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Keywords:	HIV, antiretroviral treatment, liver enzyme elevation, total bilirubin elevation

Hepatic dysfunction in patients living with HIV on antiretroviral treatment with normal baseline liver function and without HBV/HCV infection: an 11-year retrospective cohort study

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Abstract

Objective: To characterize the association between duration of exposure to ART and liver dysfunction in HIV patients with an initially normal baseline liver function and without HBV/HCV infection.

Methods: A retrospective cohort study included in HIV-infected individuals with normal liver function parameters at ART initiation and without HBV/HCV infection during April 14, 2004 to April 13, 2015 from Guigang city, Guangxi, China. The association between duration of ART and liver enzyme elevation (LEE) or total bilirubin elevation (TBE), the markers of liver damage, was analyzed. Cox regression was used to examine the factors related to LEE/TBE.

Results: Of 2230 eligible patients, 27.31% (609/2230) developed LEE/TBE and contributed 10.09/100 person-years crude incidence rate. The highest LEE/TBE incidence was observed in patients with 0-6 months' ART (51.50/100 person years). The incidence decreased to 14.45/100 person years in patients with 18-24 months' ART and maintained at a relatively low and stable level in patients with two years' ART or longer (average of 8.35/100 person years). Cox regression analysis revealed that male patients, local patients, higher baseline CD4 cell count, higher baseline ALT, current regimen 3TC+TDF+EFV, 3TC+D4T+NVP, 3TC+AZT+EFV, 3TC+TDF+LVP/r or other regimens, were the risky factors for LEE/TBE.

Conclusions: The LEE/TBE incidence is relatively high among HIV-infected patients on ART with normal baseline liver function and without HBV/HCV infection. Nevertheless, cumulative ART duration does not increase the risk of hepatic dysfunction. Therefore, ART could tend to be long-term even considering ART-related liver toxicity, however, monitoring and management of liver dysfunction among patients on ART are important in clinical therapy.

Keywords: HIV, antiretroviral treatment, liver enzyme elevation, total bilirubin elevation

Strengths and limitations of this study

1. The strength of this study is its large size and the long-term retrospective observation of HIV/AIDS patients on ART, which could provide relatively reliable findings on association between liver damage and ART.

2. Liver damage is common among patients living with HIV on ART. Cumulative ART during does not increase the risk of hepatic dysfunction. There is a need for monitoring and management of liver dysfunction among patients living with HIV on ART.

3. Because of the nature of retrospective cohort study, unmeasured confounding factors such as smoking, alcohol consumption, and other opportunistic infections besides HBV/HCV could not be appropriately estimated and ruled out.

4. Once detected as liver dysfunction, those patients would more likely to take some measures, such as quitting smoking, limiting liquor, taking some liver protective drugs, than patients who had no abnormal liver function.

5. We lacked the information about further progression of liver disease such as hepatocarcinoma as well as liver dysfuntion-related mortality, which limited our findings to be associated with clinical outcomes.

Introduction

Antiretroviral treatment (ART) has significantly reduced morbidity and mortality in persons living with human immunodeficiency virus (HIV) worldwide [1]. An estimated 19.5 million people globally had received ART by 2016 [2]. With the implementation of ART, the life expectancy of HIV-infected individuals is now approaching that of the general population [3]. However, ART has some adverse effects, especially hepatic damage, which come along with the treatment [4]. An earlier study showed that the prevalence of liver transaminase elevation among HIV-positive individuals on ART ranges from 14% to 20% [5]. Consistently, some researchers have found the incidence of hepatic injury in ART-treated patients was increased [4]. However, quite a few other studies indicated that the approved antiretroviral agents have low liver toxicity and generally are considered to be well tolerated [6,7].

Mechanisms of liver damage among HIV-1 infected patients are multiple, probably attributing to HIV infection itself [8], hepatitis viral co-infections, ART-related hepatotoxicity [9], acquired immune deficiency syndrome (AIDS) related neoplasms [10], or experiencing age-related co-morbid conditions[11]. Hepatitis viral infections, either hepatitis B virus (HBV) or hepatitis C virus (HCV), have been reported to lead to hepatotoxicity [12,13]. Elevated hepatotoxicity was observed to be associated with ART in HIV/AIDS patients co-infected with HBV or HCV [4,12]. ART-related hepatotoxicity was also reported in quite a few previous studies[6,11,13]. However, most of these studies had no initial liver function reported [14]. Therefore, although there are many studies focusing on relationship of ART and liver function amongst HIV-1 infected patients, so far few have adequately powered to assess the exact nature of the association between ART and hepatic dysfunction in patients with an initially normal hepatic function. In addition, after extended exposure to antiretroviral, whether the association between ART and hepatic dysfunction remains true is unclear. Since ART generally lasts for many years and even lifelong, the effect of long-term ART on liver function needs to be elucidated, which is crucial to ascertain whether the risks of hepatic impairment is self-limiting, and to help to make better clinical decision and monitoring.

Guangxi Zhuang Autonomous Region, a province in western China, has the second highest HIV prevalence in China, with an more than 100,000 reported HIV/AIDS infected cases by the end of 2016[15], which accounting for ~13% of total national HIV/AIDS cases. Guigang city is a prefecture-level city in Guangxi, and there were more than 5000 reported HIV/AIDS cases during 2008 to 2013, and the number listed in the top five among all cities in Guangxi [16]. Previous studies have shown that most of HIV/AIDS patients in Guigang city had receive ART. However, the mortality reached 6.13%, which is higher than South Africa around 2004~2013[17]. Of the death cases, 74.21% died of HIV-related diseases[18]. In this study, we retrospectively collected the data from the ART cohort in Guigang city, aiming to investigate the association between duration of exposure to ART and elevation of liver function parameters [liver enzyme elevation (LEE) and/or total bilirubin elevation (TBE)] in HIV patients with an initially normal baseline liver function and without HBV/HCV infection.

Methods

Patient and Public Involvement

This retrospective cohort study was conducted in Guigang City, Guagnxi, China. All HIV-positive individuals from Guigang People's Hospital or Guigang Centers for Disease Control and Prevention (CDC) were reported to the National Notifiable Disease Monitoring System. Of them, HIV patients whose CD4 count was lower than 350 cells/μL (the standard increased from 200 cells/μL in 2008) or at WHO disease stage III or IV, met the Chinese national treatment criteria and were referred for treatment with standard ART, informed consent was obtained from all participants who willing to be treated.

The individuals receiving ART were followed up at 0.5, 1, 2 and 3 months after ART initiation and then every 3 months by staff of local CDC, with clinical indexes detected. If the ART regimen changed, the follow-up schedule was restarted. The ART information and clinical data were reported to the China's National Free Antiretroviral Treatment Program (NFATP) of Information System for the Prevention

and Control of AIDS.

We retrospectively collected the data during April 14, 2004 to April 13, 2015 from NFATP. Patients were included in this study if they met the following inclusion criteria: HIV/AIDS patients on ART, 16 years old or older, had normal baseline liver function parameters [alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin in serum (TB)] at ART initiation, had at least twice liver function measurements during the follow up. Patients were excluded if the following conditions occurred: had liver dysfunction (abnormal level of AST, ALT, or TB) at ART initiation, hepatitis B virus and/or hepatitis C virus positive at baseline or during follow-up, had no baseline CD4 cell count, and had less than twice of follow-up were also excluded. Censoring occurred at loss to follow-up, referral to other hospital, death, discontinuation of ART, or end of the observation period. The study was approved by the Human Research Ethics Committee of Guangxi Medical University (Ethical Review No. 2013-130).

Definitions

For this study, ART was defined as combined use of three or more antiretroviral drugs from any drug class. Baseline was defined as various clinical indexes from the most recent record before or after ART initiation. LEE was defined as AST>40 U/L, or ALT>40 U/L, TBE was defined as TB>20 μmol/L. Any one index exceeding the standard level was considered as abnormal (LEE/TBE). AIDS progression as indicated by CD4 lymphocyte count was: stage I (≥500 cells/μL), stage II (200–499 cells/μL), stage III (50- 199 cells/μL), and stage IV (<50 cells/μL) as recommendation of WHO. Follow-up time for each individual was calculated until the last visit plus 3 months, or last liver function detection date plus 3 months, to allow for report delay. October 13, 2015 was set as the cut-off date of follow-up, to guarantee the last enrollment could have had at least twice of follow-up.

The eligible patients were divided into two groups according to the occurrence of LEE/TBE during follow-up. The non-LEE/TBE group included patients who did not have LEE/TBE or had only once elevation, and the LEE/TBE group included patients

who had twice or more times of LEE/TBE.

Statistical analysis

Categorical variables were expressed as frequency or proportion. Quantitative variables were described by mediam ± interquartile range (IQR). Chi-square test (for categorical variables) and nonparametric test (for quantitative variables) were used to compare the characteristics between LEE/TBE and non-LEE/TBE groups. The crude incidence rates of liver damage (LEE/TBE) (per 100 person-years) of different ART duration were calculated in patients sorted by 6-month interval according to their ART duration time. We assumed that there is a linear association between cumulative ART duration and LEE/TBE based on the crude incidence rate. χ^2 trend test was conducted for analysis of the change of LEE/TBE incidence rates along with the ART duration time. The Cox regression analysis was used to evaluate the related factors for LEE/TBE. The data were analyzed using Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc. Chicago, USA) and GraphPad Prism version 6.0 (GraphPad Software, San Diego, California, USA).

Results

Demographic characteristics of eligible HIV/AIDS patients at baseline

A total of 4516 patients who initiated ART during April 14, 2004 and April 13, 2015 were enrolled in this study from the Information System for the Prevention and Control of AIDS in Guigang city. Of them, 2286 patients were excluded, including 419 patients who didn't have baseline liver function parameters (358) or CD4 cell count record (61), 184 HBV/HCV positive at baseline, 18 HBV/HCV positive during follow-up, 857 had abnormal baseline liver function parameters, 734 had less than twice liver function record, and 74 without follow-up (Figure 1). Finally, 2230 patients fulfilled inclusion criteria and were included in this study (Figure 1).

The 2230 eligible individuals contributed 6033.89 person years of follow-up, Table 1 shows demographic characteristics of eligible HIV/AIDS patients at baseline. The mediam age at diagnosis was 49.0 years old (IQR: 35.0-60.0), the mediam age at

initiation of ART was 49.0 years old (IQR: 36.0-61.0). Of the 2230 patients, 62.8% were male, 75.4% were married or cohabitation, 97.0% were Guigang residents, and 90.6% acquired HIV heterosexually or homosexually. The eligible patients were divided into two groups according to the occurrence of LEE/TBE: 72.69% (1621/2230) belonged to non-LEE/TBE group, and 27.31% (609/2230) belonged to LEE/TBE group. The difference of residence, diagnosis age, ART initiation age and transmission route between LEE/TBE and non-LEE/TBE group were statistically significant ($p<0.05$) (Table 1).

Table 1. Demographic characteristics of study population at baseline

Characteristics	Total (n=2230)	Liver function parameters (ALT, AST, TB)[a]		χ^2	p
		Non-LEE/TBE (n=1621)	LEE/TBE (n=609)		
Gender				1.55	0.21
Male	1400(62.8%)	1005(62.0%)	395(64.9%)		
Female	830(37.2%)	616(38.0%)	214(35.1%)		
Marital Status				3.80	0.43
Unmarried	220(9.9%)	155(9.6%)	65(10.7%)		
Married/cohabitation	1682(75.4%)	1223(75.4%)	459(75.4%)		
Divorced/separated	58(2.6%)	39(2.4%)	19(3.1%)		
Widowed	260(11.7%)	198(12.2%)	62(10.2%)		
Others	10(0.4%)	6(0.4%)	4(0.7%)		
Residence				11.45	0.003
Guigang city	2163(97.0%)	1584(97.7%)	579(95.1%)		
Other cities in Guangxi	63(2.8%)	34(2.1%)	29(4.8%)		
Other provinces	4(0.2%)	3(0.2%)	1(0.2%)		
Age (years) at Diagnosis				23.53	<0.001
<40	705(31.6%)	470(29.0%)	235(38.6%)		
40-60	924(41.4%)	679(41.9%)	245(40.2%)		
≥60	601(27.0%)	472(29.1%)	129(21.2%)		
Age (years) at ART Initiation				23.75	<0.001
<40	684(30.7%)	455(28.1%)	229(37.6%)		
40-60	924(41.4%)	678(41.8%)	246(40.4%)		
≥60	622(27.9%)	488(30.1%)	134(22.0%)		

Transmission Route				28.19	<0.001
Blood transfusion	89(4.0%)	43(2.7%)	46(7.6%)		
Sexual transmission	2020(90.6%)	1492(92.0%)	528(86.7%)		
Other or unknown	121(5.4%)	86(5.3%)	35(5.7%)		

[a] ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin in serum

Clinical characteristics of study population

Among the eligible 2230 patients, the proportion of patients at four clinical stages at baseline was 37.2% (stage I), 18.0% (stage II), 31.3% (stage III), and 13.5% (stage IV), respectively. Baseline mediam CD4 cell count at ART initiation was 164.0 cells/μL (IQR: 54.0-265.0). Baseline AST, ALT, TB was 24.4 U/L, 17.7U/L, 8.1 μmol/L, respectively, and follow-up AST, ALT, TB was 26.2 U/L, 22.6U/L, 7.3 μmol/L, respectively. Mediam CD4 cell count during follow-up was 309.6 cells/μL (IQR: 200.0-437.9). The eligible patients had a mediam ART duration time of 2.4 years (IQR: 1.0-4.0) (Table S1). Among them, 609 had twice or more times abnormal liver function parameters during follow-up, and made up the LEE/TBE group, contributing 10.09/100 person-years crude incidence rate of follow up (95%CI: 9.31-10.87). The remaining 1621 patients had no or only once abnormal liver function parameters during follow-up, and made up the non-LEE/TBE group (Figure 1, Table 1 and S1). The difference of baseline AST and ALT, current disease stage, current ART regimen, and during of ART between LEE/TBE and non-LEE/TBE group were statistically significant ($p<0.05$).

Crude incidence rates of liver enzyme elevation/total bilirubin elevation (LEE/TBE) in patients with different ART duration times

With all patients sorted by 6-month interval according to their ART duration time, the crude incidence rates of liver damage (LEE/TBE) (per 100 person-years) in each interval of ART duration were calculated (Figure 2). The LEE/TBE occurred in all patients with different ART duration time, and the overall LEE/TBE incidence was 10.09/100 person-years (95%CI: 9.31-10.87) (Figure 2). The highest incidence was observed in patients with 0-6 months' ART (51.50/100 person years, 95%CI:

26.34-76.65), and then the incidence rapidly decreased to 14.45/100 person years (95%CI: 9.96-18.94) in patients with 18-24 months' ART (Figure 2). There is a clear decreasing trend of incidence rate of LEE/TBE along with the ART duration time in patients with less than 2 years of ART (χ^2 trend test, χ^2=13.85, p<0.001). The incidence rate was then kept at a relatively low and stable level in patients who had longer ART duration time (≥ 2 years), with an average of 8.35/100 person years (95%CI: 7.59–9.11) (Figure 2).

Related factors for liver enzyme elevations/total bilirubin elevation (LEE/TBE)

In order to identify related factors for LEE/TBE, the data of all 2230 eligible patients were included in the Cox regression analysis. Univariable analysis were performed firstly, and then variables that were statistically significant (p<0.05) were included in a multivariate Cox regression analysis. As shown in Table 2, female patients had a lower risk to develop LEE/TBE compared with male patients (AHR 0.83, 95% CI: 0.69-0.99; p=0.04), patients from other cities in Guangxi had a lower risk to develop LEE/TBE compared with local residents (AHR=0.28, 95% CI 0.17-0.47, p<0.001), patients at initial disease stage III, IV or at current disease stage II, III were less likely to have LEE/TBE compared with patients at initial disease stage I or at current disease stage I, respectively. Compared with current use of 3TC+TDF+EFV, the current regimens 3TC+AZT+NVP, 3TC+D4T+NVP, 3TC+AZT+EFV, 3TC+TDF+LVP/r or other regimens had lower risk to develop LEE/TBE (Table 2). Except the above factors that could decrease the risk of LEE/TBE, higher baseline CD4 cell count (200-350 cells/μL, AHR=1.30, 95% CI: 1.04-1.62; >350 cells/μL, AHR=2.65, 95% CI: 1.84-3.81, compared with cell count <200 cells/μL); higher baseline ALT (20-30 U/L, AHR=1.50, 95% CI: 1.25-1.80; 30-40 U/L , AHR=1.61, 95% CI: 1.27-2.04, compared with ALT< 20U/L) had higher risk for LEE/TBE (Table 2). It is worth noting that higher CD4 cell count during follow-up (> 350 cells/μL) (AHR=0.56, 95% CI: 0.43-0.73, p<0.001, compared with cell count < 200 cells/μL) had lower risk to develop LEE/TBE (Table 2).

Table 2. Factors associated with LEE/TBE among HIV/AIDS patients on ART

Variables	Total Patients (n)	LEE/ TBE (n)	Person-Years	LEE/TBE per 100 person year	HR(95% CI)	*p*	AHR(95% CI)[a]	*p*
Gender								
Male	1400	395	3595.59	10.99	1	-	1	-
Female	830	214	2438.30	8.78	0.75(0.63-0.89)	0.001	0.83(0.69-0.99)	0.04
Marital Status						0.33		
Unmarried	220	65	560.48	11.60	1	-		
Married/cohabitation	1682	459	4600.81	9.98	0.83(0.64-1.07)	0.15		
Divorced/separated	58	19	169.37	11.22	0.88(0.53-1.48)	0.64		
Widowed	260	62	678.49	9.14	0.78(0.55-1.11)	0.17		
Others	10	4	24.74	16.17	1.78(0.65-4.88)	0.27		
Residence						<0.001		<0.001
Guigang city	2163	579	5729.91	10.10	1	-	1	-
Other cities in Guangxi	63	29	290.20	9.99	0.34(0.21-0.54)	<0.001	0.28(0.17-0.47)	<0.001
Other provinces	4	1	13.78	7.26	0.59(0.08-4.21)	0.60	0.72(0.10-5.19)	0.74
Age (years) at Diagnosis						0.62		
<40	705	235	2249.34	10.45	1	-		
40-60	924	245	2415.74	10.14	0.98(0.82-1.18)	0.84		
≥60	601	129	1368.81	9.42	1.09(0.88-1.35)	0.43		
Age (years) at ART Initiation						0.68		
<40	684	229	2183.73	10.49	1	-		
40-60	924	246	2429.50	10.13	0.97(0.81-1.17)	0.78		
≥60	622	134	1420.66	9.43	1.07(0.86-1.33)	0.53		
Transmission Route						0.87		
Blood transfusion	89	46	342.95	13.41	1	-		
Sexual transmission	2020	528	5364.81	9.84	0.94(0.70-1.28)	0.71		
Others	121	35	326.13	10.73	1.02(0.65-1.58)	0.95		
Baseline Disease Stage						<0.001		0.003
I	830	219	1930.65	11.34	1	-	1	-
II	402	119	1248.67	9.53	0.68(0.55-0.86)	0.001	0.78(0.61-1.01)	0.06
III	698	186	2157.84	8.62	0.57(0.47-0.70)	<0.001	0.63(0.49-0.80)	<0.001
IV	300	85	696.73	12.20	0.85(0.66-1.10)	0.22	0.66(0.47-0.93)	0.02
Baseline CD4 count						<0.001		<0.001
<200 cells/μL	1312	358	3749.56	9.55	1	-	1	-
200-350 cells/μL	721	203	2010.22	10.10	1.16(0.97-1.37)	0.104	1.30(1.04-1.62)	0.02
≥350 cells/μL	197	48	274.11	17.51	2.75(2.02-3.74)	<0.001	2.65(1.84-3.81)	<0.001

Baseline AST						0.03		
<20 U/L	508	104	1327.79	7.83	1	-		
20-30 U/L	1180	340	3150.96	10.79	1.34(1.08-1.67)	0.01		
30-40 U/L	542	165	1555.14	10.61	1.24(0.97-1.59)	0.08		
Baseline ALT						<0.001		<0.001
<20 U/L	1352	305	3627.10	8.41	1	-	1	-
20-30 U/L	631	201	1671.60	12.02	1.37(1.15-1.64)	0.001	1.50(1.25-1.80)	<0.001
30-40 U/L	247	103	735.19	14.01	1.51(1.21-1.89)	<0.001	1.61(1.27-2.04)	<0.001
Baseline TB								
10-20 μmol/L	1540	418	4055.21	10.31	1	-		
<10 μmol/L	690	191	1978.68	9.65	0.97(0.82-1.16)	0.75		
Current Disease Stage						<0.001		<0.001
I	722	158	1343.48	11.76	1	-	1	-
II	516	162	1788.47	9.06	0.60(0.48-0.75)	<0.001	0.66(0.51-0.84)	0.001
III	680	186	2181.08	8.53	0.60(0.49-0.75)	<0.001	0.61(0.46-0.80)	<0.001
IV	312	103	720.86	14.29	1.07(0.83-1.37)	0.60	0.90(0.65-1.25)	0.52
Current ART Regimen						<0.001		<0.001
3TC+TDF+EFV	753	215	1580.68	13.60	1	-	1	-
3TC+AZT+NVP	372	80	1378.94	5.80	0.32(0.24-0.41)	<0.001	0.31(0.24-0.40)	<0.001
3TC+D4T+NVP	129	31	399.02	7.77	0.32(0.22-0.48)	<0.001	0.33(0.22-0.49)	<0.001
3TC+AZT+EFV	333	94	1027.13	9.15	0.64(0.50-0.82)	<0.001	0.61(0.48-0.78)	<0.001
3TC+D4T+EFV	76	22	146.20	15.05	1.11(0.72-1.72)	0.64	1.22(0.78-1.90)	0.39
3TC+TDF+LVP/r	263	91	775.23	11.74	0.76(0.60-0.98)	0.03	0.72(0.56-0.93)	0.01
Other regimens	304	76	726.69	10.46	0.72(0.56-0.94)	0.02	0.68(0.52-0.89)	0.004
CD4 Count during Follow-up						0.01		<0.001
<200 cells/μL	555	135	1195.03	11.30	1	-	1	-
200-350 cells/μL	739	202	2057.59	9.82	0.80(0.64-1.00)	0.05	0.84(0.67-1.07)	0.15
≥350 cells/μL	936	272	2781.27	9.78	0.72(0.58-0.89)	0.002	0.56(0.43-0.73)	<0.001

Abbreviations: HR, hazard ratio; AHR, adjusted hazard ratio, ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin in serum.

a Covariates of the adjusted model included gender, residence, baseline disease stage, baseline CD4 counts, baseline AST and ALT, current disease stage, current ART regimen, and CD4 count during follow-up .

Discussion

This retrospective cohort study was conducted on HIV-positive individuals with an initially normal hepatic function and without HBV/HCV infection. We found that LEE/TBE occurred in all patients with different period of ART, indicating that the

existence of hepatic damage was associated with ART, which has been shown in a number of previous studies [4,5]. In our study, the LEE/TBE prevalence was 27.31% (609/2230) (Figure 1), which is higher than those (14%-23%) in several previous studies conducted on HIV/AIDS patients [19,20]. In addition, the overall LEE/TBE incidence (10.09/100 person-years, 95%CI: 9.31-10.87) in our study is also higher than that (6.04/100 person-years of chronic LEE incidence) in a similar study on HIV-monoinfected persons by Kovari et al [21]. One possible reason for higher prevalence and overall incidence of LEE/TBE in our study is that we used three hepatic function indexes (AST, ALT, TB) to define liver dysfunction, whereas others generally used two indexes (AST, ALT) or only used ALT to define.

The highest LEE/TBE incidence was observed in patients with 0-6 months' ART duration (51.50/100 person years). The incidence rapidly decreased to14.45/100 person years in patients with 2 years of ART and then maintained at a relatively lower level (average of 8.35/100 person years) in patients with longer ART during times (≥ 2 years) (Figure 2). These findings are consistent with quite a few previous studies on liver toxicity caused by various antiretroviral drugs such as NVP, EFV, TDF, which also observed a strong association between drugs and the development of LEE emerging within the first 2 years after drug initiation [21]. The significant decreasing trend of incidence rate of LEE/TBE within the first 2 years of ART during might be resulted from a number of adaptation mechanisms which are initiated to counteract the inflicted damage [22]. More importantly, the above findings indicate that cumulative ART time does not increase the risk of hepatic dysfunction, which is of great clinical importance to support the stratagem of WHO to increase the number of HIV/AIDS patients on ART [23], which has been shown to greatly reduce rate of sexual transmission of HIV in several recent HIV Prevention Trails[24,25].

Several related factors for LEE/TBE were observed in the present study (Table 2). Female patients had a lower risk to develop LEE/TBE compared with male patients (AHR=0.83). Similar result was found in several previous studies [19,26]. Higher baseline ALT also showed higher risk for LEE/TBE, which is similar to the result of Carlo's study showing that baseline ALT was associated with hepatotoxicity [27]. It is

easily understood, as ALT is a biomarker of hepatic function, and its elevated level indicate hepatotoxicity or liver dysfunction.

In this study, higher CD4 cell count at baseline and lower CD4 cell count during follow-up were associated with LEE/TBE, indicating the level of immunodeficiency and the host immunity involve in liver dysfunction upon ART. Consistent to the effects of CD4 cell count, we found that patients at initial disease stage III, IV were less likely to have LEE/TBE compared with patients at initial disease stage I (Table 2). These results are consistent with some previous studies. For example, the risk of nevirapine-induced hepatitis increases 12-fold in women with more than 250 CD4 cells/μL and 5-fold in males with more than 400 CD4 cells/μL[28], and current CD4 count<200 cells/μL was associated with elevated liver enzyme[19]. However, our results are inconsistent with some other studies, showing lower risk of hepatic dysfunction come along with higher CD4 cell count in individuals on ART[8]. Actually, contradicting data were reported by different researchers in term of relationship of CD4 cell count and liver dysfunction, which may be related to the different ART regimens investigated as well as some other factors that might affect host immune status when patients were on ART[19].

Our study also showed that current regiment 3TC+TDF+EFV could lead to an increased risk of LEE/TBE compared with other regimens, including regimens 3TC+AZT+NVP, 3TC+D4T+NVP, 3TC+AZT+EFV, 3TC+TDF+LVP/r or other combinations. The liver toxicity of TDF and EFV has been previously described in several studies [21,22]. Since 3TC+TDF+EFV is currently considered as one of first-line regimens, the importance of combination of liver protection therapy needs to be stressed for this regimen.

Conclusions

Although HIV-infected patients with abnormal baseline liver function and with HBV/HCV infection had been excluded, the incidence of LEE/TBE is still rather high among HIV/AIDS patients on ART, suggesting that monitoring and management of liver dysfunction among HIV/AIDS patients on ART are important in clinical therapy.

Nevertheless, cumulative ART during does not increase the risk of hepatic dysfunction, which provides indirect evidence supporting that the ART could tend to be long-term. In addition, we have identified a number of related factors for LEE/TBE, which may contribute to ART toward less liver dysfunction and to be long-term.

Competing interests

All authors declare that they have no conflict of interest.

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Authors' contribution

HL, XQ and LY conceived and designed the study. FQ, JJ conducted the data analysis, literature review, and drafted the manuscript. CQ, YH, BL, and YX were involved in the study supervision, data collection, and interpretation of the data. JH, ZX, CN, YL, NZ, JL, WW, and JY assisted with data management and data analysis. All authors contributed to the revision of the manuscript and approved the final version.

Data sharing

No additional data available.

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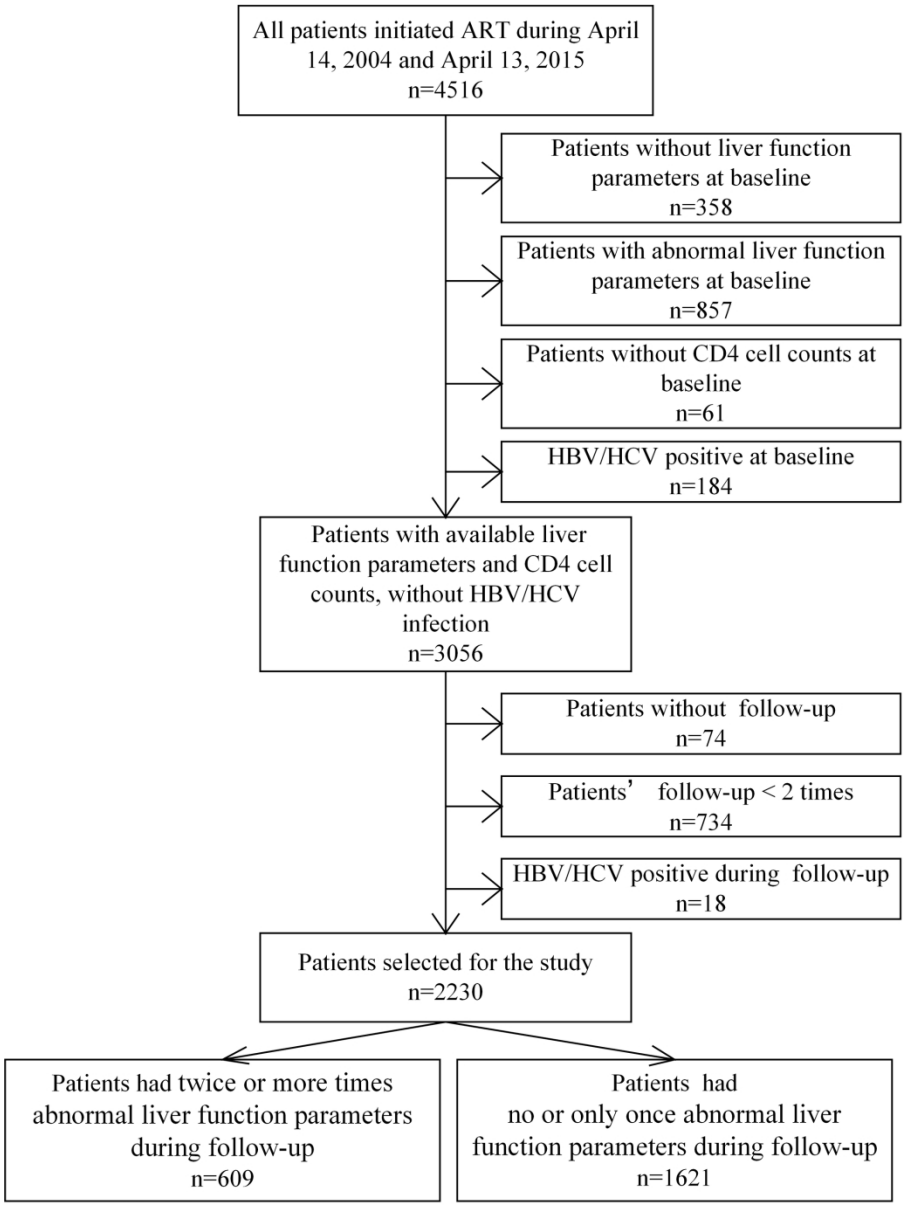
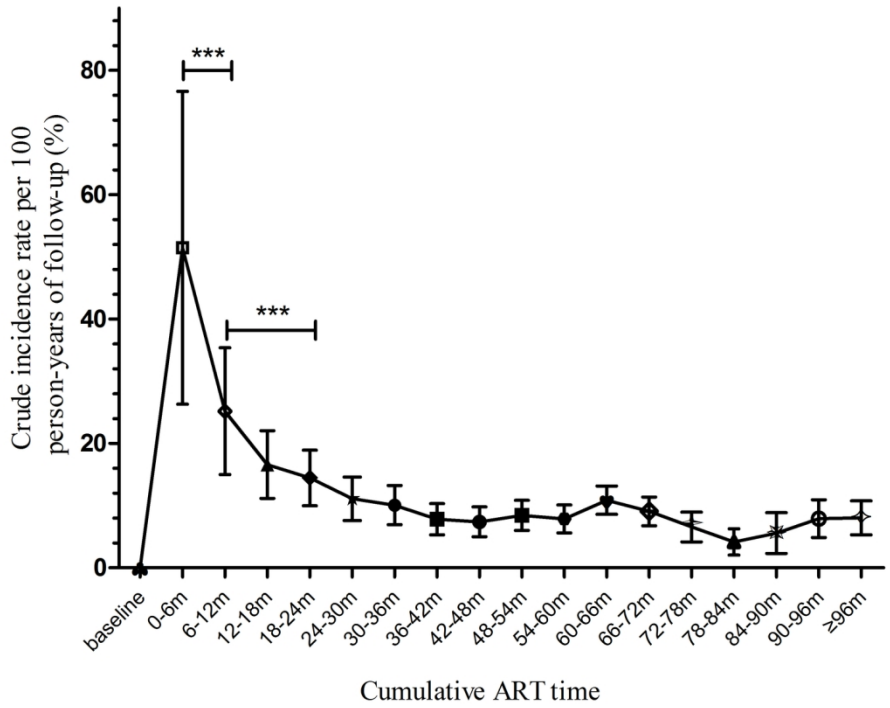


Figure1. Patients Flowchart

187x250mm (300 x 300 DPI)



Patients under observation (n)	364	211	218	169	187	148	174	168	136	93	94	73	72	53	23	26	21
LEE/TBE (n)	50	46	58	39	46	39	49	43	39	35	43	35	30	17	11	14	15

Figure 2. Crude incidence rates of liver enzyme elevation/total bilirubin elevation (LEE/TBE) with cumulative ART time (Error bars are 95% CIs. *** p<0.05).

140x128mm (300 x 300 DPI)

Supplementary

Table S1. Clinical characteristics of study population

Characteristics	Total (n=2230)	Liver function parameters (ALT, AST, TB)[a]		χ^2/Z	p
		Non-LEE/TBE (n=1621)	LEE/TBE (n=609)		
Baseline Diseases Stage				1.73	0.63
Ⅰ	830(37.2%)	611(37.7%)	219(36.0%)		
Ⅱ	402(18.0%)	283(17.5%)	119(19.5%)		
Ⅲ	698(31.3%)	512(31.6%)	186(30.5%)		
Ⅳ	300(13.5%)	215(13.3%)	85(14.0%)		
Baseline CD4 Count				1.12	0.57
<200 cells/μL	1312(58.8%)	954(58.9%)	358(58.8%)		
200-350 cells/μL	721(32.3%)	518(32.0%)	203(33.3%)		
≥350 cells/μL	197(8.8%)	149(9.2%)	48(7.9%)		
Baseline AST				15.99	<0.001
<20 U/L	508(22.8%)	404(24.9%)	104(17.1%)		
20-30 U/L	1180(52.9%)	840(51.8%)	340(55.8%)		
30-40 U/L	542(24.3%)	377(23.3%)	165(27.1%)		
Baseline ALT				47.70	<0.001
<20 U/L	1352(60.6%)	1047(64.6%)	305(50.1%)		
20-30 U/L	631(28.3%)	430(26.5%)	201(33.0%)		
30-40 U/L	247(11.1%)	144(8.9%)	103(16.9%)		
Baseline TB				0.07	0.79
<10 μmol/L	1540(69.1%)	1122(69.2%)	418(68.6%)		
10-20 μmol/L	690(30.9%)	499(30.8%)	191(31.4%)		
Current Disease Stage				20.16	<0.001
Ⅰ	722(32.4%)	564(34.8%)	158(25.9%)		
Ⅱ	516(23.1%)	354(21.8%)	162(26.6%)		
Ⅲ	680(30.5%)	494(30.5%)	186(30.5%)		
Ⅳ	312(14.0%)	209(12.9%)	103(16.9%)		
Current ART Regimen				15.70	0.02
3TC+TDF+EFV	753(33.8%)	538(33.2%)	215(35.3%)		
3TC+AZT+NVP	372(16.7%)	292(18.0%)	80(13.1%)		
3TC+D4T+NVP	129(5.8%)	98(6.0%)	31(5.1%)		
3TC+AZT+EFV	333(14.9%)	239(14.7%)	94(15.4%)		
3TC+D4T+EFV	76(3.4%)	54(3.3%)	22(3.6%)		
3TC+TDF+LPV/r	263(11.8)	172(10.6%)	91(14.9%)		

Other regimens	304(13.6%)	228(14.1%)	76(12.5%)		
CD4 Count during Follow-up				3.94	0.14
<200 cells/μL	555(24.9%)	420(25.9%)	135(22.2%)		
200-350 cells/μL	739 (33.1%)	537(33.1%)	202(33.2%)		
≥350 cells/μL	936(42.0%)	664(41.0%)	272(44.7%)		
Follow-up AST[b] U/L	26.2(21.6-33.3)	24.1(20.4-29.3)	34.0(27.0-42.7)	19.7	<0.001
Follow-up ALT[b] U/L	22.6(16.9-30.8)	20.0(15.6-25.4)	33.6(25.9-43.3)	23.4	<0.001
Follow-up Tbil[b] μmol/L	7.3(5.6-9.4)	7.2(5.6-9.1)	7.5(5.7-10.2)	3.7	<0.001
During of ART (years) [b]	2.4(1.0-4.0)	2.1(0.8-3.7)	3.2(1.5-5.1)	-9.63	<0.001

[a] ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin in serum

[b] Data are presented as medium ± interquartile range (IQR)

Contributorship statement

Hao Liang, Xionglin Qin and Li Ye conceived and designed the study. Fengxiang Qin, Junjun Jiang conducted the data analysis, literature review, and drafted the manuscript. Chunwei Qin, Yunxuan Huang, Bingyu Liang, and Yuexiang Xu were involved in the study supervision, data collection, and interpretation of the data. Jiegang Huang, Zhiliang Xu, Chuanyi Ning, Yanyan Liao, Ning Zang, Jingzhen Lai, Wudi Wei, and Jun Yu assisted with data management and data analysis. All authors contributed to the revision of the manuscript and approved the final version.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract ✔
		(*b*) Provide in the abstract an informative and balanced summary of what was done and w hat was found ✔
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported ✔
Objectives	3	State specific objectives, including any prespecified hypotheses ✔
Methods		
Study design	4	Present key elements of study design early in the paper ✔
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection ✔
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up ✔ *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed ✔ *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable ✔
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group ✔
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why ✔
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding ✔
		(*b*) Describe any methods used to examine subgroups and interactions ✔
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed ✔ *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram ✔
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders ✔
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) ✔
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time ✔
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included ✔
		(*b*) Report category boundaries when continuous variables were categorized ✔
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives ✔
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias ✔
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence ✔
Generalisability	21	Discuss the generalisability (external validity) of the study results ✔
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based ✔

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Liver damage in patients living with HIV on antiretroviral treatment with normal baseline liver function and without HBV/HCV infection: an 11-year retrospective cohort study

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	Prevention and Treatment & Guangxi Universities Key Laboratory of Prevention and Control of Highly Prevalent Disease
Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	Epidemiology
Keywords:	HIV, antiretroviral treatment, liver enzyme elevation, total bilirubin elevation

Liver damage in patients living with HIV on antiretroviral treatment with normal baseline liver function and without HBV/HCV infection: an 11-year retrospective cohort study

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Hao Liang, Xionglin Qin and Li Ye contributed equally to this paper.

Abstract

Objective: To characterize the association between duration of exposure to ART and liver damage in HIV patients with an initially normal baseline liver function and without HBV/HCV infection.

Methods: A retrospective cohort study was conducted in HIV-infected individuals with normal liver function parameters at ART initiation and without HBV/HCV infection, from April 14, 2004 to April 13, 2015 in Guigang city, Guangxi, China. The association between duration of ART and liver damage [grade II-IV liver enzyme elevation (LEE) and/or total bilirubin elevation (TBE)], was analyzed. Cox regression was used to examine the factors related to liver damage.

Results: Of 2119 eligible patients, 12.41% (263/2119) developed liver damage (grade II-IV LEE/TBE) and contributed 4.11/100 person-years crude incidence rate. The highest liver damage incidence was observed in patients with 6-12 months' ART (15.16/100 person years). The incidence decreased to 5.56/100 person years in patients with 12-18 months' ART and 3.13/100 person years in patients with 18-24 months' ART, and then maintained at a relatively low and stable level in patients with two years' ART or longer (average of 3.65/100 person years). Cox regression analysis revealed that current WHO disease stage II, III or IV (compared with stage I) were the risk factors for liver damage, while baseline disease stage II, III (compared with stage I) and current regimen 3TC+AZT+NVP were the protective factors for liver damage.

Conclusions: Liver damage always exists among HIV-infected patients on ART with normal baseline liver function and without HBV/HCV infection. Nevertheless, cumulative ART duration does not increase the risk of liver damage. ART could tend to be long-term, however, monitoring and management of liver damage among patients on ART are also important in clinical therapy.

Keywords: HIV, antiretroviral treatment, liver enzyme elevation, total bilirubin elevation

Strengths and limitations of this study

1. The strength of this study is its large size and the long-term retrospective observation of HIV/AIDS patients on ART, which could provide relatively reliable findings on association between liver damage and ART.

2. Liver damage exists among patients living with HIV on ART. However, cumulative ART during does not increase the risk of liver damage.

3. In this retrospective cohort study, confounding factors such as smoking, alcohol consumption, and other opportunistic infections besides HBV/HCV could not be estimated and ruled out.

4. Lack of information about further progression of liver disease such as hepatocarcinoma as well as liver dysfunction-related mortality limited our findings to be associated with clinical outcomes.

Introduction

Antiretroviral treatment (ART) has significantly reduced morbidity and mortality in persons living with human immunodeficiency virus (HIV) worldwide [1]. An estimated 19.5 million people globally had received ART by 2016 [2]. With the implementation of ART, the life expectancy of HIV-infected individuals is now approaching that of the general population [3]. However, ART has some adverse effects, especially hepatic damage, which comes along with the treatment [4]. An earlier study showed that the prevalence of liver transaminase elevation among HIV-positive individuals on ART ranges from 14% to 20% [5]. Consistently, some researchers have found the incidence of hepatic injury in ART-treated patients was increased [4]. However, quite a few other studies indicated that the approved antiretroviral agents have low liver toxicity and generally are considered to be well tolerated [6,7].

Mechanisms of liver damage among HIV-1 infected patients are multiple, probably attributing to HIV infection itself [8], hepatitis viral co-infections, ART-related hepatotoxicity [9], acquired immune deficiency syndrome (AIDS) related neoplasm [10], or experiencing age-related co-morbid conditions[11]. Hepatitis viral infections, either hepatitis B virus (HBV) or hepatitis C virus (HCV), have been reported to lead to hepatotoxicity [12,13]. Elevated hepatotoxicity was observed to be associated with ART in HIV/AIDS patients co-infected with HBV or HCV [4,12]. ART-related hepatotoxicity was also reported in quite a few previous studies [6,11,13]. Furthermore, didanosine (DDI) is no longer recommended as the first-line antiretroviral drugs for HIV patients because of its hepatotoxicity [14]. Although there are many studies focusing on relationship of ART and liver function amongst HIV-1 infected patients, after extended exposure to antiretroviral, whether the association between ART and hepatic dysfunction remains true is unclear. Moreover, even though most of the previous studies were controlled for baseline liver enzymes level [5,7-9], there were still some studies had no initial liver function reported [15] or co-infected with viral hepatitis [4,6,12]. Since ART generally lasts for many years and even lifelong, the effect of long-term ART on liver function needs to be elucidated, which is crucial to ascertain whether the risks of hepatic impairment are self-limiting, and to help to make better clinical

decision and monitoring.

Guangxi Zhuang Autonomous Region, a province in western China, has the second highest HIV-infected reported cases in China, with more than 100,000 reported HIV/AIDS cases by the end of 2016 [16], which accounting for ~13% of total national HIV/AIDS cases. Guigang city is a prefecture-level city in Guangxi, and there were more than 5000 reported HIV/AIDS cases during 2008 to 2013, which listed in the top five among all cities in Guangxi [17]. Previous studies have shown that most of HIV/AIDS patients in Guigang city had receive ART. However, the mortality reached 6.13%, which is higher than those in other places such as South Africa during 2004~2013 [18]. Of the HIV/AIDS death cases in Guigang city, 74.21% died of HIV-related diseases [19]. In this study, we retrospectively collected the data from the ART cohort in Guigang city, aiming to investigate the association between duration of exposure to ART and elevation of liver function parameters [grade II-IV liver enzyme elevation (LEE) and/or total bilirubin elevation (TBE)] in HIV patients with an initially normal baseline liver function and without HBV/HCV infection.

Methods

Patient and Public Involvement

This retrospective cohort study was conducted in Guigang City, Guangxi, China. All HIV-positive individuals from Guigang People's Hospital or Guigang Centers for Disease Control and Prevention (CDC) were reported to the National Notifiable Disease Monitoring System. Of them, HIV patients whose CD4 count was lower than 350 cells/μL (the standard in 2008 is 200 cells/μL) or at WHO disease stage III or IV, met the Chinese national treatment criteria and were referred for treatment with standard ART. Informed consent was obtained from all participants who willing to be treated.

The individuals receiving ART were followed up at 0.5, 1, 2 and 3 months after ART initiation and then every 3 months by staff of local CDC, with clinical indexes detected. If the ART regimen changed, the follow-up schedule was restarted. The ART information and clinical data were reported to the China's National Free

Antiretroviral Treatment Program (NFATP) of Information System for the Prevention and Control of AIDS.

We retrospectively collected the data during April 14, 2004 to April 13, 2015 from NFATP. Patients were included in this study if they met the following inclusion criteria: treatment-naïve HIV/AIDS patients on ART, aged 16 years old or older, had normal baseline liver function parameters [alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin in serum (TBIL)] at ART initiation, had at least once liver function measurements during the follow up. Patients were excluded if the following conditions occurred: had liver dysfunction (abnormal level of AST, ALT, or TBIL) at ART initiation, HBV and/or HCV positive (HBV infection was diagnosed by a positive HBsAg, HBeAg, HBcAb, or detectable HBV DNA. HCV infection was diagnosed by HCV seropositivity or detectable HCV RNA). All of the HIV/AIDS patients receiving ART in Guigang city were routinely tested for HBV/HCV infection) at baseline or during follow-up, had no baseline CD4 cell count, had none of the three liver function parameters (AST, ALT, TBIL), had no CD4 cell count records during follow-up. Censoring occurrence included lost to follow-up, referral to other hospital, death, discontinuation of ART, or end of the observation period. The study was approved by the Human Research Ethics Committee of Guangxi Medical University (Ethical Review No. 2013-130).

Definitions

For this study, ART was defined as combined use of three or more antiretroviral drugs from any drug class. Baseline was defined as various clinical indexes from the most recent record before or after ART initiation. The upper level of normality (ULN) was defined as AST=40 U/L, ALT=40 U/L, and TBIL=20 μmol/L according to the division of *AIDS Toxicity Guidelines* [20]. Any one index exceeding the ULN was considered as abnormal levels (grade I-IV LEE/TBE). Grade I-IV LEE were defined when elevation reaches 1-2.5, 2.5-5.0, 5.0-10.0, >10.0 times as high as the ULN, respectively; and grade I-IV TBE were defined when elevation reaches 1-1.5, 1.5-2.5, 2.5-5.0, >5.0 times as high as the ULN, respectively (according to the division of

AIDS Toxicity Guidelines [20]). The stages of AIDS progression were defined by CD4 lymphocyte counts: stage I (≥500 cells/μL), stage II (200 - 499 cells/μL), stage III (50 - 199 cells/μL), and stage IV (<50 cells/μL) as recommendation of WHO. Follow-up time for each individual was calculated until the last visit plus 3 months, or last liver function detection date plus 3 months, to allow for report delay.

The eligible patients were divided into two groups according to the occurrence of grade II-IV LEE/TBE during follow-up. The normal hepatic function group included patients who did not have LEE/TBE or had only grade I LEE/TBE, and the liver damage group included the patients who had grade II or III or IV LEE/TBE.

Statistical analysis

Categorical variables were expressed as frequency or proportion. Quantitative variables were described by median ± interquartile range (IQR). Chi-square test or Fisher's test (for categorical variables) and nonparametric test (for quantitative variables) were used to compare the characteristics between normal hepatic function and liver damage groups. The crude incidence rates (incidence densities) of liver damage (per 100 person-years) of different ART duration were calculated in patients sorted by 6-month interval according to their ART duration time. We assumed that there is a linear association between cumulative ART duration and LEE/TBE based on the crude incidence rate. χ^2 linear trend test was conducted for analysis of the change of incidence rates along with the ART duration time. The Cox regression analysis was used to evaluate the related factors for liver damage. The data were analyzed using Statistical Package for the Social Sciences (SPSS) version 23.0 (SPSS Inc. Chicago, USA) and GraphPad Prism version 6.0 (GraphPad Software, San Diego, California, USA).

Results

Demographic characteristics of eligible HIV/AIDS patients at baseline

A total of 4516 patients who initiated ART during April 14, 2004 and April 13, 2015 were enrolled in this study from the Information System for the Prevention and

Control of AIDS in Guigang city. Of them, 2397 patients were excluded, including 185 patients who had none of the three baseline liver function parameters (131) or CD4 cell count record (54), 1209 had abnormal baseline liver function parameters, 114 HBV/HCV positive at baseline, 48 without follow-up, 19 HBV/HCV positive during follow-up, 554 had none of the three liver function parameters record during follow-up, and 268 had no CD4 cell count record during follow-up. Finally, 2119 patients fulfilled inclusion criteria and were included in this study (Figure 1).

The 2119 eligible individuals contributed 6397.13 person years of follow-up, Table 1 shows demographic characteristics of eligible HIV/AIDS patients at baseline. The median age at diagnosis was 47.81 years old (IQR: 34.85-59.54), the median age at initiation of ART was 48.31 years old (IQR: 35.44-59.81). Of the 2119 patients, 61.5% were male, 75.8% were married or cohabitation, 96.6% were residents in Guigang city, and 90.7% acquired HIV heterosexually or homosexually [4.1% acquired HIV by blood/plasma transfusion (82 patients were infected by intravenous drug injection, and 5 patients were infected by blood/plasma transfusion)]. The eligible patients were divided into two groups according to the occurrence of grade II-IV LEE/TBE: 87.59% (1856/2119) belonged to normal hepatic function group (did not have LEE/TBE or had only grade I LEE/TBE), and 12.41% (263/2119) belonged to liver damage group (grade II-IV LEE/TBE). The differences in residence, diagnosis age, ART initiation age, and transmission route between normal hepatic function group and liver damage group were statistically significant (p<0.05) (Table 1).

Table 1. Demographic characteristics of study population at baseline

Characteristics	Total (n=2119)	Liver function parameters (ALT, AST, TBIL) [a]		χ^2	p
		Normal hepatic function group (n=1856)	Liver damage group (n=263)		
Gender				1.13	0.29
Male	1304(61.5%)	1150(62.0%)	154(58.6%)		
Female	815(38.5%)	706(38.0%)	109(41.4%)		
Marital Status				6.92	0.14
Unmarried	215(10.1%)	178(9.6%)	37(14.1%)		

Married/cohabitation	1607(75.8%)	1419(76.5%)	188(71.5%)		
Divorced/separated	51(2.4%)	44(2.4%)	7(2.7%)		
Widowed	236(11.1%)	205(11.0%)	31(11.8%)		
Others	10(0.5%)	10(0.5%)	0(0)		
Residence				13.26 [b]	<0.001
Guigang city	2047(96.6%)	1803(97.1%)	244(92.8%)		
Other cities in Guangxi	67(3.2%)	48(2.6%)	19(7.2%)		
Other provinces	5(0.2%)	5(0.3%)	0(0)		
Age (years) at Diagnosis				6.85	0.03
<40	722(34.1%)	616(33.2%)	106(40.3%)		
40-60	884(41.7%)	777(41.9%)	107(40.7%)		
≥60	513(24.2%)	463(24.9%)	50(19.0%)		
Age (years) at ART Initiation				7.66	0.02
<40	704(33.2%)	600(32.3%)	104(39.5%)		
40-60	890(42.0%)	781(42.1%)	109(41.4%)		
≥60	525(24.8%)	475(25.6%)	50(19.0%)		
Transmission Route				22.33	<0.001
Blood transfusion	87(4.1%)	62(3.3%)	25(9.5%)		
Sexual transmission	1921(90.7%)	1697(91.4%)	224(85.2%)		
Other or unknown	111(5.2%)	97(5.2%)	14(5.3%)		

[a] ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin in serum

[b] Fisher's exact test

Clinical characteristics of study population

Among the eligible 2119 patients, the proportion of patients at four WHO disease stages at baseline was 37.3% (stage I), 17.7% (stage II), 32.0% (stage III), and 12.9% (stage IV), respectively. Baseline median CD4 cell count at ART initiation was 164.0 cells/μL (IQR: 56.0-264.0). Baseline AST, ALT, TBIL was 24.0 U/L, 17.3U/L, 8.3 μmol/L, respectively, and follow-up AST, ALT, TBIL was 24.0 U/L, 20.2U/L, 7.1 μmol/L, respectively. Median CD4 cell count during follow-up was 308.0 cells/μL (IQR: 194.0-441.0). The eligible patients had a median ART duration time of 2.75 years (IQR: 1.46-4.25) (Table 2). Among them, 263 had developed grade II-IV LEE/TBE during follow-up, and made up liver damage group, contributing 4.11/100 person-years crude incidence rate of follow up (95%CI: 3.63-4.60). The remaining

1856 patients did not develop LEE/TBE or only had grade I LEE/TBE during follow-up, and made up normal hepatic function group (Figure 1, Table 1 and 2). The differences in baseline CD4 cell count, baseline AST, current disease stage, current ART regimen, median AST, ALT, TBIL during follow-up, duration of ART between normal hepatic function group and liver damage group were statistically significant (p<0.05).

Table 2. Clinical characteristics of study population

Characteristics	Total (n=2119)	Liver function parameters (ALT, AST, TBIL) [a]		χ^2/Z	p
		Normal hepatic function group (n=1856)	Liver damage group (n=263)		
Baseline Diseases Stage				1.38	0.71
Ⅰ	791(37.3%)	690(37.2%)	101(38.4%)		
Ⅱ	376(17.7%)	329(17.7%)	47(17.9%)		
Ⅲ	679(32.0%)	602(32.4%)	77(29.3%)		
Ⅳ	320(12.9%)	235(12.7%)	38(14.4%)		
Baseline CD4 Count				14.47	0.001
<200 cells/μL	1250(59.0%)	1098(59.2%)	164(57.8%)		
200-350 cells/μL	690(32.6%)	587(31.6%)	113(39.2%)		
≥350 cells/μL	179(8.4%)	171(9.2%)	10(3.0%)		
Baseline AST				10.23	0.02
<20 U/L	475(22.4%)	428(23.1%)	47(17.9%)		
20-30 U/L	1036(50.2%)	937(50.5%)	126(47.9%)		
30-40 U/L	480(22.7%)	410(22.1%)	70(26.6%)		
Missing	101(4.8%)	81(4.4%)	20(7.6%)		
Baseline ALT				3.47	0.33
<20 U/L	1294(61.1%)	1145(61.9%)	149(56.7%)		
20-30 U/L	587(27.7%)	510(27.5%)	77(29.3%)		
30-40 U/L	233(11.0%)	197(10.6%)	36(13.7%)		
Missing	5(0.2%)	4(0.2%)	1(0.4%)		
Baseline TBIL				2.38	0.30
<10 μmol/L	1409(66.5%)	1245(67.1%)	164 (62.4%)		
10-20 μmol/L	693(32.7%)	596(32.1%)	97(36.9%)		
Missing	17(0.8%)	15(0.8%)	2(0.8%)		
Current Disease Stage				12.34	0.01

Ⅰ	1894(89.4%)	1663(89.6%)	231(87.8%)		
Ⅱ	109(5.1%)	100(5.4%)	9(3.4%)		
Ⅲ	83(3.9%)	70(3.8%)	13(4.9%)		
Ⅳ	33(1.6%)	23(1.2%)	10(3.8%)		
Current ART Regimen				49.56	<0.001
3TC+TDF+EFV	750(35.4%)	681(36.7%)	69(26.2%)		
3TC+AZT+NVP	411(19.4%)	383(20.6%)	28(10.6%)		
3TC+D4T+NVP	71(3.4%)	65(3.5%)	6(2.3%)		
3TC+AZT+EFV	361(17.0%)	289(15.6%)	72(27.4%)		
3TC+D4T+EFV	65(3.1%)	56(3.0%)	9(3.4%)		
3TC+TDF+LPV/r	294(13.9%)	240(12.9%)	54(20.5%)		
Other regimens	167(7.9%)	142(7.7%)	25(9.5%)		
Median CD4 Count during Follow-up				0.26	0.88
<200 cells/μL	549(25.9%)	484(26.1%)	65(24.7%)		
200-350 cells/μL	703(33.2%)	612(33.0%)	91(34.6%)		
≥350 cells/μL	867(40.9%)	760(40.8%)	107(40.7%)		
Follow-up AST [b] U/L	24.0(20.0-30.0)	23.7(19.6-29.4)	26.0(21.4-38.8)	13.2 [c]	<0.001
Follow-up ALT [b] U/L	20.2(15.5-27.0)	20.0(15.3-26.0)	24.0(16.5-39.5)	20.7 [c]	<0.001
Follow-up TBIL[b] μmol/L	7.1(5.5-9.1)	7.0(5.4-8.9)	8.4(6.6-10.6)	31.1 [c]	<0.001
During of ART (years) [b]	2.7(1.5-4.2)	2.6(1.4-4.1)	3.8(2.2-5.2)	43.3 [c]	<0.001

[a] ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin in serum

[b] Data are presented as medium ± interquartile range (IQR)

[c] Nonparametric test

Crude incidence rates of grade II-IV liver enzyme elevation/total bilirubin elevation (LEE/TBE) in patients at different ART duration times

With all patients sorted by 6-month interval according to their ART duration time, the crude incidence rates of liver damage (grade II-IV LEE/TBE) (per 100 person-years) in each interval of ART duration were calculated. The liver damage occurred in all patients at different ART duration intervals, and the overall liver damage incidence was 4.11/100 person-years (95%CI: 3.63-4.60). The highest incidence was observed in patients with 6-12 months' ART (15.16/100 person years, 95%CI: 9.37-20.95), and then the incidence decreased to 3.13/100 person years (95%CI: 1.24-5.03) in patients with 18-24 months' ART. There is a decreasing trend of incidence rate of liver damage

along with the ART duration intervals in patients with 0.5 to 2 years of ART (χ^2 linear trend test, x^2=5.43, p=0.2). The incidence rate then maintained at a relatively low and stable level in patients who had longer ART duration time (≥ 2 years) (χ^2 linear trend test, χ^2=35.22, p<0.001), with an average of 3.65/100 person years (95%CI: 3.63–4.60) (Figure 2).

Related factors for grade II-IV liver enzyme elevations/total bilirubin elevation (LEE/TBE)

In order to identify related factors for liver damage (grade II-IV LEE/TBE), the data of all 2119 eligible patients were included in the Cox regression analysis. Univariable analysis were performed firstly, and then variables that were statistically significant (p<0.05) were included in a multivariate Cox regression analysis. As shown in Table 3, patients at initial WHO disease stage II (AHR=0.49, 95% CI: 0.34-0.70, p<0.001), or III (AHR=0.46, 95% CI: 0.33-0.64, p<0.001) were less likely to occur liver damage compared with patients at initial WHO disease stage I. Compared with current use of 3TC+TDF+EFV, the current regimens 3TC+AZT+NVP had lower risk to develop liver damage (AHR=0.27, 95% CI: 0.19-0.41). Except the above factors that could decrease the risk of LEE/TBE, current WHO disease stage II, III and IV (stage II, AHR=2.07, 95% CI: 1.04-4.13; stage III, AHR=3.90, 95% CI: 2.10-7.27; stage IV, AHR=3.36, 95%CI: 1.76-6.43, compared with stage I, respectively) had higher risk to occur liver damage (Table 3).

Table 3. Factors associated with liver damage among HIV/AIDS patients on ART

Variables	Total Patients (n)	Liver damage (n)	Person-Years	Liver damage per 100 person year	HR (95% CI)	*p*	AHR (95% CI) [a]	*p*
Gender								
Male	1304	154	3800.40	4.05	1	-		
Female	815	109	2596.74	4.20	0.94(0.74-1.21)	0.65		
Marital Status						0.08		
Unmarried	215	37	614.04	6.03	1	-		
Married/cohabitati	1607	188	4875.47	3.86	0.60(0.42-0.85)	0.01		

on								
Divorced/separated	51	7	170.18	4.11	0.63(0.28-1.41)	0.26		
Widowed	236	31	705.51	4.39	0.73(0.45-1.18)	0.20		
Others	10	0	31.95	0.00	0.00(-)	0.93		
Residence						0.50		
Guigang city	2047	244	6084.09	4.01	1	-		
Other cities in Guangxi	67	19	298.12	6.37	0.72(0.41-1.25)	0.24		
Other provinces	5	0	14.92	0.00	0.00(-)	0.93		
Age (years) at Diagnosis						0.95		
<40	722	106	2442.31	4.34	1	-		
40-60	884	107	2570.05	4.16	1.02(0.78-1.33)	0.90		
≥60	513	50	1384.78	3.61	1.06(0.75-1.48)	0.75		
Age (years) at ART Initiation						0.97		
<40	704	104	2397.52	4.34	1	-		
40-60	890	109	2585.24	4.22	1.03(0.79-1.35)	0.82		
≥60	525	50	1414.38	3.54	1.03(0.34-1.45)	0.86		
Transmission Route						0.44		
Blood transfusion	87	25	360.66	6.93	1	-		
Sexual transmission	1921	224	5682.10	3.94	0.77(0.50-1.16)	0.21		
Others	111	14	354.38	3.95	0.74(0.38-1.42)	0.36		
Baseline Disease Stage						<0.001		<0.001
I	791	101	2065.67	4.89	1	-	1	-
II	376	47	1288.25	3.65	0.55(0.39-0.78)	0.001	0.49(0.34-0.70)	<0.001
III	679	77	2283.76	3.37	0.46(0.34-0.62)	<0.001	0.46(0.33-0.64)	<0.001
IV	320	38	759.45	5.00	0.69(0.47-1.01)	0.06	0.68(0.45-1.04)	0.07
Baseline CD4 count						0.02		0.05
<200 cells/μL	1250	152	4014.64	3.79	1	-	1	-
200-350 cells/μL	690	103	2093.44	4.92	1.45(1.13-1.86)	0.004	1.42(1.07-1.89)	0.02
≥350 cells/μL	179	8	289.06	2.77	1.30(0.63-2.67)	0.48	1.03(0.49-2.14)	0.95
Baseline AST						0.30		
<20 U/L	475	47	1360.81	3.45	1	-		
20-30 U/L	1036	126	3167.70	3.98	1.14(0.82-1.60)	0.44		
30-40 U/L	480	70	1543.79	4.53	1.20(0.83-1.74)	0.34		
Missing	101	20	324.83	6.16	1.66(0.98-2.81)	0.06		
Baseline ALT						0.69		
<20 U/L	1294	149	3914.47	3.81	1	-		
20-30 U/L	587	77	1707.76	4.51	1.15(0.88-1.52)	0.31		
30-40 U/L	233	36	748.83	4.81	1.14(0.79-1.64)	0.49		
Missing	5	1	26.08	3.83	0.64(0.09-4.57)	0.65		

Baseline TBIL						0.52		
<10 μmol/L	1409	164	4166.89	3.94	1	-		
10-20 μmol/L	693	97	2165.24	4.48	1.15(0.89-1.45)	0.28		
Missing	17	2	65.01	3.08	0.82(0.20-3.32)	0.78		
Current Disease Stage						<0.001		<0.001
I	1894	231	5994.86	3.85	1	-	1	-
II	109	9	204.03	4.41	1.81 (0.93-3.54)	0.08	2.07(1.04-4.13)	0.04
III	83	13	133.19	9.76	4.30(2.44-7.57)	<0.001	3.90(2.10-7.27)	<0.001
IV	33	10	65.05	15.37	3.86(2.05-7.28)	<0.001	3.36(1.76-6.43)	<0.001
Current ART Regimen						<0.001		<0.001
3TC+TDF+EFV	750	69	1757.18	3.93	1	-	1	-
3TC+AZT+NVP	411	28	1710.75	1.64	0.27(0.18-0.43)	<0.001	0.27(0.19-0.41)	<0.001
3TC+D4T+NVP	71	6	164.18	3.65	1.21(0.52-2.79)	0.66	1.04(0.44-2.49)	0.93
3TC+AZT+EFV	361	72	1182.47	6.09	1.35(0.97-1.88)	0.08	1.23(0.88-1.72)	0.23
3TC+D4T+EFV	65	9	122.93	7.32	2.87(1.43-5.77)	0.003	1.88(0.89-3.97)	0.10
3TC+TDF+LVP/r	294	54	964.17	5.60	1.14(0.79-1.62)	0.49	1.13(0.79-1.62)	0.50
Other regimens	167	25	495.47	5.05	1.23(0.78-1.94)	0.38	1.15(0.73-1.83)	0.55
Median CD4 Count during Follow-up						0.05		
<200 cells/μL	549	65	1396.45	4.65	1	-		
200-350 cells/μL	703	91	2234.90	4.07	0.75(0.54-1.03)	0.86		
≥350 cells/μL	867	107	2765.79	3.87	0.68(0.50-0.93)	0.01		

Abbreviations: HR, hazard ratio; AHR, adjusted hazard ratio, ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin in serum.

[a] Covariates of the adjusted model included gender, residence, baseline disease stage, baseline CD4 counts, baseline AST and ALT, current disease stage, current ART regimen, and CD4 count during follow-up.

Discussion

This retrospective cohort study was conducted on HIV-positive individuals with an initially normal hepatic function and without HBV/HCV infection. We found that liver damage (grade II-IV LEE/TBE) occurred in all patients at different period of ART, indicating that hepatic damage always exists among HIV/AIDS patients receiving ART, which has been shown in a few previous studies [4,5], although the incidence rate of LEE/TBE in our study was quite stable and low in the longer ART during times. Our study showed that the liver damage prevalence was 12.41% (263/2119) (Figure 1), which is lower than those (14%-23%) in several previous

studies conducted on HIV/AIDS patients [21,22]. In addition, the overall liver damage incidence (4.11/100 person-years, 95%CI: 3.63-4.60) in our study is also lower than that (6.04/100 person-years of chronic LEE incidence) in a similar study on HIV-monoinfected persons by Kovari et al [23]. One possible reason for lower prevalence and overall incidence of liver damage in our study is that we used the grade II-IV LEE/TBE to define liver dysfunction, which is a higher threshold to define liver dysfunction compared to the similar research in several papers [20], whereas others might use different definition of LEE [12,23].

The highest liver damage incidence was observed in patients with 6-12 months' ART duration (11.56/100 person years). The incidence rapidly decreased in patients with 2 years of ART and then maintained at a relatively lower level (Figure 2). These findings are consistent with quite a few previous studies on liver toxicity caused by various antiretroviral drugs such as NVP, EFV, TDF, which also observed a strong association between drugs and the development of LEE emerging within the first 2 years after drug initiation [23]. The significant decreasing trend of incidence rate of liver damage between 0.5 to 2 years of ART duration might be resulted from a number of adaptation mechanisms which are initiated to counteract the inflicted damage [24]. Importantly, the above findings indicate that cumulative ART time does not increase the risk of liver damage, which is of great clinical importance to support the strategy of WHO to increase the number of HIV/AIDS patients on ART [25], and the strategy has been shown to greatly reduce rate of sexual transmission of HIV in several recent HIV Prevention Trails [26,27]. Meanwhile, antiretroviral examined are no longer recommended by international guidelines, current evidence favors improved safety hepatic profile for IN strand transfer inhibitors (INSTIs), which was confirmed hepatic safety [28].

Several related factors for liver damage were observed in the present study (Table 3). Patients currently at stage II, III or IV had higher risk to develop liver damage compared with patients in stage I, indicating the level of immunodeficiency and the host immunity involve in liver damage upon ART. These results are consistent with some previous studies. For example, the risk of nevirapine-induced hepatitis increases

12-fold in women with more than 250 CD4 cells/μL and 5-fold in males with more than 400 CD4 cells/μL (disease stage II) [29].

In this study, we also found that patients at initial disease stage II, III were less likely to develop liver damage compared with patients at initial disease stage I (Table 3). Possible reason is that the patients with more severe symptom might be more likely to take some positive measures, such as improved adherence, healthier lifestyle, than patients who had normal liver function. However, our results didn't show there is relationship between CD4 cell count (including baseline and follow-up) and liver damage. The results are consistent with some studies but inconsistent with some other studies, for example, one study showed lower risk of hepatic dysfunction come along with higher CD4 cell count in individuals on ART [8]. In fact, contradicting results were reported by different researchers in term of relationship of CD4 cell count and liver dysfunction, which may be related to the different ART regimens investigated as well as some other factors that might affect host immune status when patients were on ART [21].

Our study also showed that patients with current regiment 3TC+AZT+NVP had lower risk to develop liver damage (grade II-IV LEE/TBE) compared with regiment 3TC+TDF+EFV. The liver toxicity of TDF and EFV has been previously described in several studies [23,24]. Since 3TC+TDF+EFV is currently considered as one of first-line regimens, the importance of combination of liver protection may need to be stressed for this regimen.

Conclusions

In this study, although patients with abnormal baseline liver function and with HBV/HCV infection had been excluded, the incidence of liver damage (grade II-IV LEE/TBE) still occurred among HIV/AIDS patients on ART, indicating liver damage always exists among HIV-infected patients on ART. Nevertheless, cumulative ART duration does not increase the risk of liver damage. Therefore, ART could tend to be long-term, however, monitoring and management of liver damage among patients on ART are also important in clinical therapy.

Competing interests

All authors declare that they have no conflict of interest.

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Authors' contribution

HL, XQ and LY conceived and designed the study. FQ, JJ conducted the data analysis, literature review, and drafted the manuscript. CQ, YH, BL, and YX were involved in the study supervision, data collection, and interpretation of the data. JH, ZX, CN, YL, NZ, JL, WW, and JY assisted with data management and data analysis. All authors contributed to the revision of the manuscript and approved the final version.

Data sharing

No additional data available.

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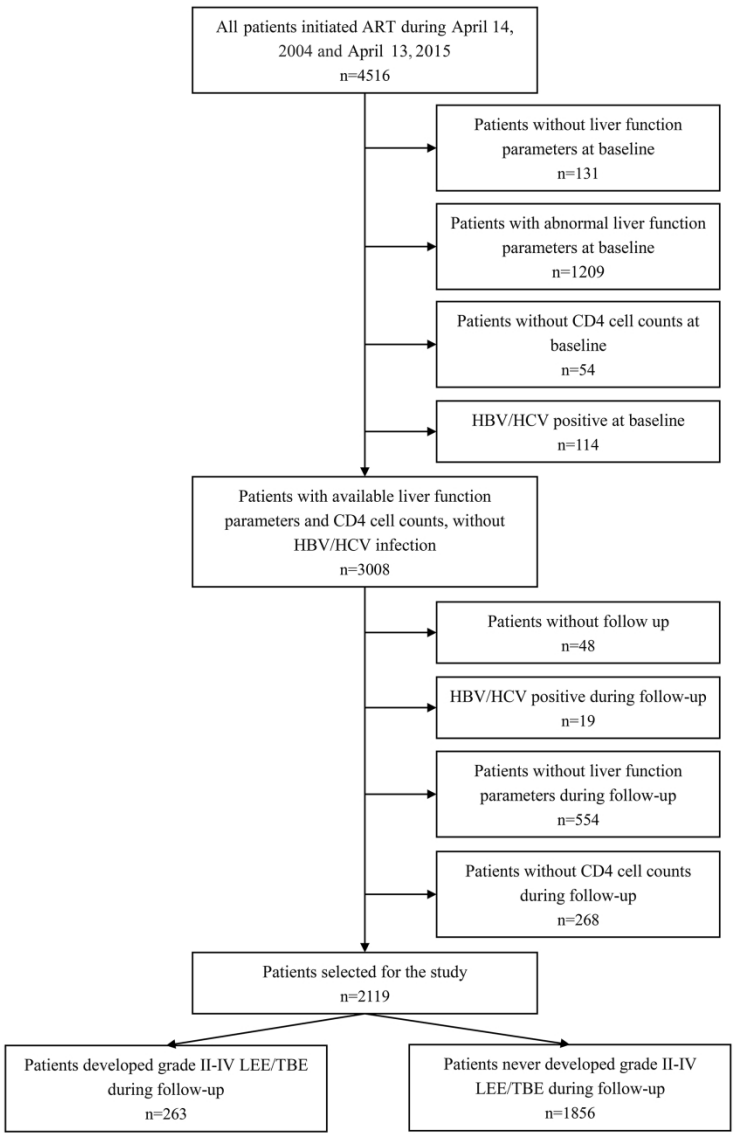

Figure1. Patients Flowchart

Figure1. Patients Flowchart

168x270mm (300 x 300 DPI)

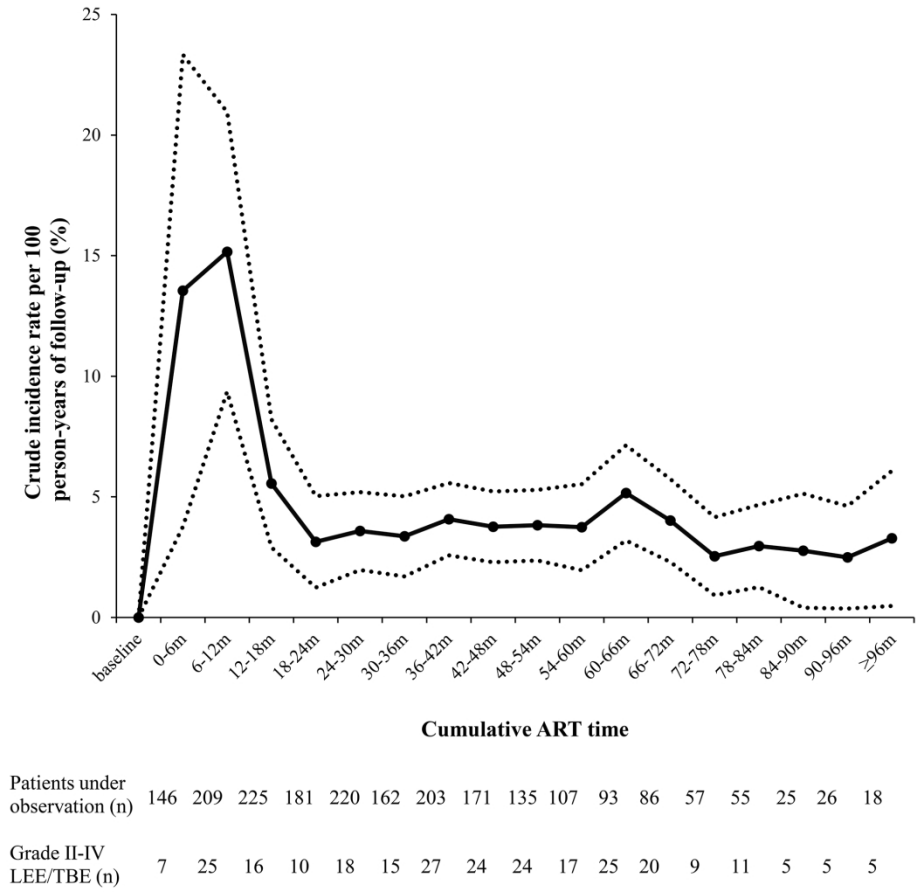

Figure 2. Crude incidence rates of liver damage [grade II-IV liver enzyme elevation (LEE) / total bilirubin elevation(TBE)] with cumulative ART time.

Figure 2. Crude incidence rates of liver damage [grade II-IV liver enzyme elevation (LEE) / total bilirubin elevation(TBE)] with cumulative.

191x202mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **Page1-2**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **Page 2**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **Page 4**
Objectives	3	State specific objectives, including any prespecified hypotheses **Page 5**
Methods		
Study design	4	Present key elements of study design early in the paper **Page 5-6**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **Page 5-6**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **Page 6-7** *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls **Not applicable** *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants **Not applicable**
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed **Not applicable** *Case-control study*—For matched studies, give matching criteria and the number of controls per case **Not applicable**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **Page 6-7**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **Page 5-7**
Bias	9	Describe any efforts to address potential sources of bias **Page 5**
Study size	10	Explain how the study size was arrived at **Page 5-6**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **Page 6-7**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **Page 7**
		(*b*) Describe any methods used to examine subgroups and interactions **Not applicable**
		(*c*) Explain how missing data were addressed **Not applicable**
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed **Not applicable** *Case-control study*—If applicable, explain how matching of cases and controls was addressed **Not applicable** *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy **Not applicable**

		(*e*) Describe any sensitivity analyses	**Not applicable**
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	**Page 7-8**
		(b) Give reasons for non-participation at each stage	**Page 7-8**
		(c) Consider use of a flow diagram	**Figure 1**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	**Page 8-11**
		(b) Indicate number of participants with missing data for each variable of interest	**Not applicable**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	**Page 8-11**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	**Page 11-12, Figure 2**
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	**Not applicable**
		Cross-sectional study—Report numbers of outcome events or summary measures	**Not applicable**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	**Page 12-14**
		(*b*) Report category boundaries when continuous variables were categorized	**Page 8-11**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	**Not applicable**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	**Not applicable**
Discussion			
Key results	18	Summarise key results with reference to study objectives	**Page 14-15**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	**Page 3**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**Page 15-16**
Generalisability	21	Discuss the generalisability (external validity) of the study results	**Page 16**
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	**Page 17**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at

http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Liver damage in patients living with HIV on antiretroviral treatment with normal baseline liver function and without HBV/HCV infection: an 11-year retrospective cohort study in Guangxi, China

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Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	Epidemiology
Keywords:	HIV, antiretroviral treatment, liver enzyme elevation, total bilirubin elevation

Liver damage in patients living with HIV on antiretroviral treatment with normal baseline liver function and without HBV/HCV infection: an 11-year retrospective cohort study in Guangxi, China

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Abstract

Objective: To characterize the association between duration of exposure to ART and liver damage in HIV patients with an initially normal baseline liver function and without HBV/HCV infection.

Methods: A retrospective cohort study was conducted in HIV-infected individuals with normal liver function parameters at ART initiation and without HBV/HCV infection, from April 14, 2004 to April 13, 2015 in Guigang city, Guangxi, China. The association between duration of ART and liver damage [grade II-IV liver enzyme elevation (LEE) and/or total bilirubin elevation (TBE)], was analyzed. Cox regression was used to examine the factors related to liver damage.

Results: Of 2119 eligible patients, 12.41% (263/2119) developed liver damage (grade II-IV LEE/TBE) and contributed 4.11/100 person-years crude incidence rate. The highest liver damage incidence was observed in patients with 6-12 months' ART (15.16/100 person years). The incidence decreased to 5.56/100 person years in patients with 12-18 months' ART and 3.13/100 person years in patients with 18-24 months' ART, and then maintained at a relatively low and stable level in patients with two years' ART or longer (average of 3.65/100 person years). Cox regression analysis revealed that current WHO disease stage II, III or IV (compared with stage I) were the risk factors for liver damage, while baseline disease stage II, III (compared with stage I) and current regimen 3TC+AZT+NVP were the protective factors for liver damage.

Conclusions: Liver damage always exists among HIV-infected patients on ART with normal baseline liver function and without HBV/HCV infection. Nevertheless, cumulative ART duration does not increase the risk of liver damage. ART could tend to be long-term, however, monitoring and management of liver damage among patients on ART are also important in clinical therapy.

Keywords: HIV, antiretroviral treatment, liver enzyme elevation, total bilirubin elevation

Strengths and limitations of this study

1. The study retrospectively collected 11-year records of HIV/AIDS patients on ART and involved in larger samples than several other similar studies.

2. Cumulative ART duration does not increase the risk of liver damage.

3. Monitoring and management of liver damage among patients on ART are still important in clinical therapy.

4. Confounding factors such as smoking, alcohol consumption, and other opportunistic infections besides HBV/HCV could not be estimated and ruled out.

5. Lack of information about further progression of liver diseases such as hepatocarcinoma and liver dysfunction-related mortality limits our findings to be associated with clinical outcomes.

Introduction

Antiretroviral treatment (ART) has significantly reduced morbidity and mortality in persons living with human immunodeficiency virus (HIV) worldwide [1]. An estimated 19.5 million people globally had received ART by 2016 [2]. With the implementation of ART, the life expectancy of HIV-infected individuals is now approaching that of the general population [3]. However, ART has some adverse effects, especially hepatic damage, which comes along with the treatment [4]. An earlier study showed that the prevalence of liver transaminase elevation among HIV-positive individuals on ART ranged from 14% to 20% [5]. Consistently, some researchers have found the incidence of hepatic injury in ART-treated patients was increased [4]. However, quite a few other studies indicated that the approved antiretroviral agents have low liver toxicity and generally are considered to be well tolerated [6,7].

Mechanisms of liver damage among HIV-1 infected patients are multiple, probably attributing to HIV infection itself [8], hepatitis viral co-infections, ART-related hepatotoxicity [9], acquired immune deficiency syndrome (AIDS) related neoplasm [10], or experiencing age-related co-morbid conditions [11]. Hepatitis viral infections, either hepatitis B virus (HBV) or hepatitis C virus (HCV), have been reported to lead to hepatotoxicity [12,13]. Elevated hepatotoxicity was observed to be associated with ART in HIV/AIDS patients co-infected with HBV or HCV [4,12]. ART-related hepatotoxicity was also reported in quite a few previous studies [6,11,13]. Furthermore, didanosine (DDI) is no longer recommended as the first-line antiretroviral drugs for HIV patients because of its hepatotoxicity [14]. Although there are many studies focusing on relationship of ART and liver function amongst HIV-1 infected patients, after extended exposure to antiretroviral therapy, whether the association between ART and hepatic dysfunction remains true is unclear. Moreover, even though most of the previous studies were controlled for baseline liver enzymes level [5,7-9], there were still some studies had no initial liver function [15] or co-infection with viral hepatitis [4,6,12] reported. Since ART generally lasts for many years and even lifelong, the effect of long-term ART on liver function needs to be elucidated, which is crucial to ascertain whether the risks of hepatic impairment are self-limiting, and to help to make better clinical decision and monitoring.

Guangxi Zhuang Autonomous Region, a province in western China, has the second highest HIV-infected reported cases in China, with more than 100,000 reported HIV/AIDS cases by the end of 2016 [16], which accounting for ~13% of total national HIV/AIDS cases. Guigang city is a prefecture-level city in Guangxi, and there were more than 5000 reported HIV/AIDS cases during 2008 to 2013, which listed in the top five among all cities in Guangxi [17]. Previous studies have shown that most of HIV/AIDS patients in Guigang city had receive ART. However, the mortality reached 6.13%, which is higher than those in other places such as South Africa during 2004~2013 [18]. Of the HIV/AIDS death cases in Guigang city, 74.21% died of HIV-related diseases [19]. In this study, we retrospectively collected the data from the ART cohort in Guigang city, aiming to investigate the association between duration of exposure to ART and elevation of liver function parameters [grade II-IV liver enzyme elevation (LEE) and/or total bilirubin elevation (TBE)] in HIV patients with an initially normal baseline liver function and without HBV/HCV infection.

Methods

Study site and population

This retrospective cohort study was conducted in Guigang City, Guangxi, China. All HIV-positive individuals from Guigang People's Hospital or Guigang Centers for Disease Control and Prevention (CDC) were reported to the National Notifiable Disease Monitoring System. Of them, HIV patients whose CD4 cell count was lower than 350 cells/μL (the standard in 2008 was changed to 200 cells/μL) or at WHO disease stage III or IV, met the Chinese national treatment criteria and were referred for treatment with standard ART. Informed consent was obtained from all participants who were willing to be treated.

The individuals receiving ART were followed up at 0.5, 1, 2 and 3 months after ART initiation and then every 3 months by staff of local CDC, with clinical indexes detected. If the ART regimen changed, the follow-up schedule was restarted. The ART information and clinical data were reported to the China's National Free Antiretroviral Treatment Program (NFATP) of Information System for the Prevention and Control of

AIDS.

We retrospectively collected the data during April 14, 2004 to April 13, 2015 from NFATP. Patients were included in this study if they met the following inclusion criteria: treatment-naïve HIV/AIDS patients on ART, aged 16 years old or older, had normal baseline liver function parameters [alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin in serum (TBIL)] at ART initiation, had at least once liver function measurements during the follow up. Patients were excluded if the following conditions occurred: had liver dysfunction (abnormal level of AST, ALT, or TBIL) at ART initiation, HBV and/or HCV positive (HBV infection was diagnosed by a positive HBsAg, HBeAg, HBcAb, or detectable HBV DNA. HCV infection was diagnosed by HCV seropositivity or detectable HCV RNA). All of the HIV/AIDS patients receiving ART in Guigang city were routinely tested for HBV/HCV infection) at baseline or during follow-up, had no baseline CD4 cell count, had none of the three liver function parameters (AST, ALT, TBIL), had no CD4 cell count records during follow-up. Censoring occurrence included lost to follow-up, referral to other hospital, death, discontinuation of ART, or end of the observation period. The study was approved by the Human Research Ethics Committee of Guangxi Medical University (Ethical Review No. 2013-130).

Definitions

For this study, ART was defined as combined use of three or more antiretroviral drugs from any drug class. Baseline was defined as various clinical indexes from the most recent record before or after ART initiation. The upper level of normality (ULN) was defined as AST=40 U/L, ALT=40 U/L, and TBIL=20 μmol/L according to the division of *AIDS Toxicity Guidelines* [20]. Any one index exceeding the ULN was considered as abnormal levels (grade I-IV LEE/TBE). Grade I-IV LEE were defined when elevation reaches 1-2.5, 2.5-5.0, 5.0-10.0, >10.0 times as high as the ULN, respectively; and grade I-IV TBE were defined when elevation reaches 1-1.5, 1.5-2.5, 2.5-5.0, >5.0 times as high as the ULN, respectively (according to the division of *AIDS Toxicity Guidelines* [20]). The stages of AIDS progression were defined by CD4 lymphocyte counts: stage I

(≥500 cells/μL), stage II (200 - 499 cells/μL), stage III (50 - 199 cells/μL), and stage IV (<50 cells/μL) as recommendation of WHO. Follow-up time for each individual was calculated until the last visit plus 3 months, or last liver function detection date plus 3 months, to allow for report delay.

The eligible patients were divided into two groups according to the occurrence of grade II-IV LEE/TBE during follow-up. The normal hepatic function group included patients who did not have LEE/TBE or had only grade I LEE/TBE, and the liver damage group included the patients who had grade II or III or IV LEE/TBE.

Statistical analysis

Categorical variables were expressed as frequency or proportion. Quantitative variables were described by median ± interquartile range (IQR). Chi-square test or Fisher's test (for categorical variables) and nonparametric test (for quantitative variables) were used to compare the characteristics between normal hepatic function and liver damage groups. The crude incidence rates (incidence densities) of liver damage (per 100 person-years) of different ART duration were calculated in patients sorted by 6-month interval according to their ART duration time. We assumed that there is a linear association between cumulative ART duration and LEE/TBE based on the crude incidence rate. χ^2 linear trend test was conducted for analysis of the change of incidence rates along with the ART duration time. The Cox regression analysis was used to evaluate the related factors for liver damage. The data were analyzed using Statistical Package for the Social Sciences (SPSS) version 23.0 (SPSS Inc. Chicago, USA) and GraphPad Prism version 6.0 (GraphPad Software, San Diego, California, USA).

Patient and Public Involvement

The patients and public did not involve in study design or conduct of the study. We just simply extracted data from records of NFATP of Information System for the Prevention and Control of AIDS.

Results

Demographic characteristics of eligible HIV/AIDS patients at baseline

A total of 4516 patients who initiated ART during April 14, 2004 and April 13, 2015 were enrolled in this study from the Information System for the Prevention and Control of AIDS in Guigang city. Of them, 2397 patients were excluded, including 185 patients who had none of the three baseline liver function parameters (131) or CD4 cell count record (54), 1209 had abnormal baseline liver function parameters, 114 HBV/HCV positive at baseline, 48 without follow-up, 19 HBV/HCV positive during follow-up, 554 had none of the three liver function parameters record during follow-up, and 268 had no CD4 cell count record during follow-up. Finally, 2119 patients fulfilled inclusion criteria and were included in this study (Figure 1).

The 2119 eligible individuals contributed 6397.13 person years of follow-up, Table 1 shows demographic characteristics of eligible HIV/AIDS patients at baseline. The median age at diagnosis was 47.81 years old (IQR: 34.85-59.54), the median age at initiation of ART was 48.31 years old (IQR: 35.44-59.81). Of the 2119 patients, 61.5% were male, 75.8% were married or cohabitation, 96.6% were residents in Guigang city, and 90.7% acquired HIV heterosexually or homosexually [4.1% acquired HIV by blood/plasma transfusion (82 patients were infected by intravenous drug injection, and 5 patients were infected by blood/plasma transfusion)]. The eligible patients were divided into two groups according to the occurrence of grade II-IV LEE/TBE: 87.59% (1856/2119) belonged to normal hepatic function group (did not have LEE/TBE or had only grade I LEE/TBE), and 12.41% (263/2119) belonged to liver damage group (grade II-IV LEE/TBE). The differences in residence, diagnosis age, ART initiation age, and transmission route between normal hepatic function group and liver damage group were statistically significant (p<0.05) (Table 1).

Table 1. Demographic characteristics of study population at baseline

Characteristics	Total (n=2119)	Liver function parameters (ALT, AST, TBIL) [a]		χ^2	p
		Normal hepatic function group (n=1856)	Liver damage group (n=263)		
Gender				1.13	0.29

Male	1304(61.5%)	1150(62.0%)	154(58.6%)		
Female	815(38.5%)	706(38.0%)	109(41.4%)		
Marital Status				6.92	0.14
Unmarried	215(10.1%)	178(9.6%)	37(14.1%)		
Married/cohabitation	1607(75.8%)	1419(76.5%)	188(71.5%)		
Divorced/separated	51(2.4%)	44(2.4%)	7(2.7%)		
Widowed	236(11.1%)	205(11.0%)	31(11.8%)		
Others	10(0.5%)	10(0.5%)	0(0)		
Residence				13.26 [b]	<0.001
Guigang city	2047(96.6%)	1803(97.1%)	244(92.8%)		
Other cities in Guangxi	67(3.2%)	48(2.6%)	19(7.2%)		
Other provinces	5(0.2%)	5(0.3%)	0(0)		
Age (years) at Diagnosis				6.85	0.03
<40	722(34.1%)	616(33.2%)	106(40.3%)		
40-60	884(41.7%)	777(41.9%)	107(40.7%)		
≥60	513(24.2%)	463(24.9%)	50(19.0%)		
Age (years) at ART Initiation				7.66	0.02
<40	704(33.2%)	600(32.3%)	104(39.5%)		
40-60	890(42.0%)	781(42.1%)	109(41.4%)		
≥60	525(24.8%)	475(25.6%)	50(19.0%)		
Transmission Route				22.33	<0.001
Blood transfusion	87(4.1%)	62(3.3%)	25(9.5%)		
Sexual transmission	1921(90.7%)	1697(91.4%)	224(85.2%)		
Other or unknown	111(5.2%)	97(5.2%)	14(5.3%)		

[a] ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin in serum

[b] Fisher's exact test

Clinical characteristics of study population

Among the eligible 2119 patients, the proportion of patients at four WHO disease stages at baseline was 37.3% (stage I), 17.7% (stage II), 32.0% (stage III), and 12.9% (stage IV), respectively. Baseline median CD4 cell count at ART initiation was 164.0 cells/μL (IQR: 56.0-264.0). Baseline AST, ALT, TBIL was 24.0 U/L, 17.3 U/L, 8.3 μmol/L, respectively, and follow-up AST, ALT, TBIL was 24.0 U/L, 20.2U/L, 7.1 μmol/L, respectively. Median CD4 cell count during follow-up was 308.0 cells/μL (IQR: 194.0-

441.0). The eligible patients had a median ART duration time of 2.75 years (IQR: 1.46-4.25) (Table 2). Among them, 263 had developed grade II-IV LEE/TBE during follow-up, and made up liver damage group, contributing 4.11/100 person-years crude incidence rate of follow up (95%CI: 3.63-4.60). The remaining 1856 patients did not develop LEE/TBE or only had grade I LEE/TBE during follow-up, and made up normal hepatic function group (Figure 1, Table 1 and 2). The differences in baseline CD4 cell count, baseline AST, current disease stage, current ART regimen, median AST, ALT, TBIL during follow-up, duration of ART between normal hepatic function group and liver damage group were statistically significant (p<0.05).

Table 2. Clinical characteristics of study population

Characteristics	Total (n=2119)	Liver function parameters (ALT, AST, TBIL) [a]		χ^2/Z	p
		Normal hepatic function group (n=1856)	Liver damage group (n=263)		
Baseline Diseases Stage				1.38	0.71
Ⅰ	791(37.3%)	690(37.2%)	101(38.4%)		
Ⅱ	376(17.7%)	329(17.7%)	47(17.9%)		
Ⅲ	679(32.0%)	602(32.4%)	77(29.3%)		
Ⅳ	320(12.9%)	235(12.7%)	38(14.4%)		
Baseline CD4 cell Count				14.47	0.001
<200 cells/μL	1250(59.0%)	1098(59.2%)	164(57.8%)		
200-350 cells/μL	690(32.6%)	587(31.6%)	113(39.2%)		
≥350 cells/μL	179(8.4%)	171(9.2%)	10(3.0%)		
Baseline AST				10.23	0.02
<20 U/L	475(22.4%)	428(23.1%)	47(17.9%)		
20-30 U/L	1036(50.2%)	937(50.5%)	126(47.9%)		
30-40 U/L	480(22.7%)	410(22.1%)	70(26.6%)		
Missing	101(4.8%)	81(4.4%)	20(7.6%)		
Baseline ALT				3.47	0.33
<20 U/L	1294(61.1%)	1145(61.9%)	149(56.7%)		
20-30 U/L	587(27.7%)	510(27.5%)	77(29.3%)		
30-40 U/L	233(11.0%)	197(10.6%)	36(13.7%)		
Missing	5(0.2%)	4(0.2%)	1(0.4%)		
Baseline TBIL				2.38	0.30
<10 μmol/L	1409(66.5%)	1245(67.1%)	164 (62.4%)		

10-20 μmol/L	693(32.7%)	596(32.1%)	97(36.9%)		
Missing	17(0.8%)	15(0.8%)	2(0.8%)		
Current Disease Stage				12.34	0.01
Ⅰ	1894(89.4%)	1663(89.6%)	231(87.8%)		
Ⅱ	109(5.1%)	100(5.4%)	9(3.4%)		
Ⅲ	83(3.9%)	70(3.8%)	13(4.9%)		
Ⅳ	33(1.6%)	23(1.2%)	10(3.8%)		
Current ART Regimen				49.56	<0.001
3TC+TDF+EFV	750(35.4%)	681(36.7%)	69(26.2%)		
3TC+AZT+NVP	411(19.4%)	383(20.6%)	28(10.6%)		
3TC+D4T+NVP	71(3.4%)	65(3.5%)	6(2.3%)		
3TC+AZT+EFV	361(17.0%)	289(15.6%)	72(27.4%)		
3TC+D4T+EFV	65(3.1%)	56(3.0%)	9(3.4%)		
3TC+TDF+LPV/r	294(13.9%)	240(12.9%)	54(20.5%)		
Other regimens	167(7.9%)	142(7.7%)	25(9.5%)		
Median CD4 cell Count during Follow-up				0.26	0.88
<200 cells/μL	549(25.9%)	484(26.1%)	65(24.7%)		
200-350 cells/μL	703(33.2%)	612(33.0%)	91(34.6%)		
≥350 cells/μL	867(40.9%)	760(40.8%)	107(40.7%)		
Follow-up AST (U/L) [b]	24.0(20.0-30.0)	23.7(19.6-29.4)	26.0(21.4-38.8)	13.2 [c]	<0.001
Follow-up ALT (U/L) [b]	20.2(15.5-27.0)	20.0(15.3-26.0)	24.0(16.5-39.5)	20.7 [c]	<0.001
Follow-up TBIL (μmol/L) [b]	7.1(5.5-9.1)	7.0(5.4-8.9)	8.4(6.6-10.6)	31.1 [c]	<0.001
During of ART (years) [b]	2.7(1.5-4.2)	2.6(1.4-4.1)	3.8(2.2-5.2)	43.3 [c]	<0.001

[a] ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin in serum

[b] Data are presented as median ± interquartile range (IQR)

[c] Nonparametric test

Crude incidence rates of grade II-IV liver enzyme elevation/total bilirubin elevation (LEE/TBE) in patients at different ART duration times

With all patients sorted by 6-month interval according to their ART duration time, the crude incidence rates of liver damage (grade II-IV LEE/TBE) (per 100 person-years) in each interval of ART duration were calculated. The liver damage occurred in all patients at different ART duration intervals, and the overall liver damage incidence was 4.11/100 person-years (95%CI: 3.63-4.60). The highest incidence was observed in patients with 6-12 months' ART (15.16/100 person years, 95%CI: 9.37-20.95), and

then the incidence decreased to 3.13/100 person years (95%CI: 1.24-5.03) in patients with 18-24 months' ART. There is a decreasing trend of incidence rate of liver damage along with the ART duration intervals in patients with 0.5 to 2 years of ART (χ^2 linear trend test, χ^2=5.43, p=0.2). The incidence rate then maintained at a relatively low and stable level in patients who had longer ART duration time (≥ 2 years) (χ^2 linear trend test, χ^2=35.22, p<0.001), with an average of 3.65/100 person years (95%CI: 3.63–4.60) (Figure 2).

Related factors for grade II-IV liver enzyme elevations/total bilirubin elevation (LEE/TBE)

In order to identify related factors for liver damage (grade II-IV LEE/TBE), the data of all 2119 eligible patients were included in the Cox regression analysis. Univariable analysis were performed firstly, and then variables that were statistically significant (p<0.05) were included in a multivariate Cox regression analysis. As shown in Table 3, patients at initial WHO disease stage II (AHR=0.49, 95% CI: 0.34-0.70, p<0.001), or III (AHR=0.46, 95% CI: 0.33-0.64, p<0.001) were less likely to occur liver damage compared with patients at initial WHO disease stage I. Compared with current use of 3TC+TDF+EFV, the current regimens 3TC+AZT+NVP had lower risk to develop liver damage (AHR=0.27, 95% CI: 0.19-0.41). Except the above factors that could decrease the risk of LEE/TBE, current WHO disease stage II, III and IV (stage II, AHR=2.07, 95% CI: 1.04-4.13; stage III, AHR=3.90, 95% CI: 2.10-7.27; stage IV, AHR=3.36, 95%CI: 1.76-6.43, compared with stage I, respectively) had higher risk to occur liver damage (Table 3).

Table 3. Factors associated with liver damage among HIV/AIDS patients on ART

Variables	Total Patients (n)	Liver damage (n)	Person-Years	Liver damage per 100 person year	HR (95% CI)	*p*	AHR (95% CI) [a]	*p*
Gender								
Male	1304	154	3800.40	4.05	1	-		
Female	815	109	2596.74	4.20	0.94(0.74-1.21)	0.65		

Marital Status						0.08		
Unmarried	215	37	614.04	6.03	1	-		
Married/cohabitation	1607	188	4875.47	3.86	0.60(0.42-0.85)	0.01		
Divorced/separated	51	7	170.18	4.11	0.63(0.28-1.41)	0.26		
Widowed	236	31	705.51	4.39	0.73(0.45-1.18)	0.20		
Others	10	0	31.95	0.00	0.00(-)	0.93		
Residence						0.50		
Guigang city	2047	244	6084.09	4.01	1	-		
Other cities in Guangxi	67	19	298.12	6.37	0.72(0.41-1.25)	0.24		
Other provinces	5	0	14.92	0.00	0.00(-)	0.93		
Age (years) at Diagnosis						0.95		
<40	722	106	2442.31	4.34	1	-		
40-60	884	107	2570.05	4.16	1.02(0.78-1.33)	0.90		
≥60	513	50	1384.78	3.61	1.06(0.75-1.48)	0.75		
Age (years) at ART Initiation						0.97		
<40	704	104	2397.52	4.34	1	-		
40-60	890	109	2585.24	4.22	1.03(0.79-1.35)	0.82		
≥60	525	50	1414.38	3.54	1.03(0.34-1.45)	0.86		
Transmission Route						0.44		
Blood transfusion	87	25	360.66	6.93	1	-		
Sexual transmission	1921	224	5682.10	3.94	0.77(0.50-1.16)	0.21		
Others	111	14	354.38	3.95	0.74(0.38-1.42)	0.36		
Baseline Disease Stage						<0.001		<0.001
I	791	101	2065.67	4.89	1	-	1	-
II	376	47	1288.25	3.65	0.55(0.39-0.78)	0.001	0.49(0.34-0.70)	<0.001
III	679	77	2283.76	3.37	0.46(0.34-0.62)	<0.001	0.46(0.33-0.64)	<0.001
IV	320	38	759.45	5.00	0.69(0.47-1.01)	0.06	0.68(0.45-1.04)	0.07
Baseline CD4 cell count						0.02		0.05
<200 cells/μL	1250	152	4014.64	3.79	1	-	1	-
200-350 cells/μL	690	103	2093.44	4.92	1.45(1.13-1.86)	0.004	1.42(1.07-1.89)	0.02
≥350 cells/μL	179	8	289.06	2.77	1.30(0.63-2.67)	0.48	1.03(0.49-2.14)	0.95
Baseline AST						0.30		
<20 U/L	475	47	1360.81	3.45	1	-		
20-30 U/L	1036	126	3167.70	3.98	1.14(0.82-1.60)	0.44		
30-40 U/L	480	70	1543.79	4.53	1.20(0.83-1.74)	0.34		
Missing	101	20	324.83	6.16	1.66(0.98-2.81)	0.06		
Baseline ALT						0.69		

<20 U/L	1294	149	3914.47	3.81	1	-		
20-30 U/L	587	77	1707.76	4.51	1.15(0.88-1.52)	0.31		
30-40 U/L	233	36	748.83	4.81	1.14(0.79-1.64)	0.49		
Missing	5	1	26.08	3.83	0.64(0.09-4.57)	0.65		
Baseline TBIL						0.52		
<10 μmol/L	1409	164	4166.89	3.94	1	-		
10-20 μmol/L	693	97	2165.24	4.48	1.15(0.89-1.45)	0.28		
Missing	17	2	65.01	3.08	0.82(0.20-3.32)	0.78		
Current Disease Stage						<0.001		<0.001
I	1894	231	5994.86	3.85	1	-	1	-
II	109	9	204.03	4.41	1.81 (0.93-3.54)	0.08	2.07(1.04-4.13)	0.04
III	83	13	133.19	9.76	4.30(2.44-7.57)	<0.001	3.90(2.10-7.27)	<0.001
IV	33	10	65.05	15.37	3.86(2.05-7.28)	<0.001	3.36(1.76-6.43)	<0.001
Current ART Regimen						<0.001		<0.001
3TC+TDF+EFV	750	69	1757.18	3.93	1	-	1	-
3TC+AZT+NVP	411	28	1710.75	1.64	0.27(0.18-0.43)	<0.001	0.27(0.19-0.41)	<0.001
3TC+D4T+NVP	71	6	164.18	3.65	1.21(0.52-2.79)	0.66	1.04(0.44-2.49)	0.93
3TC+AZT+EFV	361	72	1182.47	6.09	1.35(0.97-1.88)	0.08	1.23(0.88-1.72)	0.23
3TC+D4T+EFV	65	9	122.93	7.32	2.87(1.43-5.77)	0.003	1.88(0.89-3.97)	0.10
3TC+TDF+LVP/r	294	54	964.17	5.60	1.14(0.79-1.62)	0.49	1.13(0.79-1.62)	0.50
Other regimens	167	25	495.47	5.05	1.23(0.78-1.94)	0.38	1.15(0.73-1.83)	0.55
Median CD4 cell Count during Follow-up						0.05		
<200 cells/μL	549	65	1396.45	4.65	1	-		
200-350 cells/μL	703	91	2234.90	4.07	0.75(0.54-1.03)	0.86		
≥350 cells/μL	867	107	2765.79	3.87	0.68(0.50-0.93)	0.01		

Abbreviations: HR, hazard ratio; AHR, adjusted hazard ratio, ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin in serum.

[a] Covariates of the adjusted model included gender, residence, baseline disease stage, baseline CD4 cell counts, baseline AST and ALT, current disease stage, current ART regimen, and CD4 cell count during follow-up.

Discussion

This retrospective cohort study was conducted on HIV-positive individuals with an initially normal hepatic function and without HBV/HCV infection. We found that liver damage (grade II-IV LEE/TBE) occurred in all patients at different period of ART, indicating that hepatic damage always exists among HIV/AIDS patients receiving ART, which has been shown in a few previous studies [4,5], although the incidence rate of

LEE/TBE in our study was quite stable and low in the longer ART during times. Our study showed that the liver damage prevalence was 12.41% (263/2119) (Figure 1), which is lower than those (14%-23%) in several previous studies conducted on HIV/AIDS patients [21,22]. In addition, the overall liver damage incidence (4.11/100 person-years, 95%CI: 3.63-4.60) in our study is also lower than that (6.04/100 person-years of chronic LEE incidence) in a similar study on HIV-monoinfected persons by Kovari et al [23]. One possible reason for lower prevalence and overall incidence of liver damage in our study is that we used the grade II-IV LEE/TBE to define liver dysfunction, which is a higher threshold to define liver dysfunction compared to the similar research in several papers [20], whereas others might use different definition of LEE [12,23].

The highest liver damage incidence was observed in patients with 6-12 months' ART duration (11.56/100 person years). The incidence rapidly decreased in patients with 2 years of ART and then maintained at a relatively lower level (Figure 2). These findings are consistent with quite a few previous studies on liver toxicity caused by various antiretroviral drugs such as NVP, EFV, TDF, which also observed a strong association between drugs and the development of LEE emerging within the first 2 years after drug initiation [23]. The significant decreasing trend of incidence rate of liver damage between 0.5 to 2 years of ART duration might be resulted from a number of adaptation mechanisms which are initiated to counteract the inflicted damage [24]. Importantly, the above findings indicate that cumulative ART time does not increase the risk of liver damage, which is of great clinical importance to support the strategy of WHO to increase the number of HIV/AIDS patients on ART [25], and the strategy has been shown to greatly reduce rate of sexual transmission of HIV in several recent HIV prevention trails [26,27]. Meanwhile, antiretroviral examined are no longer recommended by international guidelines, current evidence favors improved safety hepatic profile for IN strand transfer inhibitors (INSTIs), which was confirmed hepatic safety [28].

Several related factors for liver damage were observed in the present study (Table 3). Patients currently at stage II, III or IV had higher risk to develop liver damage compared with patients in stage I, indicating the level of immunodeficiency and the host immunity

involve in liver damage upon ART. These results are consistent with some previous studies. For example, the risk of nevirapine-induced hepatitis increases 12-fold in women with more than 250 CD4 cells/μL and 5-fold in males with more than 400 CD4 cells/μL (disease stage II) [29].

In this study, we also found that patients at initial disease stage II, III were less likely to develop liver damage compared with patients at initial disease stage I (Table 3). Possible reason is that the patients with more severe symptom might be more likely to take some positive measures, such as improved adherence, healthier lifestyle, than patients who had normal liver function. However, our results didn't show there is relationship between CD4 cell count (including baseline and follow-up) and liver damage. The results are consistent with some studies but inconsistent with some other studies, for example, one study showed lower risk of hepatic dysfunction come along with higher CD4 cell count in individuals on ART [8]. In fact, contradicting results were reported by different researchers in term of relationship of CD4 cell count and liver dysfunction, which may be related to the different ART regimens investigated as well as some other factors that might affect host immune status when patients were on ART [21].

Our study also showed that patients with current regiment 3TC+AZT+NVP had lower risk to develop liver damage (grade II-IV LEE/TBE) compared with regiment 3TC+TDF+EFV. The liver toxicity of TDF and EFV has been previously described in several studies [23,24]. Since 3TC+TDF+EFV is currently considered as one of first-line regimens, the importance of combination of liver protection may need to be emphasized for this regimen. Furthermore, according to the National Guide for Free Antiviral Treatment of HIV Patients, NVP is not recommended for men with baseline CD4 cell count ≥ 400 cells/μL and women with baseline CD4 cell count ≥ 250 cells/μL because of its hepatotoxicity [30]. In our study, the median baseline CD4 cell counts were 142.0 (IQR: 40-255) in men and 194.0 (IQR: 103-279) in women, which were much lower than the above levels. Therefore, the clinical recommendation of NVP might also contribute to the lower liver damage rate in patients with the current regiment 3TC+AZT+NVP.

Conclusions

In this study, although patients with abnormal baseline liver function and with HBV/HCV infection had been excluded, the incidence of liver damage (grade II-IV LEE/TBE) still occurred among HIV/AIDS patients on ART, indicating liver damage always exists among HIV-infected patients on ART. Nevertheless, cumulative ART duration does not increase the risk of liver damage. Therefore, ART could tend to be long-term, however, monitoring and management of liver damage among patients on ART are also important in clinical therapy.

Competing interests

All authors declare that they have no conflict of interest.

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Authors' contribution

HL, XQ and LY conceived and designed the study. FQ, JJ conducted the data analysis, literature review, and drafted the manuscript. CQ, YH, BL, and YX were involved in the study supervision, data collection, and interpretation of the data. JH, ZX, CN, YL, NZ, JL, WW, and JY assisted with data management and data analysis. All authors contributed to the revision of the manuscript and approved the final version.

Data sharing

All data included in this study are available upon request by contact with the corresponding author.

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Figure Legends

Figure 1. Patients Flowchart.

Figure 2. Crude incidence rates of liver damage [grade II-IV liver enzyme elevation (LEE) / total bilirubin elevation (TBE)] with cumulative ART time.

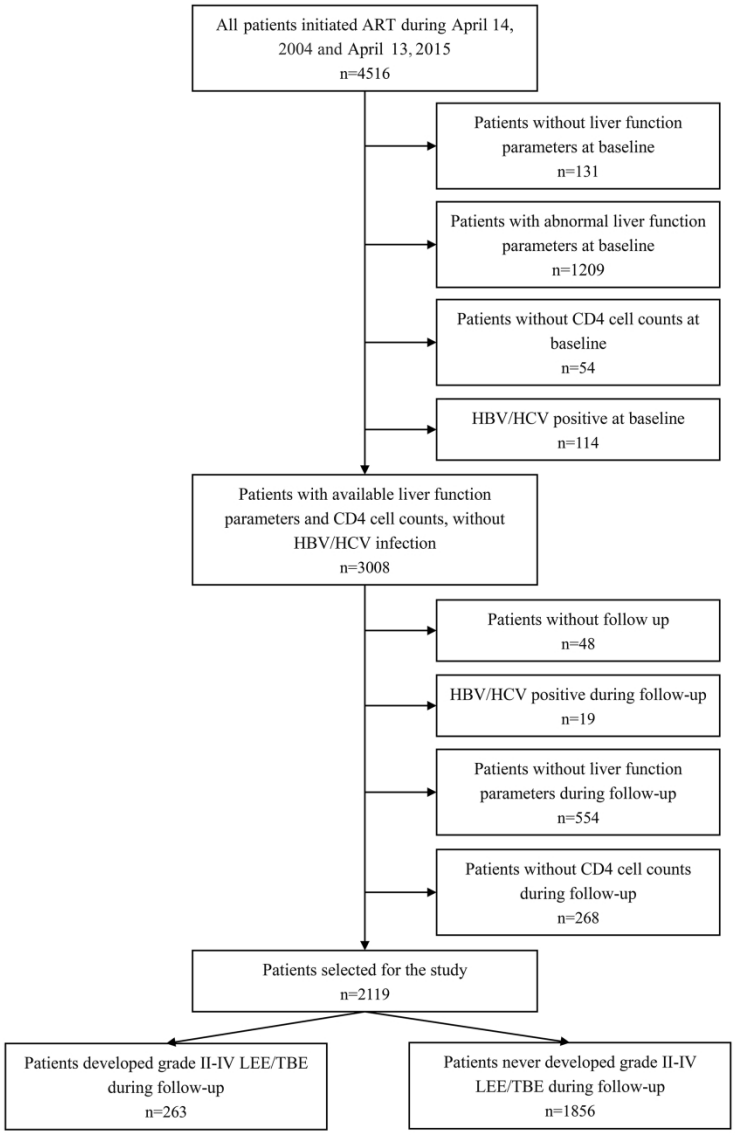

Figure1. Patients Flowchart

Figure1. Patients Flowchart

168x270mm (300 x 300 DPI)

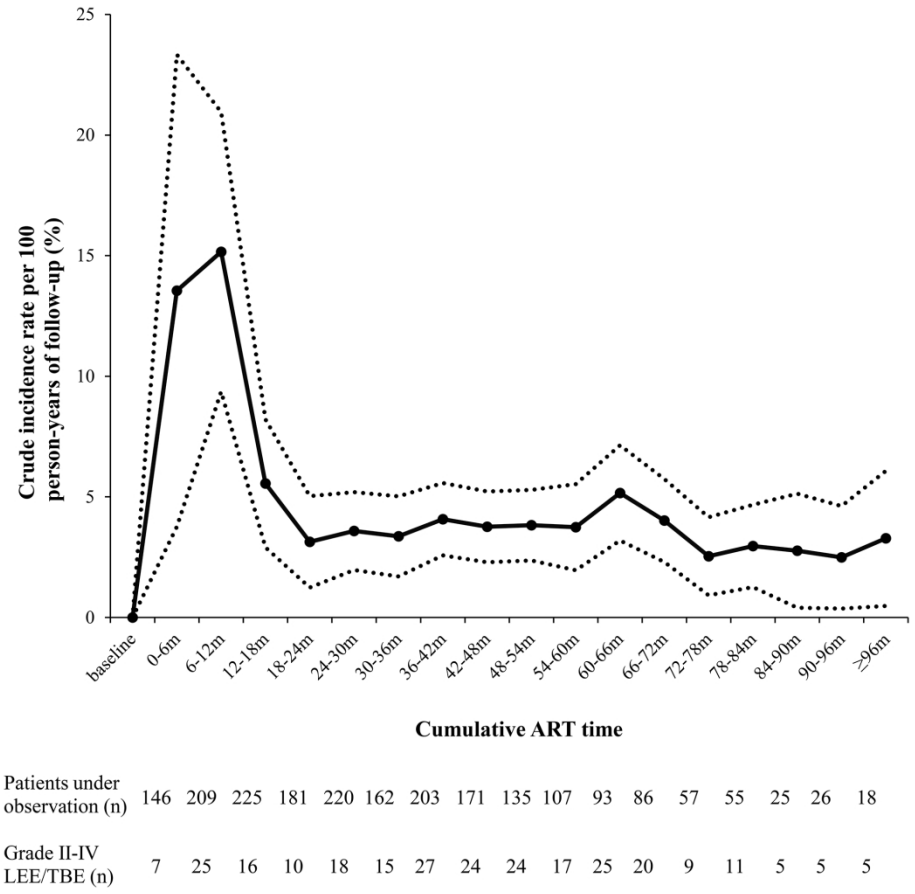

Figure 2. Crude incidence rates of liver damage [grade II-IV liver enzyme elevation (LEE) / total bilirubin elevation(TBE)] with cumulative.

191x202mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **Page1-2**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **Page 2**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **Page 4-5**
Objectives	3	State specific objectives, including any prespecified hypotheses **Page 5**
Methods		
Study design	4	Present key elements of study design early in the paper **Page 5-6**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **Page 5-7**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **Page 5-6** *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls **Not applicable** *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants **Not applicable**
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed **Not applicable** *Case-control study*—For matched studies, give matching criteria and the number of controls per case **Not applicable**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **Page 6-7**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **Page 5-7**
Bias	9	Describe any efforts to address potential sources of bias **Page 5**
Study size	10	Explain how the study size was arrived at **Page 5-7**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **Page 6-7**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **Page 7**
		(*b*) Describe any methods used to examine subgroups and interactions **Not applicable**
		(*c*) Explain how missing data were addressed **Not applicable**
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed **Not applicable** *Case-control study*—If applicable, explain how matching of cases and controls was addressed **Not applicable** *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy **Not applicable**

		(*e*) Describe any sensitivity analyses	**Not applicable**
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	**Page 8**
		(b) Give reasons for non-participation at each stage	**Page 8**
		(c) Consider use of a flow diagram	**Figure 1**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	**Page 8-11**
		(b) Indicate number of participants with missing data for each variable of interest	**Not applicable**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	**Page 8**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	**Page 11-12, Figure 2**
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	**Not applicable**
		Cross-sectional study—Report numbers of outcome events or summary measures	**Not applicable**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	**Page 12-14**
		(*b*) Report category boundaries when continuous variables were categorized	**Page 8-11**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	**Not applicable**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	**Not applicable**
Discussion			
Key results	18	Summarise key results with reference to study objectives	**Page 14-15**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	**Page 3**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**Page 15-16**
Generalisability	21	Discuss the generalisability (external validity) of the study results	**Page 16**
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	**Page 17**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at

http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.