Original Article

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Liver fibrosis after antiretroviral therapy in a longitudinal cohort of sexually infected HIV patients in eastern China

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Summary **We assessed the factors that influenced improvement or progression in human immunodeficiency virus (HIV)-infected patients who were receiving combination antiretroviral therapy (cART). This was a retrospective cohort study of HIV-infected patients receiving cART in Taizhou, Zhejiang, China, 2009-2015. Liver fibrosis was assessed by Fibrosis-4 (FIB-4) score. Improvement of liver fibrosis was defined as having > 30% decrease in FIB-4 from baseline, whereas progression of liver fibrosis was defined as having > 30% increase in FIB-4 score from baseline. A total of 955 HIV-infected patients were included. Of these, 808 (84.6%) were HIV-monoinfection, 125 (13.1%) were HIV/hepatitis B virus (HBV) coinfection and 29 (3.0%) were HIV/hepatitis C virus (HCV) coinfection. The median duration of treatment was 15 months. After treatment, 37.1% participants had > 30% decreases in FIB-4 index, 14.8% had > 30% increases in FIB-4 index, while the remaining 48.2% had stabilized FIB-4 index. In multivariate analysis, improvement of liver fibrosis was negatively associated with an older age, but was positively associated with baseline FIB-4 index and > 30% increases in CD4 cell count after ART. Progression of liver fibrosis was positively associated with an older age, but was negatively associated with gender and HIV transmission mode (male homosexual *vs*. male heterosexual, female heterosexual *vs*. male heterosexual), and baseline FIB-4 index. Our findings indicate that improvement of liver fibrosis could be achieved by early initiation of ART through better CD4 cell recovery. Liver fibrosis and hepatotoxicity associated with ART should be monitored as early as possible and throughout till the end of treatment, with special attention to the elderly and heterosexual men.**

Keywords: Antiretroviral therapy, HIV, HBV, liver fibrosis, sexual transmission

1. Introduction

The liver is a major target of human immunodeficiency virus (HIV) infection and a wide spectrum of liver disease can be seen in patients with HIV infection, ranging from steatosis, steatohepatitis, cirrhosis, non-cirrhotic portal hypertension and hepatocellular carcinoma (*1*). HIV infection adversely impacts on the progression of liver diseases. For example, HIV/hepatitis B virus (HBV) co-infected patients had significantly decreased rate of hepatitis B surface antigen (HBsAg) clearance and increased HBV replication than HBV monoinfected individuals (*2-3*), and HIV/hepatitis C virus (HCV) coinfected patients had lower rate of HCV viral suppression than HCV monoinfected controls (*4*). Consequently, progression to cirrhosis is more rapid in HIV-infected patients with chronic liver diseases (*5-6*). End stage liver disease (ESLD) has become a major cause of mortality in HIV-infected individuals receiving antiretroviral therapy (ART), accounting for a greater proportion of deaths than cardiovascular disease or non-

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acquired immune deficiency syndrome (AIDS)-related cancers (*7-8*).

In China, common risk factors for liver disease such as alcohol use, HBV and HCV infections are more prevalent in HIV-infected patients than those without HIV infection (*9-12*). All-cause mortality was also higher in HIV-infected patients with HBV and/or HCV co-infection(s) than in those with HIV only, although ART has generally increased life expectancy of HIV-infected patients (*13*). Nevertheless, little is known about the impact of ART on the development and progression of liver diseases among people living with HIV and AIDS (PLWHA) in China. In a recently published study, Li *et al*. examined progression of liver fibrosis among ART receiving HIV-infected patients, and found that ART was associated with reduction in liver fibrosis scores in the majority of HIV-hepatitis co-infected and HIV-mono-infected Chinese participants (*14*). Unfortunately, this study had only a 48 weeks observation period and did not allow for long-term evaluation of progression of liver fibrosis among HIV patients with ART. Given that Chinese PLWHA are now living much longer owing to life-long ART and are at high risk of non-AIDS deaths particularly liver diseases (*15-17*), research to solicit long-term effects of ART on the development and the outcome of liver diseases especially liver fibrosis is urgently needed.

Liver biopsy is the gold standard technique for diagnosis of liver fibrosis, but it is an invasive procedure and does carry a small risk for complications (*18*). Thus, there is a need for a noninvasive method to assess liver fibrosis for monitoring liver disease progression and for pursuing epidemiological analysis. Fibrosis-4 (FIB-4) index has been developed and validated as an inexpensive and accurate noninvasive marker for liver fibrosis in HIV-infected patients (*19-21*), and is increasingly used for studying hepatic fibrosis in HIV-infected patients (*14, 22-23*).

In this retrospective cohort study, we proposed to assess the long-term impact of ART on liver fibrosis measured by FIB-4 index among HIV-infected patients in a rural prefecture of Eastern China.

2. Materials and Methods

2.1. *Study design and subject selection*

This was a retrospective cohort study of HIV-infected patients receiving ART in Taizhou prefecture of Zhejiang province, Eastern China. To be eligible for the study, participants must fulfill the following selection criteria: *i*) were sexually infected with HIV, *ii*) started ART during January 2009 through December 2015, *iii*) aged ≥ 18 years at ART initiation, *iv*) had CD4 cell count prior to ART initiation, and *v*) had available information of age, platelet (PLT) count, alanine transaminase (ALT) and aspartate transaminase (AST) levels for calculation of FIB-4 index at both ART initiation and at least one follow-up visit.

For all participants, the observation period was from the date of ART initiation (*i.e*. baseline) to December 31, 2016. According to China National Guidelines for Free Antiretroviral Treatment for HIV/AIDS, the first line treatment regimen comprises 2 nucleotide reverse transcriptase inhibitors (NRTIs) plus 1 non- nucleotide reverse transcriptase inhibitors (NNRTI). Most commonly, the 2 NRTIs are either zidovudine (AZT) or stavudine (d4T, replaced by tenofovir disoproxil fumarate (TDF) since 2010) and either lamivudine (3TC) or didanosine (DDI) (stopped in 2008), and the 1 NNRTI is either nevirapine (NVP) or efavirenz (EFV).

This study was approved by the Institutional Review Board (IRB) of the Chinese National Center for AIDS/STD Control and Prevention and the IRB of Fudan University, Shanghai, China.

2.2. *Data collection*

Demographical, epidemiological and clinical data were extracted from the China AIDS Comprehensive Response Information Management System (CRIMS) which is a unified web-based national information system. All eligible participants were tested for HBsAg and anti-HCV antibody (HCVAb) using enzyme linked immunoabsorbent assay (ELISA) (Kehua Biotechnology Co. Ltd., Shanghai, China). Patients with CD4 cell count decreased from baseline more than 30% were defined as being decreased in CD4 cell count and with increased from baseline higher than 30% were defined as increased in CD4+ (*24*).

2.3. *Study outcomes*

Liver fibrosis was determined by FIB-4 score, using Sterling's formula calculated as (age [years] × AST [IU/L]) / (PLT count [10^9/L] × ($ALT^{1/2}$[IU/L])) (*19*). FIB-4 was ranked into 3 classes according to standard cut-off values: < 1.45 for class 1 (no significant fibrosis); 1.45-3.25 for class 2 (moderate fibrosis) and > 3.25 for class 3 (severe fibrosis) (*25*). Improvement of liver fibrosis was defined as having > 30% decrease in FIB-4 from baseline, whereas progression of liver fibrosis was defined as having > 30% increase in FIB-4 score from baseline (*26-27*).

2.4. *Statistical analysis*

All statistical analyses were performed using SPSS version 22.0. Bivariate statistical analyses were performed with the Chi-square test or Fisher's exact test for categorical variables, and Mann-Whitney *U* test or Kruskal-Wallis test for continuous variables. Two separate logistic regression analyses were performed to determine risk factors for improvement (> 30% decrease

in FIB-4 index) or progression (> 30% increase in FIB-4 index) of liver fibrosis after ART, respectively. For each of the two logistic regression models, independent variables were selected based on existing knowledge of potential causal relationships between risk factors and liver fibrosis. In this regard, variables of appropriate baseline and follow-up characteristics such as demographic variables, HIV transmission mode, HBV and HCV coinfection status, ART regimen and duration as well as CD4 cell count were subject to univariate logistic regression analysis and were included in the final multiple logistic regression models using the "forced entry" criteria for variable selection. A p-value < 0.05 was chosen as the significance level.

3. Results

3.1. *Baseline characteristics*

3.1.1. *Sociodemographic characteristics and CD4 cell count*

A total of 955 HIV-infected patients participated in the study. Of them, 75.2% were male, 75.1% were married, 69.2% were HIV-infected through heterosexual transmission and 30.8% through homosexual transmission. The median age of the study participants was 37.0 years, and the median CD4 cell count was 211 cells/μL (Table 1).

3.1.2. *HBV and HCV coinfection*

As shown in Table 1, 125 participants (13.1%) were seropositive for HBsAg and 29 (3.0%) were seropositive for HCVAb. Seven patients (0.7%) were seropositive for both HBsAg and HCVAb. The proportion of HIV monoinfection, HIV/HBV coinfection, HIV/HCV

Table 1. Baseline and follow-up characteristics of study participants by FIB-4 changes after ART

Characteristics	Total, N (%)*	FIB-4 Unchanged (within ± 30%), N (%)**	FIB-4 Decreased > 30%, N (%)**	FIB-4 Increased > 30%, N (%)**	p[a]
n	955	460	354	141	
Baseline characteristics					
Age (years)					
Median (IQR)	37.0 (28.0-47.0)	36.0 (28.0-48.0)	39.0 (29.0-47.0)	37.0 (28.0-48.0)	0.935
18-24	131 (13.7)	67 (51.2)	48 (36.6)	16 (12.2)	0.565
25-34	263 (27.5)	137 (52.1)	87 (33.1)	39 (14.8)	
35-44	268 (28.1)	119 (44.4)	109 (40.7)	40 (14.9)	
≥ 45	293 (30.7)	137 (46.8)	110 (37.5)	46 (15.7)	
Gender					
Male	718 (75.2)	341 (47.5)	261 (36.4)	116 (16.2)	0.107
Female	237 (24.8)	119 (50.2)	93 (39.2)	25 (10.6)	
Marital status					
Unmarried	238 (24.9)	117 (49.2)	81 (34.0)	40 (16.8)	0.417
Married	717 (75.1)	343 (47.8)	273 (38.1)	101 (14.1)	
HIV transmission mode					
Homosexual	294 (30.8)	152 (51.7)	105 (35.7)	37 (12.6)	0.262
Heterosexual	661 (69.2)	308 (46.6)	249 (37.7)	104 (15.7)	
CD4 count (cells/μL)					
Median (IQR)	211 (133-287)	230 (150-303)	194 (125-271)	212 (112-286)	0.634
HBV coinfection					
Yes	125 (13.1)	52 (41.6)	53 (42.4)	20 (16.0)	0.281
No	830 (86.9)	408 (49.2)	301 (36.3)	121 (14.6)	
HCV coinfection					
Yes	29 (3.0)	10 (34.5)	14 (48.3)	5 (17.2)	0.303
No	926 (97.0)	450 (48.6)	340 (36.7)	136 (14.7)	
FIB-4 Index					
Median (IQR)	1.1	0.99 (0.7-1.5)	1.4 (0.99-2.3)	0.8 (0.5-1.3)	< 0.001
< 1.45	(0.7-1.7)	343 (53.6)	181 (28.3)	116 (18.1)	< 0.001
1.45-3.25	640 (67.0)	106 (41.6)	125 (49.0)	24 (9.4)	
> 3.25	255 (26.7)	11 (18.3)	48 (80.0)	1 (1.7)	
Follow-up characteristics	60 (6.3)				
ART regimen					
AZT+3TC+EFV/NVP	883 (92.5)	422 (47.8)	331 (37.5)	130 (14.7)	0.635
TDF+3TC+EFV/NVP	72 (7.5)	38 (52.8)	23 (31.9)	11 (15.3)	
Duration of ART (months)					
< 3	220 (23.0)	115 (52.3)	74 (33.6)	31 (14.1)	0.192
3-12	232 (24.3)	118 (50.9)	76 (32.8)	38 (16.4)	
13-36	279 (29.2)	134 (48.0)	105 (37.6)	40 (14.3)	
> 36	224 (23.5)	93 (41.5)	99 (44.2)	32 (14.3)	
CD4 change (last follow-up *vs*. baseline)					
Unchanged (within ± 30%)	303 (31.7)	171 (56.4)	93 (30.7)	39 (12.9)	0.002
Increased > 30%	609 (63.8)	267 (43.8)	250 (41.1)	92 (15.1)	
Decreased > 30%	43 (4.5)	22 (51.2)	11 (25.3)	10 (23.3)	

*The proportion was calculated in row; **The proportion was calculated in column; [a]Chi-square test for categorical variables or Kruskal-Wallis test for continuous variables, as appropriate; IQR, interquartile range; 3TC, lamivudine; TDF, tenofovir.

coinfection and HIV/HBV/HCV coinfection was 84.6% (808/955), 12.4% (118/955), 2.3% (22/955) and 0.7% (7/955), respectively.

3.1.3. *FIB-4 index or liver fibrosis*

The prevalence of liver fibrosis, *i.e.*, FIB-4 ≥ 1.45, was 33.0% (315/955) overall, 31.6% (255/808) for HIV monoinfections, 39% (46/118) for HIV/HBV coinfections, 45.5% (10/22) for HIV/HCV coinfections, and 57.1% (4/7) for HIV/HBV/HCV coinfections (Table 1). The prevalence of liver fibrosis was significantly different by HBV and/or HCV coinfection status, with participants co-infected with HBV and/or HCV being more likely to be living with liver fibrosis (Fisher's exact test, $p = 0.043$). Furthermore, the proportion of participants with severe liver fibrosis, *i.e.*, FIB-4 > 3.25, was 6.3% (60/955) overall, 5.3% (43/808) for HIV monoinfections, 11.9% (14/118) for HIV/HBV coinfections, 9.1% (2/22) for HIV/HCV coinfections, and 14.3% (1/7) for HIV/HBV/HCV coinfections.

3.2. *Antiretroviral therapy*

As shown in Table 1, 92.5% of the participants were prescribed with AZT+3TC+EFV/NVP while the other 7.5% were prescribed with TDF+3TC+EFV/NVP. The combination use of TDF+3TC, both of which are efficacious for HBV suppression, was more prevalent among HIV/HBV co-infected participants (15.2%, or 19/125) than among HIV mono-infected participants (6.4%, or 53/830) ($\chi^2 = 12.11$, $p < 0.001$). About 23% of the participants had received ART for less than three months, whereas 52.7% had received ART for more than one year (Table 1).

3.3. *Follow-up characteristics*

3.3.1. *CD4 cell count*

After antiretroviral therapy, 63.8% of the participants had > 30% increases in CD4 cell count or CD4 cell recovery, whereas 4.5% had > 30% decreases in CD4 cell count. CD4 cell count remained constant from the baseline to the last follow-up after ART among 31.7% of the participants (Table 1).

3.3.2. *FIB-4 index or liver fibrosis*

After antiretroviral therapy, 354 participants (37.1%) had > 30% decreases in FIB-4 index (*i.e.*, improvement of liver fibrosis), 141 (14.8%) had > 30% increases in FIB-4 index (*i.e.*, progression of liver fibrosis), while the remaining 460 participants (48.2%) had stabilized FIB-4 index. As shown in Table 1, The FIB-4 changing status after ART was significantly associated with the participant's baseline FIB-4 score but not significantly associated with other baseline characteristics. Participants with different CD4 cell count changing status also showed different FIB-4 changing status after ART, with a higher proportion of FIB-4 increase observed among those with decreased CD4 cell count (Table 1).

3.4. *Predictors for improvement of liver fibrosis after ART*

According to univariate logistic regression analysis, improvement of liver fibrosis after ART was significantly associated with baseline FIB-4 index, duration of ART and CD4 cell count changing status (Table 2). After adjusted for potential confounding effects of the other variables using multiple logistic regression model, improvement of liver fibrosis after ART was negatively associated with an older age ($aOR_{\geq 45 \text{ vs. } 18\text{-}24} = 0.42$, 95% CI: 0.23-0.76), but was positively associated with baseline FIB-4 index ($aOR_{1.45\text{-}3.25 \text{ vs. } <1.45} = 3.53$, 95% CI: 2.44-5.10; $aOR_{>3.25 \text{ vs. } <1.45} = 16.25$, 95%CI: 7.93-33.29) and > 30% increases in CD4 cell count after ART ($aOR_{\text{increased}>30\% \text{ vs. unchanged}} = 1.46$, 95% CI: 1.05-2.04) (Table 2).

3.5. *Predictors for progression of liver fibrosis after ART*

According to univariate logistic regression analysis, progression of liver fibrosis after ART was significantly associated with gender and HIV transmission mode, and baseline FIB-4 index (Table 3). After adjusted for potential confounding effects of the other variables using multiple logistic regression model, progression of liver fibrosis after ART was positively associated with an older age ($aOR_{\geq 45 \text{ vs. } 18\text{-}24} = 2.62$, 95% CI: 1.21-5.65), but was negatively associated with gender and HIV transmission mode ($aOR_{\text{male homosexual vs. male heterosexual}} = 0.50$, 95% CI: 0.32-0.80; $aOR_{\text{female heterosexual vs. male heterosexual}} = 0.54$, 95% CI: 0.32-0.90), and baseline FIB-4 index ($aOR_{1.45\text{-}3.25 \text{ vs. } <1.45} = 0.31$, 95% CI: 0.19-0.53; $aOR_{>3.25 \text{ vs. } <1.45} = 0.04$, 95% CI: 0.01-0.34) (Table 3).

4. Discussion

This study, to the best of our knowledge, is the first community-based longitudinal cohort study to assess impact of ART on liver fibrosis among HIV-infected patients in Eastern China. We observed that one-third of the HIV-infected patients were living with liver fibrosis before ART and the majority of them had attenuated or stabilized liver fibrosis after ART. In a multicenter cross-sectional study of HIV-positive ART-naïve patients across twelve provinces in China, the proportion of participants with liver fibrosis or FIB-4 ≥ 1.45 was 26.2% (*12*). Recently, Ding *et al* reported that the prevalence of liver fibrosis was as high as 42.5% among 3900 HIV-infected ART-naïve patients in

Table 2. Logistic regression analyses of predictors for > 30% FIB-4 decrease or improvement of liver fibrosis after ART

Characteristics	Crude *OR* (95% CI)	*p*	Adjusted *OR* (95% CI)	*p*
Baseline characteristics				
Age (years)				
18-24	1.00		1.00	
25-34	0.86 (0.55-1.32)	0.483	0.83 (0.52-1.33)	0.431
35-44	1.18 (0.77-1.82)	0.439	0.74 (0.44-1.25)	0.258
≥ 45	1.04 (0.68-1.59)	0.859	0.42 (0.23-0.76)	0.004
Marital status				
Unmarried	1.00		1.00	
Married	1.19 (0.87-1.62)	0.263	1.09 (0.72-1.65)	0.681
Gender and HIV transmission mode				
Male, heterosexual	1.00		1.00	
Male, homosexual	0.95 (0.70-1.30)	0.767	1.26 (0.88-1.80)	0.214
Female, heterosexual	1.10 (0.80-1.53)	0.533	1.16 (0.80-1.68)	0.441
FIB-4 Index				
< 1.45	1.00		1.00	
1.45-3.25	2.43 (1.80-3.29)	< 0.001	3.53 (2.44-5.10)	< 0.001
> 3.25	10.1 (5.26-19.5)	< 0.001	16.25 (7.93-33.29)	< 0.001
HBV coinfection				
No	1.00		1.00	
Yes	1.29 (0.88-1.89)	0.186	1.12 (0.74-1.71)	0.595
HCV coinfection				
No	1.00		1.00	
Yes	1.61 (0.77-3.37)	0.208	1.37 (0.61-3.10)	0.444
Follow-up characteristics				
ART regimen				
AZT+3TC+EFV/NVP	1.00		1.00	
TDF+3TC+EFV/NVP	0.78 (0.46-1.30)	0.350	0.74 (0.42-1.32)	0.314
Duration of ART (months)				
< 3	1.00		1.00	
3-12	0.96 (0.64-1.42)	0.842	0.95 (0.62-1.44)	0.799
13-36	1.19 (0.82-1.72)	0.355	1.11 (0.74-1.68)	0.608
> 36	1.56 (1.06-2.29)	0.022	1.29 (0.83-2.00)	0.257
CD4 change (last follow-up *vs* baseline)				
Unchanged (within ± 30%)	1.00		1.00	
Increased > 30%	1.57 (1.17-2.11)	0.002	1.46 (1.05-2.04)	0.024
Decreased > 30%	0.78 (0.38-1.61)	0.495	0.75 (0.34-1.65)	0.480

OR, odds ratio; CI, confidence interval.

Table 3. Logistic regression analyses of predictors for a > 30%FIB-4 increase or progression of liver fibrosis after ART

Characteristics	Crude *OR* (95% CI)	*p*	Adjusted *OR* (95% CI)	*p*
Baseline characteristics				
Age (years)				
18-24	1.00		1.00	
25-34	1.25 (0.67-2.33)	0.481	1.30 (0.67-2.50)	0.437
35-44	1.26 (0.67-2.34)	0.465	1.63 (0.79-3.35)	0.184
≥ 45	1.33 (0.72-2.46)	0.349	2.62 (1.21-5.65)	0.014
Marital status				
Unmarried	1.00		1.00	
Married	0.81 (0.54-1.21)	0.305	0.65 (0.39-1.10)	0.109
Gender and HIV transmission mode				
Male, heterosexual	1.00		1.00	
Male, homosexual	0.62 (0.41-0.95)	0.031	0.50 (0.32-0.80)	0.004
Female, heterosexual	0.51 (0.31-0.83)	0.006	0.54 (0.32-0.90)	0.018
FIB-4 Index				
< 1.45	1.00		1.00	
1.45-3.25	0.46 (0.29-0.74)	0.001	0.31 (0.19-0.53)	< 0.001
> 3.25	0.07 (0.01-0.55)	0.011	0.04 (0.01-0.34)	0.002
HBV coinfection				
No	1.00		1.00	
Yes	1.11 (0.66-1.86)	0.676	1.17 (0.68-2.03)	0.565
HCV coinfection				
No	1.00		1.00	
Yes	1.21 (0.45-3.22)	0.702	1.32 (0.47-3.71)	0.605
Follow-up characteristics				
ART regimen				
AZT+3TC+EFV/NVP	1.00		1.00	
TDF+3TC+EFV/NVP	1.04 (0.53-2.03)	0.898	0.96 (0.47-1.97)	0.910
Duration of ART (months)				
< 3	1.00		1.00	
3-12	1.19 (0.71-1.99)	0.499	1.11 (0.65-1.91)	0.698
13-36	1.02 (0.61-1.69)	0.937	0.94 (0.54-1.61)	0.813
> 36	1.01 (0.59-1.73)	0.953	0.97 (0.54-1.74)	0.926
CD4 change (last follow-up *vs* baseline)				
Unchanged (within ± 30%)	1.00		1.00	
Increased > 30%	1.20 (0.80-1.80)	0.365	1.30 (0.84-2.01)	0.241
Decreased > 30%	2.05 (0.93-4.48)	0.072	1.79 (0.78-4.10)	0.170

OR, odds ratio; CI, confidence interval.

a rural prefecture of Yunnan province in Southwestern China (*28*). Obviously, these studies observed varied prevalence of liver fibrosis among HIV patients, which are mostly attributable to different sources and characteristics of the study participants. Nevertheless, all the aforementioned studies including the present study reported that liver fibrosis was more prevalent among patients with HBV and/or HCV coinfection than those with HIV monoinfection, which is also consistent with findings from other countries (*22,29-31*).

The existing literature extensively evaluates liver disease progression among HIV/HCV or HIV/HBV coinfected patients receiving ART (*27,32-33*), with only a few studies examining liver disease progression and potential risk factors among HIV-monoinfected patients. In the present study, we found that a substantial proportion of HIV-infected patients, regardless of HBV and/or HCV coinfection status, had remarkable reduction or improvement of liver fibrosis after ART, further corroborating long-term benefits of ART on liver diseases in Chinese HIV patients (*28*). Such improvement of liver fibrosis was significantly associated with age, baseline FIB-4 score and post-ART CD4 cell recovery. It is by definition that patients with higher baseline FIB-4 score had more chances to present a reduced FIB-4 score after ART while having less chances to present an increased FIB-4 score during the follow-up, as long as ART had beneficiary effects on liver fibrosis. In this study, HIV patients aged over 45 years were less likely to gain improvement of liver fibrosis than younger patients. This is not surprise given that the development of liver cirrhosis is generally a slow disease progression and takes over 20-30 years. In fact, a study of HBV infected patients in China revealed that the protective effect of female gender against hepatic cirrhosis gradually lost with increasing age (*34*). Therefore, the less improvement of liver fibrosis among the elders underscores the importance of more closely monitoring and intervening liver disease progression in HIV-infected elderly people. Furthermore, the significant association between increased CD4 cell count and improved liver fibrosis after ART observed by the present study as well as studies in China and other countries further indicates the importance of promotion of CD4 cell recovery and maintenance of normal immune status through combination antiretroviral therapy (cART) for HIV patients (*23,35*). Since early initiation of cART remarkably improves CD4 cell recovery and generally slows down the disease progression (*14,36-38*), it is highly recommended that HIV-infected patients should get treated soonest possible after HIV diagnosis.

On the other hand, about 14.8% of the HIV patients had development or progression of liver fibrosis during ART. In fact, rapid fibrosis development from ART as well as from the chronic HIV infection itself is of serious concern to HIV patients (*28,39-41*). The pathophysiology of liver fibrosis in patients with HIV is a multifactorial process whereby chronic immune activation and inflammation that are unable to be fully suppressed by ART promote production of liver fibrosis. In addition, mitochondrial toxicity, triggered by both ART and HIV, contributes to intrahepatic damage, which is even more severe in patients coinfected with viral hepatitis (*42*).

More progression of liver fibrosis was observed among the elders. As mentioned above, HIV patients suffered more and rapid progression in various chronic diseases including liver fibrosis as they are getting old. In the meantime, we also found that female gender was a protective predictor of liver fibrosis progression among treated HIV patients, especially compared with heterosexual men. This is consistent with observations among both HIV-infected and HIV-uninfected populations (*34,43*). Although this is most likely due to pathophysiological differences and differential responses to ART between males and females (*44-46*), the less exposure of female patients to behaviors that favorite liver disease progression such as smoking and drinking cannot be ruled out (*10,47-48*). Interestingly, we found that homosexual men or men who have sex with men (MSM) were less likely to encounter liver fibrosis progression than heterosexual men. The mechanisms are unknown and remain to be defined.

This study has a couple of limitations. First, misclassification of liver fibrosis is unavoidable when a noninvasive marker instead of liver biopsy was used to define liver fibrosis. However, the misclassification should be non-differential and in turn made the association estimates biased to be more conservative. Moreover, liver biopsy was not feasible in this large epidemiologic study. Second, life style data including alcohol use and physical exercise that have potential impacts on liver diseases were not collected in this study, further limiting our capacity to draw a powerful causal inference.

Despite the limitations, this large longitudinal study has several important clinical and public health implication. First, improvement of liver fibrosis is achievable among HIV patients with better CD4 cell recovery which can be expectable through early initiation of ART. Second, liver fibrosis and hepatotoxicity associated with ART should be monitored as early as possible and throughout till the end of treatment, with special attention to the elderly and heterosexual men.

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