Asian Journal of Research in Medical and Pharmaceutical Sciences

6(4): 1-9, 2019; Article no.AJRIMPS.48297
ISSN: 2457-0745

Prevalence of Liver-fibrosis Using FIB-4 in HIV-patients on Anti-retroviral Therapy in Nigeria and CD4 and TNF as Predictors

Josephine Iruolagbe[1], Robinson Ohanador[2*], Beatrice Amene-Imananagha[1], Audu Abubakar[3], Obetta Jessica[4] and A. W. Obianemmie[1]

[1]Department of Pharmacology, College of Health, University of Port Harcourt, Nigeria.
[2]Department of Biochemistry, University of Port Harcourt, Nigeria.
[3]School of Community Health and Policy, Morgan State University, Baltimore, Maryland, USA.
[4]Department of Biomedical, University of Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author JI designed the study, author RO performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AIB and AA managed the analyses of the study. Author OJ managed the literature searches and laboratory bench work, author AWO supervised the work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRIMPS/2019/v6i430105
Editor(s):
(1) Dr. Alex Xiucheng Fan, Department of Biochemistry and Molecular Biology, University of Florida, USA.
Reviewers:
(1) J. Y. Peter, University of Abuja, Nigeria.
(2) Meer Ahmad A. Mydin Meera, MAHSA University, Malaysia.
(3) Bhushan Gandhare, Institute of Pharmaceutical Science and Research, India.
Complete Peer review History: http://www.sdiarticle3.com/review-history/48297

Original Research Article

Received 17 January 2019
Accepted 04 April 2019
Published 16 April 2019

ABSTRACT

Introduction: Liver disease remains a severe complication in HIV patients despite advances in treatment with anti-retroviral drugs.
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, we also try to explore the level of liver enzymes dysfunction using liver enzyme biomarkers, Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and categorizing participants using gender

**Corresponding author: E-mail: robinsonohanador@yahoo.com;*

(male, female) and age to ascertain if gender and age will be associated with liver fibrosis in Nigerian HIV-infected patients) on Anti-retroviral (ARV) drugs 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. Data on patients' age and gender were collected, and blood samples were obtained for CD4, TNF, and LFT profiles. Extent of liver fibrosis was determined using the FIB-4, a non-invasive measuring index, to determine the presence and extent of fibrosis by categorizing as follows: FIB-4 value >3.25 as a proxy for advanced or severe fibrosis, FIB-4 value between 1.45 and 3.25 in which fibrosis status is considered as significant fibrosis; FIB-4 value<1.45 considered as no fibrosis or absence of significant fibrosis, 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.

Keywords: TNF; FIB 4; CD4 ALT; AST; ALP; liver fibrosis.

1. INTRODUCTION

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 developing countries. HIV/AIDS has defied the global trend in epidemiologic transition from infectious to non-communicable disease as it continues to pose a challenge with increased 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 other non- AIDS-related causes have been reported [3]. Liver disease appears to be the most common cause of death in adults infected with HIV; 14 – 18% of deaths recorded in HIV positive adults in America and Europe were attributed to liver disease [4]. In HIV-infected patients liver disease may result directly from the virus itself or antiretroviral toxicity [5]. While the mechanisms that cause liver injury and fibrosis in the HIV-infected patient are distinct, a detailed description of these mechanisms is beyond the scope of this study. However, numerous publications that provide a detailed insights in a review by Kaspar and Sterling [5], Tsuchida and Friedman [6].

While reports have shown that HIV- infection causes elevated liver-enzymes, (Liver-enzyme increase is due to hepatic-cell damage, but the exact mechanism this may be brought about may still need to be ascertained) 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 interacting with hepatic stellate cells which stimulate collagen production [7] resulting in liver-cell 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 [8]. 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 [9,10]. 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 [11], 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 tenofovir-disoproxilfumarate (TDF) [12]. These outcomes include noncirrhotic portal hypertension, which is significantly associated with the use of didanosine [13].

The Gold standard for evaluating the occurrence and degree of liver inflammation and fibrosis is liver biopsy [14], 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 [15].

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.

2. 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).

2.2.1 Sample size and sampling technique

Simple random sampling was used in this study and Sample size was determined using The Cochran formula is:

$$n_0 = \frac{Z^2pq}{e^2}$$

Z= 1.96
E = 0.07
P= 50% (0.5)
Q = 50% (0.5)

2.2.2 Inclusion and exclusion criteria

For the purpose of this study, participants on any mind altering medication were excluded, participants who could not give consent were also excluded.

We included participants who were 18 years and above and could give informed consent.

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% [16,17].

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 [18]. 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. Procedure for Aspartate Amino Transferase (AST), briefly, five hundred micro litre of reagent 1 (DL-Aspartate 200mmol/L and α- ketoglutarate 2mmol/L) was pipetted into a clean test tube, mixed and incubated for five minutes at 37˚C, One hundred microlitres of the serum were added, mixed and incubated at 37˚C for 30 minutes, then five hundred micro litres of developer (2,4- dinitrophenylhydrazine) were added to the reacting tube, mixed and allowed to stand for 20minutes at room temperature. Five millilitres of 0.4N NaOH were added to the tube, mixed and allowed for 5 minutes at room temperature. Absorbance of the test samples were read against water blank at 500ηm in a light path cuvette.

For Alanine Amino Transferase (ALT), procedure is the same as AST above but only varies in the substrate. The substrate here is DL-Alanine and α- ketoglutarate.

For alkaline phosphatse (ALP**),** 0.05ml of the sample was pipetted against 3ml of the reagent (R1a. Buffer Diethanolamine buffer 1 mol/l, pH 9.8, MgCl2 0.5 mmol/l, R1b. Substrate p-nitrophenylphosphate 10 mmol/l). It was mixed, and read initial absorbance was recorded and stop watch started. It was read again after 1, 2 and 3 min. The assay was read at a wavelength of Hg 405 nm.

2.4 Statistical Analysis

Analysis of the data gathered from the study was done using the Statistical Package for the Social Sciences (SPSS) version 20 software program (IBM Corporation, USA, 2011). 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).

2.5 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.

3. RESULTS

Two hundred and ten (210) participants were enrolled in this study. Both fibrosis and severe fibrosis was observed in 66% of participants, although this accounted for both male and female participants as shown in Table 1.

Table 1. Percentage of fibrosis present in participants

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.

Liver enzymes were categorized as high and normal; then observed in male and female participants. In both male and female participants, 45 % of them had high AST while high ALT level was observed in greater than 20% of participants (Table 3). The ALP and ALB of most of the participants were normal (Table 3).

Table 2. 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 3. Percentage of AST and ALT dysfunction among male and female participants

	AST n (%)		ALT 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 4. 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 5. Percentage of AST and ALT dysfunction among different age categories

	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 6. 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)*

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 5).

Table 7 shows their baseline characteristics; no significant differences were observed when t-test comparison was done between male and female participants in our variables of interest which were CD4, TNF alpha, and liver enzymes. 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) were significantly different.

Table 7. Baseline characteristics of participants

	Female	Male	P value
Age	37.63±9.29	40.29±9.52	0.122
CD4	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	2.02±1.54	2.32±1.56	.294
TNF alpha	13.5±10.18	13.05±11.74	.819

Table 8. ANOVA comparison of means of each variables at each stage of fibrosis using FIB-4

	No fibrosis (<1.45)	fibrosis (1.46-3.25)	severe fibrosis (>3.25)	P-value
CD4	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

Table 9. Correlation and multiple regression between fibrosis and other variables

Variable	mean	STD	Correlation with fib- 4	Multiple regression b	Multiple regression β
Agee	38.39	9.40	.49**	0.06	0.39
Sex	1.28	0.45	.08	0.17	0.052
CD4	397.44	212.85	-.08	-0.001**	-.12
ALP(U/L)	110.17	30.30	.22**	0.005**	0.11
AST (IU/L)	93.76	33.89	.60**	0.02**	0.53
ALT(IU/L)	75.71	34.62	-.37**	-0.01**	-0.24
ALB(g/dl)	4.17	1.119	-.11	-0.09	-0.06
TNF	13.37	10.60	.12	0.012**	0.08

*Superscript "**" shows significance (p<0.05) for correlation and regression*

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 8. AST and ALT were significantly different as fibrosis progressed.

Correlation and multiple regression analysis were used to analyze our data to examine the relationship between liver fibrosis and other variables (age, sex CD4, etc.). The table below 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 (Table 9).

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. DISCUSSION

The data obtained from our study revealed a high burden of liver fibrosis (at 66%) for HIV infected patients in the South-South region of Nigeria on ART. FIB- 4 data from this study showed the

prevalence of severe liver fibrosis at 21% (9% for female and 12% for male). Amongst this value, liver fibrosis was (20% female, 26% male) this is indicative of a male predominance in the prevalence of fibrosis, the prevalence of severe fibrosis was relatively high compared to previous studies done. The presence of severe or fibrosis was observed in 66% of participants. In Nigeria [19] reported severe fibrosis of 4.7% while another study in rural Uganda by Stabinski et al. [20] reported 17% of severe fibrosis in patients infected with HIV. The high prevalence 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 [21]. HIV infection is known to attenuate the acceleration of liver fibrosis and liver-related mortality [21]. It might also be a resultant effect of anti-retroviral therapy (ART). Antiretroviral drugs, through viral suppression, may directly destroy the liver resulting in liver disease as suggested by a Northern American cohort-study by Kim et al. [22]. The data from this study also showed that the higher the age of participants the more likely the severity of the fibrosis.

Highly-active antiretroviral therapy (HAART) has improved the prognosis of HIV infection, AIDS-related deaths, therefore, have been reduced due to the initiation of HAART [23]. Reisler et al. [24] 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 [25].

Studies have shown that HIV infection alters the liver enzyme directly or indirectly. Megan et al. [26] 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 Lebovics et al. [27]; Cappell [28] and Lefkowitch [29] 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. [30] 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. [30]. 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 [31]. This may serve as the reason for the consistency observed in CD4 and TNF alpha of different categories of participants (no fibrosis, fibrosis and severe fibrosis) in our study.

5. 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 mean levels of CD4 and TNF in different categoriesof participants (no fibrosis, fibrosis and sever 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.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The University of Port Harcourt teaching hospital ethics committee approved the study (UPH/CEREMAD/REC/04).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2197–2223.
2. Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: A systematic analysis for the Global Burden of Disease Study 2010. The lancet. 2012;380(9859):2163-96.
3. Palella Jr FJ, Baker RK, Moorman AC, Chmiel JS, Wood KC, Brooks JT, Holmberg SD. HIV outpatient study investigators. Mortality in the highly active antiretroviral therapy era: Changing causes of death and disease in the HIV outpatient study. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2006;43(1):27-34.
4. Smith CJ, Sabin CA, Lundgren JD, Thiebaut R, Weber R, Law M, Monforte AD, Kirk O, Friis-Moller N, Phillips A, Reiss P. Factors associated with specific causes of death amongst HIV-positive individuals in the D: A: D study Aids. 2011;25(6):883.
5. Kaspar MB, Sterling RK. Mechanisms of liver disease in patients infected with HIV. BMJ Open Gastroenterology. 20171;4(1): e000166.
6. Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. Nature Reviews Gastroenterology & Hepatology. 2017;14(7):397.
7. Aurpibul L, Bunupuradah T, Sophan S, Boettiger D, Wati DK, Nguyen LV, Saphonn V, Hansudewechakul R, Chokephaibulkit K, Lumbiganon P, Truong KH. Prevalence and incidence of liver dysfunction and assessment of biomarkers of liver disease in HIV-infected Asian children. The Pediatric Infectious Disease Journal. 2015;34(6):e153.
8. Bruno R, Galastri S, Sacchi P, Cima S, Caligiuri A, DeFranco R, Milani S, Gessani S, Fantuzzi L, Liotta F, Frosali F. gp120 modulates the biology of human hepatic stellate cells: A link between HIV infection and liver fibrogenesis. Gut. 2010;59(4): 513-20.
9. Joshi D, O'Grady J, Dieterich D, Gazzard B, Agarwal K. Increasing burden of liver disease in patients with HIV infection. Lancet. 2011;377(9772):1198–1209.
10. Graham CS, Baden LR, Yu E, et al. Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: A meta-analysis. Clin Infect Dis. 2001;33(4):562–569.
11. Neuman MG, Sha K, Esguerra R, et al. Inflammation and repair in viral hepatitis C. Dig Dis Sci. 2008;53(6):1468–1487.
12. Gramenzi A, Andreone P, Loggi E, et al. Cytokine profile of peripheral blood mononuclear cells from patients with different outcomes of hepatitis C virus infection. J Viral Hepat. 2005;12(5):525–530.
13. Ryom L, Lundgren JD, De Wit S, Kovari H, Reiss P, Law M, et al. D:A:D Study Group. Use of antiretroviral therapy and risk of end-stage liver disease and hepatocellular carcinoma in HIV-positive persons. AIDS. 2016;30:1731-1743.
14. Schiano TD, Uriel A, Dieterich DT, Fiel MI. The development of hepatoportal sclerosis and portal hypertension due to didanosine use in HIV. Virchows Arch. 2011;458:231-235
15. Dezsofi A, Baumann U, Dhawan A, Durmaz O, Fischler B, Hadzic N, Hierro L, Lacaille F, McLin VA, Nobili V, Socha P. Liver biopsy in children: Position paper of the ESPGHAN Hepatology Committee. Journal of Pediatric Gastroenterology and Nutrition. 2015;60(3):408-20.
16. Castera L. Noninvasive methods to assess liver disease in patients with hepatitis B or C. Gastroenterology. 2012;142(6):1293-302.
17. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology. 2006;43(6):1317–1325.
18. Neuman MG, Sha K, Esguerra R, et al. Inflammation and repair in viral hepatitis C. Dig Dis Sci. 2008;53(6):1468–1487.
19. Hawkins C, Agbaji O, Ugoagwu P, Thio CL, Auwal et al. Assessment of liver fibrosis by transient elastography in patients with HIV and hepatitis B virus coinfection in Nigeria. Clinical Infectious Diseases. 2013;57(12):e189-92.

20. Stabinski L, Reynolds SJ, Ocama P, Laeyendecker O, Boaz I, Ndyanabo A, et al. High prevalence of liver fibrosis associated with HIV infection: A cross-sectional study in rural Rakai, Uganda. Antiviral Therapy. 2011;16(3):405.
21. Thio CL, Seaberg EC, Skolasky Jr R, Phair J, Visscher B, Muñoz et al. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). The Lancet. 2002;360(9349):1921-1926.
22. Kim HN, Nance R, Van Rompaey S, Delaney JC, Crane HM, Cachay ER, et al. Poorly controlled HIV infection: An independent risk factor for liver fibrosis. Journal of Acquired Immune Deficiency Syndromes. 2016;72(4):437.
23. Holtzer CD, Roland M. The use of combination antiretroviral therapy in HIV-infected patients. Annals of Pharmacotherapy. 1999;33(2):198-209.
24. Reisler R, Liou S, Servoss J, Robbins G, Theodore D, Murphy R, Chung R. Incidence of hepatotoxicity and mortality in 21 adult antiretroviral treatment trials. InProgram and abstracts of The 1st IAS Conference on HIV Pathogenesis and Treatment. 2001;43:8-11.
25. Núñez M. Clinical syndromes and consequences of antiretroviral-related hepatotoxicity. Hepatology. 2010;52(3): 1143-55.
26. Crane M, Iser D, Lewin SR. Human immunodeficiency virus infection and the liver. World Journal of Hepatology. 2012; 4(3):91.
27. Lebovics E, Dworkin BM, Heier SK, Rosenthal WS. The hepatobiliary manifestations of human immunodeficiency virus infection. American Journal of Gastroenterology. 1988;83(1).
28. Cappell MS. Hepatobiliary manifestations of the acquired immune deficiency syndrome. American Journal of Gastroenterology. 1991;86(1).
29. Lefkowitch JH. Pathology of AIDS-related liver disease. Digestive Diseases. 1994; 12(6):321-30.
30. Alimonti JB, Ball TB, Fowke KR. Mechanisms of CD4+ T lymphocyte cell death in human immunodeficiency virus infection and AIDS. Journal of General Virology. 2003;84(7):1649-61.
31. Murphy RA, Sunpath H, Kuritzkes DR, Venter F, Gandhi RT. Antiretroviral therapy—associated toxicities in the resource-poor world: The challenge of a limited formulary. The Journal of Infectious Diseases. 2007;196(Supplement_3):S449-56.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/48297