the level of liver enzymes dysfunction categorizing participants using gender (male, female) and age to ascertain if gender and age will be associated with liver fibrosis in Nigerian HIV-infected patients.

Materials and Methods

2.1 Design

The present research is a cross-sectional study to examine the association between immune cells (CD4 and TNF alpha) and liver fibrosis and the prevalence of advanced liver fibrosis in HIV- infected patients on antiretroviral therapy.

2.2 Subjects

Recruitment of patients was carried out at the University of Port Harcourt HIV clinic, a total of 210 patients were recruited into the study. The study occurred between August 2015 and December 2015. Patients from 18 years and above who could give informed consent were included in the study while patients with a history of alcohol and substance abuse were excluded from the study. The University of Port Harcourt teaching hospital ethics committee approved the study (UPH/CEREMAD/REC/04).

2.3 Measures

2.3.1 Dependent variable

The two primary outcomes of this study were the absence of liver fibrosis and the presence of advanced liver fibrosis based on FIB-4 (a noninvasive index). FIB-4 values were calculated as follows:

FIB-4=[age×AST (IU/liter)/platelet count (10^9/liter)×ALT (IU/liter)$^{1/2}$]. As validated in a cohort of HIV/HCV-coinfected patients, FIB-4 values <1.45 are consistent with the absence of liver fibrosis with a negative predictive value of 90% and a sensitivity of 70%. Also, FIB-4 values >3.25 are consistent with significant liver fibrosis with a positive predictive value 67% and a specificity of 97%[15].

2.3.2 Independent variables

The inflammatory cytokines TNF-α was chosen based on research demonstrating that serum levels are significantly different and are related to the immune response in HIV infection, liver inflammation and liver fibrosis[16]. TNF-α was measured using ELISA kits obtained from UCY tech, Netherlands; Other variables include age, sex, and CD4 count/mm^3, CD4 count was measured using parteccy flow, Liver enzymes (ALT, AST, ALP) and Albumin was measured using Randox Kit.

2.4 Statistical analysis

Descriptive statistics were used to describe the study sample overall and were stratified by liver fibrosis status as [FIB-4 <1.45 (absence of liver fibrosis), FIB-4 1.45–3.25 (intermediate values), and FIB-4 >3.25 (presence of advanced liver fibrosis)]. Baseline characteristics were compared across groups using ANOVA.

Multiple regression was used to evaluate the association between age, sex, TNF alpha, CD4 count liver enzymes, albumin and the outcomes (i.e., the absence of liver fibrosis and the presence of advanced liver fibrosis).

3.0 Results

TABLE 1: Baseline characteristics of participants

Two hundred and ten (210) participants were enrolled in this study. Table 1 shows their baseline characteristics; no significant differences were observed in our variables of interest. The differences between the mean values of liver enzymes of male and female participants were not significant. Also, neither the mean values of Immunological parameters (CD4 and TNF alpha) nor Fibrosis score (FIB- 4 and AST platelet ratio index (APRI) were significantly different.

TABLE 1: Baseline characteristics of participants

	Female	Male	P value
Age	37.63±9.29	40.29±9.52	0.122
CD4 visit 0	415.64±210.95	351.98±213.32	.102
ALP(U/L)	112.39±33.37	104.64±20.05	.162
AST (IU/L)	94.71±34.86	91.43±31.67	.598
ALT(IU/L)	77.71±34.56	70.71±34.67	.270
ALB(g/dl)	4.11±0.95	4.36±1.45	.215
FIB-4 score visit	2.02±1.54	2.32±1.56	.294
APRI score	1.12±0.63	1.2±0.69	.531
TNF alpha	13.5±10.18	13.05±11.74	.819

Data were expressed in?

P value Considered as significance?

Put *, **, NS as per significance in table

TABLE 2: Mean distribution of liver enzymes, CD4 and TNF of different stages of Fibrosis stage using FIB-4

The levels of liver enzymes and immunological parameters were further observed at different stages of liver fibrosis (none, significant and severe fibrosis) and results presented in table 2. AST and ALT were significantly different as fibrosis progressed.

TABLE 2:

	none (<1.45)	significant fibrosis (1.46-3.25)	severe fibrosis (>3.25)	P-value
CD4 visit 0	367.02±192.11	420.77±205.3	376.25±246.97	.343
ALP(U/L)	109.4±31.24	107.48±25.83	122.04±38.41	.128
AST (IU/L)	68.36±30.54	102.03±20.01	127.92±30.78	.000
ALT(IU/L)	89.45±34.66	75.47±32.8	47.5±21.11	.000
ALB(g/dl)	4.1±0.92	4.39±1.43	3.84±0.35	.098
APRI score	0.66±0.27	1.25±0.32	1.94±0.95	.098
TNF alpha	14.52±10.66	12±10.23	14.6±11.91	.370

Data were expressed in?

P value Considered as significance?

Put *, **, NS as per significance in table

Both significant and severe fibrosis was observed in 66% of participants, although this accounted for both male and female participants as shown in table 3.

TABLE 3:

FIB-4 Fibrosis yes no	Frequency	Percent
No fibrosis	71	34
Fibrosis	139	66
	210	100

The prevalence of severe fibrosis was higher in male participants than female participants, with a value of 12% and 9% in male and female participants respectively.

Severe fibrosis observed was highest amongst participants from the age of 40 and above while significant fibrosis was highest amongst participants between 30 – 49 years.

TABLE 4: Distribution of Fibrosis using Sex and age of participants

		none (<1.45) n (%)	significant fibrosis (1.46-3.25) n (%)	severe fibrosis (>3.25) n (%)	Total
Sex	**Female**	45 (21)	43 (20)	17 (9)	105
	Male	26 (12)	55 (26)	24 (12)	105
	Total	71	98	41	210
Age	**20-29**	20 (9)	7 (4)	3 (1)	30
	30-39	39 (19)	45 (21)	7 (4)	91

40-49	5 (2)	36 (18)	14 (6)	55
50 and above	7 (4)	5 (2)	17 (8)	29
Total	71	98	41	210

TABLE 5: Percentage of AST and ALT dysfunction among male and female participants.

Liver enzymes were categorized as high and normal; then observed in male and female participants. In both male and female participants, the percentage of high AST and ALT level was greater than 45% (Table 5).

The ALP and ALB of most of the participants were normal (Table 6).

TABLE:5

	AST		**ALT**	
	N (%)		**N (%)**	
	Normal (0-42 U/L)	**High (>42 U/L)**	**Normal (0-48 IU/L)**	**High (>48 IU/L)**
Female	10 (5)	94 (45)	37 (18)	68 (32)
Male	13 (6)	92 (44)	60 (29	45 (21)
	23	186	97	113

TABLE 6: Percentage of ALP and ALB levels among male and female participants

	ALP		**ALB**		
	Normal (20-125 U/L)	**High (>125 U/L)**	**Normal (3.5 - 6 g/dl)**	**High (> 6g/dl)**	**Low (< 3.5)**
Female	81 (39)	24 (11)	93 (44)	5 (2)	7 (3)
Male	88 (42)	17 (8)	92 (44)	8 (4)	5 (2)
	169	41	185	13	12

TABLE 7: Percentage of AST and ALT dysfunction among different age categories

High AST and ALT levels were observed participants from age 30 and above, while participants below 29 years showed normal ALT and AST levels (Table 8).

TABLE:7

	AST		ALT	
	Normal (0-42 U/L)	**High (0-42 U/L)**	**Normal (0-48 IU/L)**	**High (>48 IU/L)**
20-29	4 (2)	28 (13)	18 (9)	15 (7)
30-39	11 (5)	80 (38)	36 (17)	55 (26)
40-49	4 (2)	53 (25)	22 (10)	35 (17)
50 and above	4 (2)	25 (12)	21 (10)	8 (4)

TABLE 8: Percentage of ALP and ALB levels among different age categories

	ALP		ALB		
	Normal (20-125 U/L)	**High (>125 U/L)**	**Normal (3.5 - 6 g/dl)**	**High (> 6g/dl)**	**Low (< 3.5)**
20-29	31 (15)	2 (1)	28 (13)	2 (1)	3 (1)
30-39	76 (36)	15 (7)	74 (35)	10 (5)	7 (3)
40-49	38 (18)	19 (9)	55 (26)	1 (0. 5)	1 (0.5)
50 and above	24 (11)	5 (2)	28 (13)	0 (0.0)	1 (0.5)

**ALB (serum albumin)*

** ALP (serum alkaline phosphatase)*

Table 9

Variable	mean	STD	Correlation with fib- 4	Multiple regression b	Multiple regression β
Agee	38.39	9.402	.496**	.066	.393
Sex	1.2857	.45330	.088	.178	.052
CD4 visit 0	397.4490	212.85886	-.080	-.001	-.119
ALP(U/L) visit 0	110.1769	30.30206	.227**	.005	.104

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AST (IU/L) visit 0	93.7671	33.89925	.606**	.024	.532
ALT(IU/L) visit 0	75.7143	34.62124	-.378**	-.011	-.241
ALB(g/dl) visit 0	4.1782	1.11905	-.115	-.087	-.064
TNF	13.3708	10.60878	.123	.012	.085

Correlation and multiple regression analysis were conducted on our data to examine the relationship between liver fibrosis and other variables (age, sex CD4, etc.). The table above summarizes the descriptive statistics and analysis of the results. As can be seen each variable; Age, ALP, AST and TNF alpha correlated positively and significantly to liver fibrosis, this indicated a direct proportionality between higher levels of these measured variables with a higher likelihood for the presence and extent of fibrosis. CD4, ALT, and ALP correlated negatively to fibrosis indicating that a lower CD4 count could be an indicator of the presence of liver fibrosis.

The Multiple regression model with all predictors produced R^2 = 0.664, F(8,134) = 33.15. p< 0.001, as can be seen, CD4 and ALT had negative significant regression weights indicating that lower values of these predictors will account for fibrosis. Furthermore, age, ALP, AST, and TNF alpha had positive significant regression weights indicating that participants with increased values were likely to have fibrosis. Albumin and sex did not contribute to the model.

4.0 Discussion

The data obtained from our study revealed a high burden of liver fibrosis HIV infected patients in the South-South region of Nigeria on ART. FIB- 4 data from this study showed the prevalence of liver fibrosis at 21%. Amongst this value, significant liver fibrosis was (9% female, 12% male) this is indicative of a male predominance in the prevalence of significant fibrosis, the prevalence of severe fibrosis was relatively high compared to previous studies done. The presence of severe or significant fibrosis was observed in 66% of participants. In Nigeria [17] Hawkings et al. reported severe fibrosis of 4.7% while another study in rural Uganda by [18] Stabinski et al., 2011) reported 17% of severe fibrosis in patients infected with HIV. The high burden of severe liver fibrosis in this study may be attributed to the effects of a complex interplay of HIV infection itself and direct action of the virus on the liver parenchyma cells. HIV infection is known to attenuate the acceleration of liver fibrosis and liver-related mortality (Thio et al., 2002)[19]. It might also be a resultant effect of anti-retroviral therapy (ART), ART through viral suppression, may directly destroy the liver resulting in liver disease as suggested by a Northern American cohort by Kim et al., (2016)[20]. The data from this study also showed that the higher the age of participants the more likely the severity of the fibrosis.

Comment [B2]: Follow journal guidelines

Highly antiretroviral therapy (HAART) has improved the prognosis of HIV infection, AIDS-related deaths, therefore, have been reduced due to the initiation of HAART (Holtzer and Roland, 1999)[21]. Reisler et al., (2001)[22] noted that a significant side effect of antiretroviral drugs is hepatotoxicity.

In this study, while AST and ALT levels were increased in male and female participants, ALP and ALB levels were reduced. Also, age was used to observe liver enzymes; we observed an increase in AST and ALT as age increased while ALP reduced as age increased.

Hepatotoxicity initiated by antiretroviral drugs may be linked to some number of agents in ART drug classes which include; nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs), the severity of hepatotoxicity ranges from a rise in transaminase to hepatic failure and even death (Nunez, 2010)[23].

Studies have shown that HIV infection alters the liver enzyme directly or indirectly. Megan et al. (2012)[24] reported that studies have shown that HIV infects different non-hematopoietic cells, such as the liver parenchyma cells. This may be the reason our results showed increased liver enzyme activities; our results also conform to those obtained by Lebovies et al. (1988[25]); Cappell (1991)[26] and Lefkowitch (1994)[27], they observed and reported elevated liver enzymes in HIV infected patients.

The CD4 T- cells and another component of the immune system were among the principal cells targeted by HIV. Alimonti et al. (2003)[28] also reported that antiretroviral drugs were known to inhibit the growth and replication of HIV, thereby hindering the adverse effect of the virus to the CD4 T-cells and other cells of the immune system. This may serve as a reason for the substantial decrease in the CD4 cell count of HIV non-treated group compared to the control subjects, but at the initiation of ART in the treated group the level of CD4 cell increased significantly compared to the non-treated group.

CD 4 cells and other components of the immune system (TNF alpha) are major cell line and cellular immune derivatives respectively that HIV targets and manipulates (Alimonti et al., 2003[28]). Reports have shown that antiretroviral drugs were known to inhibit the growth and replication of HIV, by doing so, the adverse effect of the virus on CD 4 cells and other immune cellular components and its consequent inflammatory pathways are inhibited to some extent (Alimonti et al., 2003)[28]. This may serve as the reason for the consistency observed in CD4 and TNF alpha of participants in our study.

Limitation

This study had some limitations. Firstly, we measured the levels of only one cytokine, and this measurement was only at baseline, there is the possibility that a complex interplay of various cytokines and inflammatory mediators may work in synergy to cause a more significant impact on liver injury observed. Also, serum TNF may not reflect the actual levels in other body compartments such as the liver tissue. Secondly, it was not practicable to obtain histological evidence of liver fibrosis through biopsies of the liver tissues from the various test subjects hence the need for a non-invasive index. Finally, the cross-sectional design of this study limits the ability to evaluate changes in the variables over time.

Conclusion

Our study found a prevalence of liver fibrosis; the study also found that Age, sex, CD4, TNF, ALP, AST and ALT were predictors of liver fibrosis. Although this study did not find any significant changes in the level of CD4 and TNF about the severity of fibrosis, we found an association between liver fibrosis, TNF-α, and CD4. We also found that older participants had higher levels of liver enzymes, while gender had no relationship with increased liver enzymes.

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