scenario, regional prevalence of CKD may influence the approach to screening and monitoring of HIV-infected patients initiated on ART. In particular, most nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), such as TDF and 3TC/FTC, are excreted by the kidney and may require dose adjustment in individuals with reduced glomerular filtration rate (GFR), and may require more intensive monitoring in patients with pre-existing CKD.

Following the 2010 WHO guidelines, the use of TDF has been increasing rapidly in Vietnam, where patients are more likely to have smaller body weight compared to Caucasians. At this stage, little is known about CKD among Vietnamese HIV-infected patients. In this context, it is important to determine the prevalence of CKD and its risk factors including TDF exposure and low body weight in this region. The present study was conducted to evaluate the above factors in Vietnamese HIV-infected patients.

Methods

Study design

We performed a cross-sectional study with an observational single-center cohort of Vietnamese HIV-infected patients on ART. This cohort was established since 2007 at the National Hospital of Tropical Disease in Hanoi, one of the largest outpatient clinics for HIV infected-patients in Vietnam. Clinical data are collected twice a year (in April and October) in this cohort. The population of this cohort comprised HIV-infected patients on ART aged more than 17 years. To evaluate CKD in this group, serum creatinine had been examined since October 2011. Serum creatinine was measured in October 2011 and April 2012. Patients whose creatinine was not obtained at both time points were excluded from the study. Other clinical data were collected twice a year (in April and October) as well. The study was approved by the Human Research Ethics Committee of National Hospital of Tropical Disease and Hanoi city. Each patient included in this study provided a written informed consent for the clinical and laboratory data to be used for publication. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Measurements

Data included demographic variables (height, weight, sex and age); a complete history of ART; use of cotrimoxazole; CD4 cell count (cell/mm^3, measured by flow cytometry); plasma HIV-RNA (copies/ml, measured by the Roche COBAS TaqMan HIV monitor assay); serum creatinine (mg/dl, measured by Jaffe method); date of HIV diagnosis and other comorbidities. CKD was defined as creatinine clearance (Ccl) estimated by the Cockcroft-Gault formula of <60 ml/min at October 2011 and April 2012 (6 months apart). Renal dysfunction at each time point was also classified into five stages according to the guidelines of the National Kidney Foundation [18]: normal renal function: Ccl ≥90 ml/min; mild renal dysfunction, Ccl between 60-89 ml/min; moderate, Ccl 30-59 ml/min; severe renal dysfunction, Ccl 15-29 ml/min; and renal failure or dialysis, with Ccl of <15 ml/min.

Statistical analysis

Statistical analysis included descriptive (mean and standard deviation), univariate and multivariate analyses. Absolute and relative frequencies were utilized for continuous and categorical variables, respectively. To evaluate the association between CKD and categorical variables, the chi-square test or Fisher exact test was applied as required. Independent T test or one-way analysis of variance (ANOVA) was used to compare mean values of normally distributed data and the Mann Whitney test or Kruskal-Wallis test for parameters with skewed data distribution. Variables significantly associated with renal dysfunction in univariate analysis ($p<0.05$) were entered into multivariate analysis. Logistic regression was used to determine the factors associated with CKD in univariate and multivariate analyses. Statistical significance was defined at two-sided p value <0.05. We used the odds ratio (OR) and 95% confidence interval (95% CI) to estimate the association of each variable with renal dysfunction. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

Patients on TDF

The percentage of TDF use in our cohort increased from 11.9% in April 2011 to 40.3% in April 2012. In contrast, stavudine (d4T) use decreased from 37.8% in April 2011 to 14.6% in April 2012. The patterns of use of TDF and d4T well reflected the recommendation of the 2010 WHO ART guidelines; recommendation for the use of TDF or zidovudine (AZT) and phasing out of d4T.

Prevalence of CKD and renal dysfunction at each time point

To determine the prevalence of CKD, serum creatinine was measured in 771 patients in October 2011 and April 2012. CKD was diagnosed in 56 (7.3 %) patients and classified as moderate in 54 and severe in 2 (Table **1**). The number of patients with moderate and severe renal dysfunction increased from 74 (9.6%) in October 2011 to 111 (14.4%) in April 2012. The data of serum creatinine by CKD stage are shown in Table **1**.

Baseline demographics and laboratory data

Table **2** compares the baseline demographics and clinical variables of patients with or without CKD for the data of October 2011. Patients with CKD were significantly older, more likely to be diabetic females treated with TDF and lopinavir boosted with ritonavir, and of significantly lower body weight with higher serum creatinine, and with history of AIDS-defining disease, compared to those without CKD. CD4 count, HIV RNA viral load, and duration of ART were not significantly different between the two groups. The mean CD4 count was >300/mm^3 and the mean HIV RNA load was <100 copies/ml in both groups.

Table 1. Prevalence of CKD and renal function at two time points in 771 HIV-infected Vietnamese on ART.

		CKD	Oct 2011	Apr 2012
Renal function	Ccl (ml/min)		n (%)	
Normal	90 or more	-	178 (23.0)	159 (20.6)
Mild reduction	60-89	-	519 (67.4)	501 (65.0)
Moderate reduction	30-59	54 (7.0)	72 (9.3)	108 (14.0)
Severe reduction	15-29	2 (0.3)	2 (0.3)	3 (0.4)
Renal failure	less than 15	0	0	0

Renal dysfunction was classified according to the guidelines of the National Kidney Foundation (18)

CKD was defined as Ccls of <60 ml/min at both time points (October 2011 and April 2012).

CKD; chronic kidney disease, ART; antiretroviral therapy

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Table 2. Baseline demographics and laboratory data of 771 patients measured at October 2011.

variables	Entire group	CKD (+)	CKD(-)	P value
Number of patients	771	56 (7.3%)	715 (92.7%)	
Age, years	36.4±7. 86	46.5±11.5	35.6±6.9	<0.001
Female, n (%)	296 (38.4%)	36 (64.3)	260 (36.4)	<0.001
Body weight, kg	55.0±8.4	47.1±6.3	55.6±8.2	<0.001
Diabetes mellitus, n (%)	32 (4.2%)	6 (10.7)	26 (3.6)	0.023
Serum creatinine, mg/dl	0.95±0. 15	1.11±0.22	0.94±0.13	<0.001
CD4+ count, /μl	349.0±202.8	337.0±215.2	349.9±201.9	0.648
HIV RNA, $\log_{10}$ c/ml	1.79±0.52	1.80±0.47	1.79±0.52	0.833
Duration of ART, years	1.34±1.54	1.69±1.96	1.32±1.51	0.083
Use of TDF, n (%)	171 (22.2%)	23 (41.1)	148 (20.7)	<0.001
Use of Lopinavir, n (%)	97 (12.6%)	13 (23.2)	43 (6.0)	0.013
Use of cotrimoxazole, n (%)	171 (22.2%)	18 (32.1)	153 (21.4)	0.062
AIDS defining disease, n (%)	69 (8.9%)	10 (17.9)	59 (8.3)	0.015

Data are mean±SD or n (%).

CKD; chronic kidney disease, ART; antiretroviral therapy, TDF; tenofovir

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Factors associated with CKD

Univariate analysis identified older age per year-increase, female sex, body weight per 1 kg-decrement, use of TDF, use of lopinavir boosted with ritonavir, diabetes mellitus, and AIDS-defining diseases as factors significantly associated with CKD. After adjustment by multivariate analysis, older age per year-increase (OR=1.229; 95%CI, 1.170-1.291; p<0.001), body weight per 1 kg-decrement (OR=1.286; 95%CI, 1.193-1.386; p<0.001), and use of TDF (OR=2.715; 95%CI, 1.028-7.168; p=0.044) were associated significantly with CKD (Table **3**).

Discussion

We documented in the present study the prevalence of CKD and the associated risk factors in our Vietnamese cohort. CKD was identified in 7.3% of the patients between October 2011 and April 2012. Although severe renal dysfunction was

Table 3. Factors associated with CKD based on uni- and multivariate analyses (n=771).

	Univariate analysis		Multivariate analysis		
Variables	OR	95% CI	OR	95% CI	p value
Age per year-increase	1.135	1.102 - 1.168	1.229	1.170 - 1.291	<0.001
Female	3.150	1.786 - 5.556	2.124	0.892 - 5.056	0.089
Body weight per 1 kg-decrement	1.170	1.119 - 1.223	1.286	1.193 - 1.386	<0.001
Use of TDF	2.670	1.522 - 4.685	2.715	1.028 - 7.168	0.044
Use of Lopinavir	2.257	1.165 - 4.370	1.439	0.460 - 4.497	0.531
Diabetes mellitus	3.180	1.251 - 8.084	1.614	0.353 - 7.383	0.537
AIDS defining disease	2.417	1.160 - 5.035	2.042	0.628 - 6.643	0.236
CD4+ cell count per cell/μl	1.000	0.998 - 1.001			
HIV-RNA level per log 10 copies/ml	1.055	0.641 - 1.736			
Duration of ART per year	1.138	0.982 - 1.318			
Use of cotrimoxazole	1.740	0.966 - 3.134			

OR = Odds ratio; CI = confidence interval; CKD; chronic kidney disease, ART; antiretroviral therapy, TDF; tenofovir

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observed in only 2 cases, we consider this finding quite alarming in our study setting, since it is more than double that reported in a previous study (3.1%) on the prevalence of CKD among Vietnamese healthy volunteers aged more than 40 years [19]. Our cohort comprised relatively younger and stable patients on ART with a mean age of 36.4 years.

In addition to the high prevalence of CKD, a striking finding in this study was that TDF use has increased steeply since the 2010 WHO ART guidelines that recommended the use of TDF; TDF use was also an independent risk for CKD in Vietnamese, in addition to low body weight. We reported previously that Japanese patients with small body weight (<59 kg) treated with TDF were at high risk of renal dysfunction [16], whereas those with body weight of >67 kg had negligible risk, similar to the patients reported by Cooper et al [13]. One experimental study of rhesus macaques also reported that TDF-associated nephrotoxicity was dose-dependent [20]. The mean body weight of the patients enrolled in the present study was 55 kg, which is about 30 kg less than that of American males of similar age (88 kg) (URL:http://www.cdc.gov/nchs/data/nhsr/nhsr010.pdf). To prevent TDF-related CKD in patients with a small body weight, the efficacy and safety of low-dose TDF adjusted to low body weight should be evaluated in a clinical trial.

One study argued that the initial decline in eGFR following the commencement of TDF therapy stabilized later after the first 6 months [21]. However, whether or not the initial decline stabilizes later in patients with low body weight remains to be documented in a longitudinal study of our cohort. It is true that the future risk of TDF-related CKD is still uncertain. In this study, almost all patients who experienced renal dysfunction continued the same ART regimen because renal dysfunction was relatively moderate as shown in Table **1**. Although one severe case showed improvement of renal function after cessation of TDF, normalization of renal function after

withdrawal of TDF was reported to be incomplete in some cases [22]. Previous studies recommended dose reduction of drugs that are cleared by the kidney, such as lamivudine and TDF, when Ccl falls below 50 ml/min [23], to avoid further worsening of renal dysfunction. Early detection of eGFR decline is important for switching from TDF to AZT or abacavir to preserve renal function. Despite those concerns, however, there is no doubt that TDF is still an important drug with enough anti-HIV potency and less mitochondrial toxicity among NRTIs. In this regard, serum creatinine should be monitored even in resource-limited situations.

Furthermore, another study that compared patients with or without TDF use depicted that TDF was more likely to be used in the salvage regimen so far; patients on TDF had the longer duration of ART and more positive viral load (Table **2**). Based on this analysis, patients on TDF were more likely to develop CKD, although the mean body weight was not significantly different between the two groups. In addition, in terms of another antiretroviral agent, protease inhibitor (PI), also known as a risk factor for CKD [11], 97 (12.6%) patients used PIs (all PIs were ritonavir boosted lopinavir). Of 97 patients, 83 (85.6%) were co-administered with TDF. Although univariate analysis suggested that the use of PIs was associated significantly with CKD, multivariate analysis did not (Table **3**). The reason of this result could be explained by the short duration of co-administration and its effect as a confounding factor for TDF use.

The present study has several limitations. Due to its cross-sectional nature, we can only draw association of events and not demonstrate causative relationship between TDF and renal dysfunction. Further longitudinal studies are required to determine the impact of the aforementioned factors on renal function. Second, co-infection with HCV, a known risk factor for CKD, was not included in this analysis due to lack of available data in our cohort. The prevalence of HCV in Vietnamese is relatively high because injecting drug use is one of the main routes of infection in Vietnam. We are adding data for a longitudinal study on TDF toxicity in our cohort. Lastly, the Modification of Diet in Renal Disease formula (MDRD) or Chronic Kidney Disease Epidemiology Collaboration (CKD-epi) is commonly used for evaluation of renal function at present [24-26], however, the racial coefficient for Vietnamese is currently not available. In addition, serum creatinine was measured by the Jaffe method in our study, which is difficult to apply to MDRD or CKD-EPI since those formulations are based on measurement of serum creatinine by the more widely used enzyme method. For this reason, our study utilized Ccl to assess renal function.

Despite these limitations, the results of the present study call for attention to active pharmacovigilance of TDF. The results identified TDF exposure as a significant and independent risk for CKD in Vietnam, although the duration of TDF use is still relatively short. Further longitudinal study is required to evaluate the impact of TDF on renal function in Vietnam and other countries with small-body weight patients.

Supporting Information

Table S1. Median and inter-quartile range of serum creatinine of 771 patients at October 2011 and April 2012.
(DOCX)

Table S2. Baseline (October 2011) demographics and laboratory data of 771 patients with or without TDF use in whom serum creatinine was measured at October 2011 and April 2012.
(DOC)

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Author Contributions

Conceived and designed the experiments: DM JT TN HG SO. Performed the experiments: NL ND NK YK. Analyzed the data: DM TN FK. Contributed reagents/materials/analysis tools: YK HG. Wrote the manuscript: DM TN HG SO.

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Ritonavir-Boosted Darunavir Is Rarely Associated with Nephrolithiasis Compared with Ritonavir-Boosted Atazanavir in HIV-Infected Patients

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Abstract

Background: Although ritonavir-boosted atazanavir (ATV/r) is known to be associated with nephrolithiasis, little is known about the incidence of nephrolithiasis in patients treated with ritonavir-boosted Darunavir (DRV/r), the other preferred protease inhibitor.

Methods: In a single-center cohort, the incidence of nephrolithiasis was compared between HIV-infected patients who commenced DRV/r-containing antiretroviral therapy and those on ATV/r. The effects of ATV/r use over DRV/r were estimated by univariate and multivariate Cox hazards models.

Results: Renal stones were diagnosed in only one patient (0.86 per 1000 person-years) of the DRV/r group (n=540) and 37 (20.2 per 1000 person-years) of the ATV/r group (n=517). The median [interquartile (IQR)] observation period in the DRV/r group was 27.1 months (IQR 18.1-38.4 months), and 40.6 months (IQR 17.5-42.7) for the ATV/r group. The total observation period was 1,163.6 person-years and 1,829.6 person-years for the DRV/r group and for the ATV/r group, respectively. In the 37 patients on ATV/r who developed nephrolithiasis, the median time from commencement of ATV/r to diagnosis was 28.1 months (IQR 18.4–42.7), whereas nephrolithiasis in the single patient of the DRV/r group occurred 11.2 month after the introduction of DRV/r. ATV/r use over DRV/r was significantly associated with nephrolithiasis by uni- and multivariate analyses (HR=26.01; 95% CI, 3.541–191.0; p=0.001) (adjusted HR=21.47; 95% CI, 2.879–160.2; p=0.003).

Conclusion: The incidence of nephrolithiasis was substantially lower in patients on DRV/r than those on ATV/r. The results suggest that DRV/r should be selected for treatment of HIV-infected patients at risk of chronic kidney disease.

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Introduction

Ritonavir-boosted darunavir (DRV/r) and ritonavir-boosted atazanavir (ATV/r) are the only two protease inhibitors (PI) selected as the preferred choices in the American Department of Health and Human Services (DHHS) guidelines for the initial treatment of patients infected with human immunodeficiency virus-1 (HIV-1) (http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf). Both drugs are widely used in combination with other antiretroviral drugs, based on their high efficacy, tolerability, favorable lipid profile, and once-daily dosing [1-4]. However, nephrolithiasis has been reported in patients receiving ATV/r-containing antiretroviral therapy (ART) [5,6]. Several case reports documented high concentrations of ATV in renal stones, suggesting the involvement of ATV in nephrolithiasis [5-8]. We recently reported in a single center cohort study that the incidence of renal stones is approximately 10 times higher among patients on ATV/r-containing

antiretroviral therapy (ART) than those on other PIs-containing ART [9].

Our study on the effects of ART on renal stone formation included only a small number of patients on DRV/r-containing ART [9,10], and no data are available at present on the incidence of nephrolithiasis in patients treated with DRV/r. Of note, de Lastours et al [11] recently reported higher ATV and DRV levels in urine samples than in plasma, whereas plasma and urinary levels of lopinavir, another commonly used PI, were comparable. They also reported the presence of PI-containing crystals in the urine of a small proportion of patients on ATV and on DRV, but not on lopinavir/ritonavir (LPV/r). The data presented by de Lastours et al suggest that DRV can crystallize in urine leading to nephrolithiasis.

The aim of the present study was to determine the incidence of DRV/r- and ATV/r-related nephrolithiasis. Such comparison is important for two reasons: 1) These two PIs are most frequently prescribed PIs in resource-rich settings, and 2) nephrolithiasis is a risk factor for chronic kidney diseases (CKD) and end-stage renal disease (ESRD), which are important comorbidities associated with AIDS and death [12-16].

Methods

Ethics statement

This study was approved by the Human Research Ethics Committee of the National Center for Global Health and Medicine, Tokyo. Each participant provided a written informed consent for the clinical and laboratory data to be used and published for research purposes. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Study Subjects

We performed a retrospective, single-center cohort study of HIV-1-infected patients using the medical records kept at the National Center for Global Health and Medicine, Tokyo, Japan. Our facility is one of the largest clinics for patients with HIV infection in Japan with more than 2,700 registered patients. The study population was HIV-infected patients, aged >17 years, who commenced treatment with DRV/r or ATV/r-containing ART between January 1, 2004 and June 30, 2012. Both treatment-naïve and treatment-experienced patients were included. The follow-up period started at the time of commencement of ART containing the abovementioned drugs for the first time during the study period, and patients were followed until June 30, 2013. Patients were excluded if they had; 1) commenced the abovementioned ART during the study period at other facilities, 2) been prescribed unboosted ATV, or 3) been under treatment for nephrolithiasis at the time of commencement of the abovementioned ART. ATV/r became available in Japan in January 2004, and DRV/r in December 2007.

The attending physician selected either ATV/r or DRV/r at baseline. The use of these drugs was based on the Japanese guidelines, which placed ATV/r and DRV/r as the preferred choice, at least for 5 years during the study period (http://www.haart-support.jp/pdf/guideline2013.pdf. in Japanese). The attending physician also selected the concurrent drugs including nucleoside reverse transcriptase inhibitors (NRTI), non-NRTI, integrase inhibitors, and CCR5 inhibitors. None of the patients received two PIs during the study period.

Measurements

The main investigator reviewed the medical records of all study patients to identify those with renal stones. Then two other investigators reviewed the set of medical records of each patient with renal stones to determine whether the case fitted into the following pre-defined criteria for nephrolithiasis: cases with a clinical diagnosis by the attending physician based on new onset of acute flank pain plus one of the following: 1) new-onset hematuria confirmed by urine dipstick test, 2) documented presence of stones or radiological findings suggestive of renal stones, such as hydronephrosis or obstruction or dilatation of the ureter, by either abdominal ultrasonography or computed tomography, 3) stone passage confirmed by either the patient or attending physician [9]. Patients with acute flank pain due to etiologies other than nephrolithiasis were excluded. At the time of diagnosis of nephrolithiasis, the attending physician selected either discontinuation or modification of ART. In our clinic, it is customary for the patient to visit the clinic once a month before the initiation of ART and until the suppression of HIV-1 viral load, but the visit interval is extended up to every three months after viral load suppression.

In this study, the primary exposure variable was ATV/r use over DRV/r. The potential risk factors for nephrolithiasis were determined according to previous studies and collected from the medical records, together with the basic demographics [7,8,17]. They included age, sex, body weight, body mass index (BMI)={bodyweight (kg) / [(height (m)]^2}, baseline laboratory data [CD4 cell count, HIV viral load, estimated glomerular filtration rate (eGFR), serum uric acid], and presence or absence of other medical conditions [concurrent use of tenofovir (TDF), past history of nephrolithiasis, previous exposure to indinavir (IDV), co-infection with hepatitis B defined by positive hepatitis B surface antigen, and co-infection with hepatitis C defined by positive hepatitis C viral load]. eGFR was calculated using the equation of the 4-variable Modification of Diet in Renal Diseases (MDRD) study [18]. For patients on ATV/r-containing ART, the value of serum total bilirubin was collected in two ways: for stone cases, total bilirubin value on the day was collected, and for non-stone cases, the value of total bilirubin 2 years after initiation of ATV/r was collected. For patients who discontinued ATV/r within 2 years, the value closest to the day of discontinuation was used. At our clinic, weight was measured on every visit whereas other variables were measured in the first visit and at least once annually. We used the data on or closest to and preceding the day of starting ART by no more than 180 days, except for serum uric acid level, which were collected within 180 days from the day of starting ART.

Statistical analysis

Baseline characteristics were compared using the Student's *t*-test or χ^2 test (Fisher exact test) for continuous or categorical variables, respectively. The time to the diagnosis of nephrolithiasis was calculated from the date of commencement of DRV/r- or ATV/r-containing ART to the date of diagnosis of nephrolithiasis. Censored cases represented those who discontinued ATV/r or DRV/r, dropped out, were referred to other facilities, or at the end of follow-up period. The time from the start of ART to the diagnosis of nephrolithiasis was analyzed by the Kaplan Meier method for patients who started DRV/r (DRV/r group) and ATV/r (ATV/r group), and the log-rank test was used to determine the statistical significance. The Cox proportional hazards regression analysis was used to estimate the impact of ATV/r use over DRV/r on the incidence of nephrolithiasis. The impact of each basic demographic parameter, baseline laboratory data, and other medical conditions listed above was also estimated with univariate Cox proportional hazards regression. To estimate the unbiased prognostic impact of ATV/r use over DRV/r for nephrolithiasis, we conducted three models using multivariate Cox proportional hazards regression analysis. Model 1 was the aforementioned univariate analysis for ATV/r use over DRV/r. Model 2 included age, sex, and weight plus model 1 in order to adjust for basic characteristics. In model 3, we added variables with P values <0.05 in univariate analysis after adjustment (these included tenofovir use, serum uric acid per 1 mg/dl, and past history of renal stones). Possible risk factors for ATV/r-related nephrolithiasis identified in previous studies were also added to model 3 (these included prior exposure to IDV) [7,8].

In addition, to examine the impact of serum total bilirubin on ATV/r-containing ART and the incidence of nephrolithiasis, the median serum total bilirubin values were compared between the renal stone and non-renal stone groups using the Mann-Whitney U test.

Statistical significance was defined as two-sided *p* values <0.05. We used hazard ratios (HRs) and 95% confidence intervals (95%CIs) to estimate the impact of each variable on nephrolithiasis. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 20.0 (SPSS, Chicago, IL).

Results

A total of 1,189 patients commenced either DRV/r- or ATV-containing ART between January 1, 2004 and June 30, 2012. Of the 1,057 patients who were included in the analysis, 540 (51%) started DRV/r-containing ART while 517 (48.9%) started ATV/r-containing ART (Figure 1). Table 1 shows the baseline characteristics of the study population. The ATV/r group included significantly younger (p=0.019), more patients of East Asian origin (p=0.009) with higher BMI (p=0.014), higher CD4 count (p=0.038), higher baseline serum uric acid (p=0.007), and a larger proportion of patients with past history of urinary stones (p=0.017) and previous exposure to IDV (p=0.036). In contrast, patients of the DRV/r group were significantly more likely to use tenofovir (p <0.001) and with higher viral load (p=0.002) (Table 1).

Thirty eight patients fulfilled the pre-defined criteria for nephrolithiasis. Nephrolithiasis was identified in 1 (0.2%) of the DRV/r group and 37 patients (7.1%) of the ATV/r group, with an estimated incidence of 0.86 and 20.2 per 1,000 person-years, respectively. The incidence of nephrolithiasis in the ATV/r group was approximately 20 times higher than that in the DRV/r group.

Of the patients with nephrolithiasis, 9 and 12 were diagnosed by hematuria and stone passage, respectively, as defined above. Furthermore, 17 were diagnosed by radiological studies, of which renal calcification was identified in 5 patients. Figure 2 shows the time from initiation or switching of DRV/r or ATV/r to the diagnosis of nephrolithiasis by the Kaplan Meier method. Patients of the ATV/r group were significantly more likely to develop renal stones, compared to those of the DRV/r group (p<0.001, Log-rank test).

The median time from the commencement of ART to the diagnosis of nephrolithiasis was 28.1 months [interquartile range (IQR), 18.4–42.7 months] for the ATV/r group and only one patient with nephrolithiasis in the DRV/r group was diagnosed 11.2 month after the introduction of DRV/r-containing ART. The total observation period was 1,163.6 patient-years [median, 27.1 months, IQR, 18.1–38.4 months] for the DRV/r group, and 1,829.6 patient-years [median, 40.6 months, IQR, 17.5–42.7 months] for the ATV/r group. Among the ATV/r group, the median total bilirubin value of the renal stone group was marginally higher than that of the non-renal stone group [2.7 (IQR 2-3.8) and 2.2 mg/dl (IQR 1.6-3.0), respectively, P=0.051].

Univariate analysis showed a significant relationship between ATV/r use and nephrolithiasis (HR=26.01; 95% CI, 3.541–191.0; p=0.001) (Table 2). Higher serum uric acid (HR=1.415; 95% CI, 1.173–1.705; p<0.001) and past history of nephrolithiasis (HR=2.658; 95% CI, 1.111–6.359; p=0.028) were also significantly associated with the nephrolithiasis. On the other hand, tenofovir use was negatively associated with nephrolithiasis (HR=0.435; 95% CI, 0.210–0.899; p=0.025) (Table 2). Multivariate analysis identified ATV/r use over DRV/r as an independent risk for nephrolithiasis after adjustment for age, male sex, and weight (adjusted HR=27.08 95% CI, 3.680–199.3; p=0.001) (Table 3, Model 2), and also after adjustment for other risk factors (adjusted HR= 21.47; 95% CI, 2.879–160.2; p=0.003) (Table 3, Model 3).

The chemical composition of the renal stones of the single case on DRV/r was analyzed with high performance liquid chromatography with ultraviolet detection (HPLC-UV) method as described elsewhere [19,20], but the analysis did not identify DRV. Renal stones of patients on ATV/r were not analyzed.

Discussion

To our knowledge, this is the first study that investigated the incidence of DRV/r-associated nephrolithiasis. Only a single case of nephrolithiasis was detected among 540 patients on DRV/r-containing ART with total observation period of 1,163.6 patient-years. The incidence of nephrolithiasis in the DRV/r group was only 0.86 per 1,000 person-years, comparable to that in the general population in Japan (1.14 per 1,000 person-

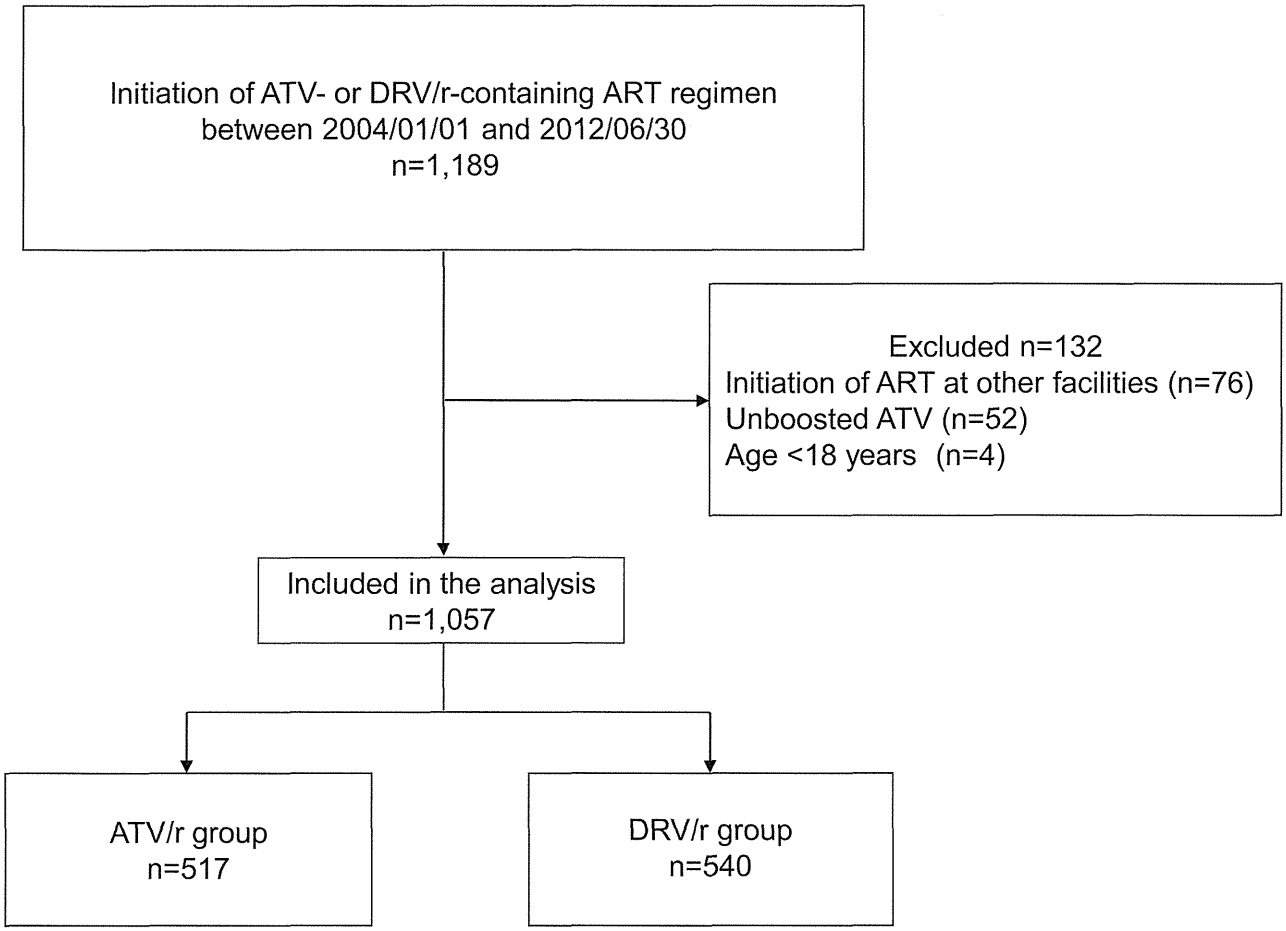

Figure 1. Flow diagram of patient selection. ART, antiretroviral therapy; ATV, atazanavir; DRV/r, ritonavir-boosted darunavir; ATV/r, ritonavir-boosted atazanavir.
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years) [21], whereas that in the ATV/r group was 20.2 per 1,000 person-years, approximately 20 times higher. Univariate and multivariate analyses identified ATV/r use over DRV/r as an independent risk factor for nephrolithiasis with a high hazard ratio. Furthermore, in the single patient with nephrolithiasis on DRV/r, DRV was not detected as a component of renal stones.

This study showed that the risk of nephrolithiasis is substantially lower among patients on DRV/r- than those on ATV/r-containing ART based on clinically feasible criteria. This finding is important considering DRV/r and ATV/r are the two PIs considered the preferred regimen for the treatment-naïve patients (http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf). Both PIs have similar characteristics; they are highly effective and tolerable with favorable lipid profile, and possess a high barrier to drug resistance [1-4]. One of the strengths of ATV/r is more abundant clinical evidence due to longer market availability than that of DRV/r. On the other hand, ATV/r often causes indirect hyperbilirubinemia, and requires acidic gastric environment for optimal absorption that requires some consideration on drug-drug interactions (http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf) (http://packageinserts.bms.com/pi/pi_reyataz.pdf). The substantially lower incidence of renal stones in patients on DRV/r than ATV/r adds another dimension to patient management in relation to the selection of a PI.

The development of renal stones, even a single episode, is a risk factor for CKD, doubling of serum creatinine level, and ESRD [12,13,16]. Many studies have also demonstrated that ATV/r use is a risk for renal dysfunction and CKD [22-25]. The high incidence of nephrolithiasis with ATV/r use identified in the present study may in part explain the risk of ATV/r for CKD. Thus, ATV/r should be introduced carefully in patients with concomitant predisposing factors for CKD. In this regard, there are no studies that show the association of DRV/r use with renal dysfunction or CKD, although this may in part be due to more recent introduction of DRV/r compared with ATV/r.

Why is nephrolithiasis less likely to occur with DRV/r compared to ATV/r? Although the mechanism of PI-induced nephrolithiasis is not fully understood, precipitation of pure PI is suggested as a possible etiology [8]. Up to 20% of IDV (an old PI well-known for its precipitation and renal stone formation) is

Table 1. Baseline demographics and laboratory data of patients who received ritonavir-boosted darunavir- or ritonavir-boosted atazanavir-containing antiretroviral therapy.

	DRV/r (n=540)	ATV/r (n=517)	P[a]
Age, years*	39 (33-46)	36 (31-44)	0.019
Male sex	498 (92.2)	480 (92.8)	0.727
Race (East Asian origin)	494 (91.5)	494 (95.6)	0.009
Body weight, kg*	62.1 (55.8-70)	64.0 (57.6-72)	0.074
body mass index, kg/m^2*	21.7 (19.8-24.1)	22.4 (20.4-24.6)	0.014
CD4 cell count, cells/μL*	251 (90-399)	260 (190-383)	0.038
HIV load, $\log_{10}$ copies/mL*	4.27 (1.70-5.17)	3.94 (1.70-4.66)	0.002
Treatment naïve	309 (57.2)	280 (54.2)	0.322
Tenofovir use	342 (63.3)	196 (37.9)	<0.001
eGFR, mL/min/1.73 m^2*	116 (102-131)	115 (103-130)	0.842
Serum uric acid, mg/dL*	5.7 (4.7-6.5)	5.9 (5.1-6.7)	0.007
HBV or HCV coinfection	78 (14.4)	64 (12.4)	0.367
Past history of nephrolithiasis	22 (4.1)	39 (7.5)	0.017
Previous exposure to IDV	25 (4.6)	41 (7.9)	0.030

Data are number (%) of patients or * median (interquartile range).

DRV/r, ritonavir-boosted darunavir; ATV/r, ritonavir-boosted atazanavir; eGFR, estimated glomerular filtration rate; HBV, hepatitis B virus, HCV, hepatitis C virus, HIV, human immunodeficiency virus; IDV, indinavir.

a. The χ^2 test or Fisher exact test was used for categorical data, and the Student *t* test was used for continuous variables.

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excreted unchanged in the urine, a property that contributes to the high incidence of nephrolithiasis in patients treated with IDV [26] (http://www.merck.com/product/usa/pi_circulars/c/crixivan/crixivan_pi.pdf). Unchanged DRV and ATV are reported to be excreted in urine at similar proportions of 7.7% and 7% of the administered dose, respectively (http://packageinserts.bms.com/pi/pi_reyataz.pdf) (http://www.merck.com/product/usa/pi_circulars/c/crixivan/crixivan_pi.pdf). However, strong acidity (e.g., pH of 1.9) is required to achieve optimal dissolution of ATV, and its solubility in urine is known to decrease with increase in pH (http://packageinserts.bms.com/pi/pi_reyataz.pdf). Because urine is usually mildly acidic [9], the difference in the solubility of DRV and ATV in urine might explain the different incidence of nephrolithiasis in patients using these two PIs. Although de Lastours et al [11] described the presence of DRV crystals in the urine of 4 (7.8%) out of 51 patients on DRV/r and suggested that DRV/r use might be a risk for renal stones, the number of enrolled patients in their study was relatively small to allow firm conclusions.

The present study has several limitations. First, due to the retrospective nature of the study, the baseline characteristics of the enrolled patients were not controlled. It is possible that more patients with potential risks for nephrolithiasis were included in the ATV/r group. In the ATV/r group, more patients were hyperuricemic, had history of renal stones, and previous exposure to IDV, which are known risk factors for nephrolithiasis. However, multivariate analysis clearly showed that ATV/r use is an independent risk factor with high hazard ratio even after adjustment for variables including the above three. Second, the median observation period was longer in the ATV/r group than in the DRV/r group (40.6 versus 27.1 months), suggesting that the risk of nephrolithiasis in the ATV/r group could be overestimated. Further studies are warranted to elucidate whether much longer use of DRV/r induces nephrolithiasis. However, it is noteworthy that in patients with nephrolithiasis, the median time from the commencement of ATV/r or DRV/r to the diagnosis of nephrolithiasis was 28.1 months (IQR: 18.4-42.7 months), which was similar to that of the DRV/r group [median 27.1 (IQR: 18.1-38.4)], backing up the result of the present study: the risk of nephrolithiasis is substantially lower among patients on DRV/r than those on ATV/r. Third, stone composition analysis was conducted in only one patient with renal stones (treated with DRV/r), therefore, it is possible that renal stones caused by other etiologies are included.

In conclusion, the present study demonstrated that the risk of nephrolithiasis, an important risk factor of CKD, is approximately 20 times lower among patients on DRV/r- than those on ATV/r-containing ART, providing DRV/r one advantage over ATV/r in the selection of PI. ATV/r use was identified as a significant and independent risk factor for nephrolithiasis in a robust statistical model that included ATV/r use over DRV/r as a primary exposure. ATV/r should be prescribed with caution in patients with predisposing factors for nephrolithiasis and those with CKD.

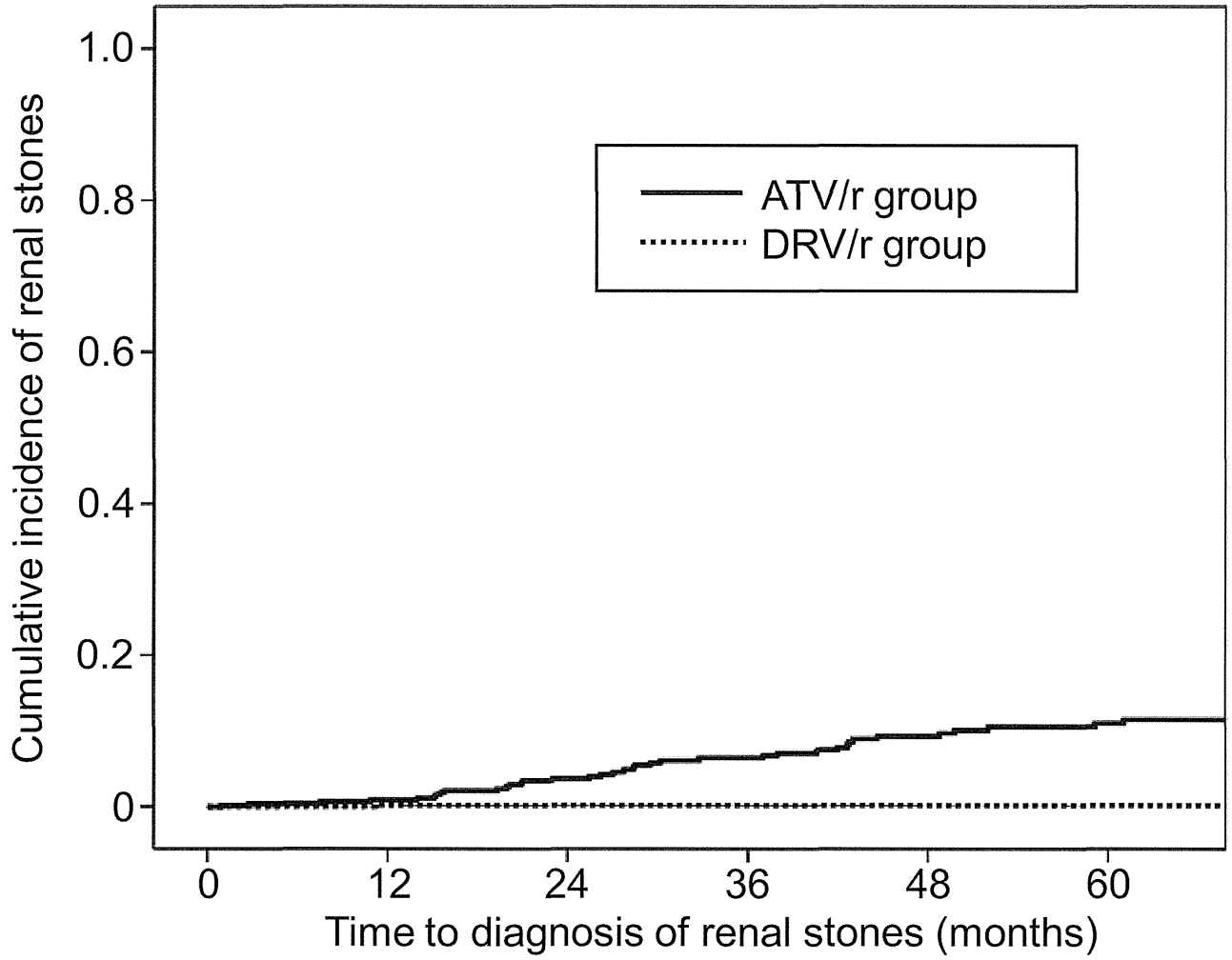

Figure 2. Kaplan-Meier curve showing time to the diagnosis of nephrolithiasis. ATV/r, ritonavir-boosted atazanavir; DRV/r, ritonavir-boosted darunavir.

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Table 2. Univariate analysis to estimate the risk of various factors for nephrolithiasis.

	Hazard ratio	95%CI	P value
ATV/r use over DRV/r	26.01	3.541-191.0	0.001
Age per 1 year	1.002	0.973-1.031	0.907
Male sex	1.665	0.401-6.919	0.483
Race (East Asian origin)	2.287	0.314-16.68	0.414
Weight per 1 kg increment	0.998	0.970-1.027	0.872
body mass index per 1 kg/m^2 increment	0.996	0.905-1.095	0.927
CD4 count per 10 /μl increment	0.999	0.983-1.016	0.901
HIV viral load per log10/ml	1.063	0.859-1.316	0.575
Treatment naïve	1.020	0.538-1.936	0.950
Tenofovir use	0.435	0.210-0.899	0.025
Baseline eGFR per 10 ml/min/1.73 m^2 decrement	1.103	0.980-1.242	0.105
Baseline serum uric acid per 1 mg/dl increment	1.415	1.173-1.705	<0.001
Hepatitis B or Hepatitis C	1.418	0.592-3.393	0.433
Past history of renal stone	2.658	1.111-6.359	0.028
Previous exposure to IDV	1.192	0.366-3.879	0.771

ATV/r, ritonavir-boosted atazanavir; DRV/r, ritonavir-boosted darunavir; HIV, human immunodeficiency virus; eGFR, estimated glomerular filtration rate; IDV, indinavir.

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Table 3. Multivariate analysis to estimate the risk of ATV/r- over DRV/r-containing antiretroviral therapies for nephrolithiasis.

	Model 1 crude (n=1,057)		Model 2 adjusted (n=1,056)		Model 3 adjusted (n=1,021)	
	HR	95%CI	HR	95%CI	HR	95%CI
ATV/r use over DRV/r	27.05	3.687-198.5	27.08	3.680-199.3	21.47	2.879-160.2
Age per 1 year			1.009	0.980-1.039	1.006	0.976-1.037
Male sex			1.939	0.441-8.528	1.202	0.262-5.512
Weight per 1 kg increment			0.988	0.956-1.021	0.979	0.947-1.012
Tenofovir use					0.678	0.313-1.470
Baseline serum uric acid per 1mg/dl increment					1.418	1.150-1.750
Past history of renal stone					1.661	0.520-5.307
Past exposure to IDV					0.491	0.100-2.403

HR, Hazard ratio; CI, confidence interval; ATV/r, ritonavir-boosted atazanavir; DRV/r, ritonavir-boosted darunavir; HIV, IDV, indinavir.

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Author Contributions

Conceived and designed the experiments: TN YH HG SO. Performed the experiments: TN YH KW K. Teruya. Analyzed the data: TN YH HK EK. Contributed reagents/materials/ analysis tools: K. Tsukada YK. Wrote the manuscript: TN YH HG SO.

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Incidence and Risk Factors for Incident Hepatitis C Infection Among Men Who Have Sex With Men With HIV-1 Infection in a Large Urban HIV Clinic in Tokyo

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Background: The epidemiology of hepatitis C virus (HCV) infection among HIV-infected men who have sex with men (MSM) who do not inject drugs in Asia remains unknown.

Method: The incidence and risk factors for incident HCV infection among HIV-infected MSM at a large HIV clinic in Tokyo were elucidated. Poisson regression compared the incidence of HCV seroconversion at different observation periods.

Results: Of 753 HIV-1 infected MSM patients negative for HCV antibody (HCVAb) at baseline and available follow-up HCVAb test, 21 patients (2.8%) seroconverted to HCVAb positive over 2246 person-years (PY), for an incidence of 9.35 per 1000 PY. The incidence increased over time from 0 per 1000 PY in 2005–2006, 3.0 per 1000 PY in 2007–2008, 7.7 per 1000 PY in 2009–2010, to 24.9 per 1000 PY in 2011–2012 ($P = 0.012$). Of 21 incident cases, only 4 (19%) were injection drug users, and sensitivity analysis that excluded injection drug users yielded similar findings. Multivariate analysis identified illicit drug use to be an independent risk for HCV infection (hazard ratio = 3.006; 95% confidence interval: 1.092 to 8.275; $P = 0.033$).

Conclusions: Incident HCV infection is increasing among HIV-1-infected MSM noninjection drug users at resource-rich setting in Asia. Illicit drug use is an independent risk factor for incident HCV infection in this population.

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INTRODUCTION

Coinfection with hepatitis C virus (HCV) in patients with HIV-1 infection is one of the major comorbidities and associates with worsened mortality.[1–6] Although HCV infection is especially common among injection drug users because of transparental infection,[7] noninjection illicit drug use and traumatic sexual behavior in the absence of injection drug use are reported in the United States and Europe to enhance noninjection drug-related transmission of HCV.[8–14]

In Japan, because of the low prevalence of injection drug use in the general population (lifetime prevalence of illicit drug use as a whole in 2009 was only 2.9%), coinfection with HCV in patients with HIV-1 infection is rare.[15–18] Recently, however, at our clinic, the AIDS Clinical Center, Tokyo, there has been a surge in the rate of acute HCV infection among men who have sex with men (MSM) with HIV-1 infection who deny using injection drugs. The AIDS Clinical Center is one of the largest referral centers for HIV care in Japan with patients mostly comprising MSM.[19]

This study was designed to determine the incidence and risk factors of incident HCV infection in MSM with HIV-1 infection at a resource-rich setting in Asia, with a special focus on noninjection illicit drug use.

METHODS

Study Subjects

The study population was HIV-1-infected MSM, aged older than 17 years, who visited our clinic for the first time from January 2005 to December 2010, and with negative HCV antibody (HCVAb) at baseline and at least 1 subsequent HCVAb test result. The following exclusion criteria were applied: (1) patients who visited the clinic for a second opinion, (2) patients referred to other facilities on their first or second visit. The study patients were followed up until December 31, 2012.

Measurements

At our clinic, HCVAb, CD4 cell count, HIV-1 viral load, hepatitis B surface antigen (HBsAg), and *Treponema pallidum* latex agglutination (TPHA) test are routinely conducted on the first visit. Patients positive for HCVAb subsequently undergo another HCV-RNA measurement. Patients visit our clinic at least every 3 months for monitoring CD4 cell count, HIV-1 viral load, and liver function tests, because the prescription period under the Japanese health care system is limited to 3 months. Repeat HCVAb tests were performed at the discretion of the treating physician. Laboratory data and baseline demographics on the first visit were collected from the medical records.

Social demographics including sexuality and history and type of illicit drug used (injection or noninjection) were collected through a structured interview conducted on the first visit.[20] Because the interview could underestimate the prevalence of illicit drug use, we also searched the medical records for information on illicit drug use and related variables covering the period from the first visit to December 2012.

At our clinic, written informed consent is obtained from each patient to store serum samples drawn at the first visit and subsequent visits at the discretion of the treating physician.[21] Either latest HCVAb test result conducted in clinical practice or from the latest stored serum sample was used to determine the status of HCV infection, whichever the follow-up time was longer. This study was approved by the Human Research Ethics Committee of the hospital.

Statistical Analysis

The incidence of HCV was calculated by dividing the number of HCVAb seroconversion by person-time at risk. Person-time represented the time from the first visit to the first positive HCVAb in patients diagnosed with incident HCV infection and the time from the first visit to the last negative HCVAb result in patients without incident infection. Poisson regression was used to compare the incidence of HCV seroconversion among 4 observation periods.

The uni- and multivariate Cox proportional hazards model was used to estimate the impact of illicit drug use as a whole over nonusers on the incidence of HCV infection as a primary exposure.

In multivariate analysis, age was added to the univariate analysis for illicit drug use over nonuse to form model 2. In model 3, injection drug use and insurance status were added because of their significant association with incident HCV infection in univariate analysis. Methamphetamine use and incarceration because of drugs were not added because of their multicollinearity with injection drug use (most injection drug users in Japan use methamphetamine injection).[18] Baseline CD4 count, HBsAg, and TPHA were added to the model because previous studies showed that low CD4 count and these infections are risk factors.[6,22]

Statistical significance was defined at 2-sided P values of <0.05. We used the hazard ratios (HRs) and 95% confidence intervals (95% CIs) to estimate the impact of each variable on the incidence of HCV infection. All statistical analyses were performed with the Statistical Package for Social Sciences version 20.0 (SPSS, Chicago, IL).

RESULTS

A total of 1182 MSM patients with HIV-1 infection visited the AIDS Clinical Center for the first time during the study inclusion period. Of these, 114 patients were excluded from the analysis (Fig. 1). The remaining 1068 patients had baseline HCVAb results, of whom 22 were positive for

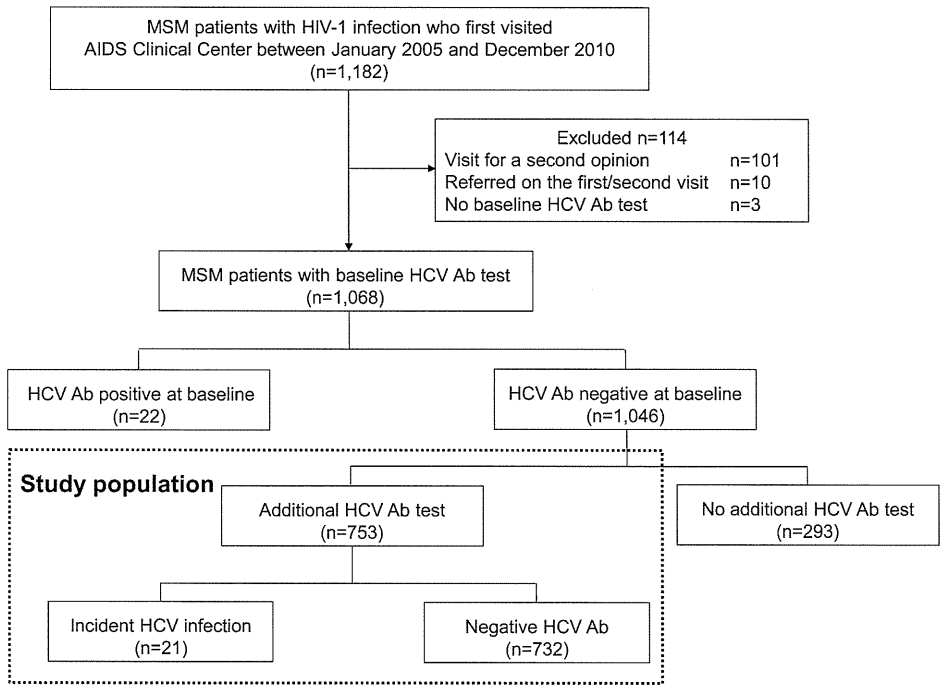

FIGURE 1. Patient enrollment process.

TABLE 1. Baseline Characteristics of Patients With and Without Incident HCV Infection

	All (n = 753)	Incident HCV (n = 21)	HCV-free (n = 732)	*P*
Median (IQR) age (yrs)	35 (29–41)	35 (32–42)	35 (29–41)	0.951
Illicit drug use, n (%)	298 (40)	15 (71)	283 (39)	0.003
Injection drug use, n (%)	37 (5)	4 (19)	33 (5)	0.016
Methamphetamine use, n (%)	48 (6)	4 (19)	44 (6)	0.039
Arrest due to illicit drug use, n (%)	24 (3)	3 (14)	21 (3)	0.026
Median (IQR) CD4 count (/μL)	244 (100–365)	311 (159–391)	244 (98–364)	0.539
Median (IQR) HIV-1 viral load (log_{10}/mL)	4.69 (4.00–5.20)	4.54 (3.51–4.97)	4.69 (4.00–5.22)	0.110
AIDS, n (%)	223 (30)	7 (33)	216 (30)	0.809
Positivity for hepatitis B surface antigen	48 (6)	2 (10)	46 (6)	0.638
Positivity for TPHA	236 (31)	8 (38)	228 (31)	0.483
On antiretroviral therapy, n (%)	66 (9)	3 (14)	63 (9)	0.419
Ethnicity, n (%)				0.537
Japanese	712 (95)	21 (100)	691 (94)	
Other Asian	20 (3)	0	20 (3)	
White	21 (3)	0	21 (3)	
Health insurance status, n (%)				0.003
With insurance	704 (94)	16 (76)	688 (94)	
No insurance	15 (2)	1 (5)	14 (2)	
On social benefits	34 (5)	4 (19)	30 (4)	
Median (IQR) follow-up days	984 (539–1557)	852 (324–1491)	997 (543–1562)	0.334

Patients' characteristics and social demographics were compared between those with incident HCV infection and those without such infection by the Student *t* test for continuous variables and by either the χ^2 test or Fisher exact test for categorical variables.

HCVAb. Of 1046 HIV-1 infected MSM patients with negative baseline HCVAb, the follow-up HCVAb test was available for 753 (72%) patients, either with clinical data (n = 295) or with stored blood samples (n = 458). The study population comprised the latter group of 753 patients.

Twenty-one patients (2.8%) incidentally seroconverted to HCVAb positive over 2246 person-years (PY) of total observation period, with an incidence of 9.35 per 1000 PY (95% CI: 6.12 to 14.2). The median time from the first visit to HCV seroconversion was 852 days [interquartile range (IQR), 324–1491 days]. The incidence increased over time from 0 per 1000 PY (0 case/270 PY) in 2005–2006, 3.0 per 1000 PY (2/672 PY) in 2007–2008, 7.7 per 1000 PY (6/779 PY) in 2009–2010, to 24.9 per 1000 PY (13/522 PY) in 2011–2012 ($P = 0.012$). As sensitivity analysis, the same calculations were conducted for the study population excluding injection drug users, and the results were similar; 17 patients became HCVAb positive over 2146 PY for an incidence of 7.92 per 1000 PY, and the incidence increased over time [0/1000 PY (0/258 PY) in 2005–2006, 1.5/1000 PY (1/650 PY) in 2007–2008, 8.0/1000 PY (6/747 PY) in 2009–2010, and 20.2/1000 PY (10/495 PY) in 2011–2012 ($P = 0.045$)].

The study patients were mostly Japanese men of relatively young age, covered with health insurance (Table 1); 40% of the study patients were illicit drug users based on the interview and medical records. Amyl nitrite, 5-methoxy-diisopropyltryptamine, 3,4-methylenedioxymethamphetamine, cannabis, heroin, cocaine, and opium were named by the patients. Patients with incident HCV infection were significantly more likely to be illicit drug users ($P = 0.003$). In addition, incident HCV cases were more likely to be injection drug users ($P = 0.016$), methamphetamine users ($P = 0.039$), and incarcerated owing to illicit drug use ($P = 0.026$) (Table 1). In contrast to the high prevalence of illicit drug use (40%), the prevalence of injection drug use was low (5%).

Among the 21 HCV incident cases, only 4 (19%) patients were injection drug users. Of the 21, 18 patients were screened for HCVAb because of high serum alanine aminotransferase levels, including all 17 positive for HCV RNA.

Among illicit drug users (n = 298) and nonusers (n = 455), 15 (5.0%) and 6 (1.3%) patients, respectively, were infected with HCV, with incidence of 16.6 and 4.48 per 1000 PY, respectively. Illicit drug users were significantly more likely to be infected with HCV, compared with nonusers ($P = 0.004$, Log-rank test). The total observation period was 906 PY (median, 1012 days; IQR, 543–1607 days) for illicit drug users and 1340 PY (median, 963 days; IQR, 538–1542 days) for nonusers.

Univariate analysis showed a significant relationship between illicit drug use and incident HCV infection (HR = 3.662; 95% CI: 1.420 to 9.439; $P = 0.007$) (see **Table S1, Supplemental Digital Content**, http://links.lww.com/QAI/A479). Furthermore, injection drug use (HR = 5.387; 95% CI: 1.804 to 16.09; $P = 0.003$), methamphetamine use (HR = 3.220; 95% CI: 1.083 to 9.573; $P = 0.035$), incarceration owing to illicit drugs (HR = 4.857; 95% CI: 1.429 to 16.51; $P = 0.011$), and on social benefits (government financial assistance) (HR = 6.982; 95% CI: 2.308 to 21.12; $P = 0.001$) were significantly associated with incident HCV infection. Conversely, age, low baseline CD4 count, positive HBsAg, and positive TPHA were not associated with incident HCV infection.

Multivariate analysis identified illicit drug use as a significant risk for incident HCV infection after adjustment for age (adjusted HR = 3.814; 95% CI: 1.447 to 10.05; P = 0.007) and for other variables (adjusted HR = 3.006; 95% CI: 1.092 to 8.275; P = 0.033) (see **Table S2, Supplemental Digital Content**, http://links.lww.com/QAI/A479). Injection drug users (HR = 4.672; 95% CI: 1.425 to 15.31; P = 0.011) and on social benefits (HR = 7.942; 95% CI: 2.370 to 26.62; P = 0.001) were also risk factors for incident HCV infection in multivariate analysis.

DISCUSSION

At our large urban HIV clinic in Tokyo, 21 (2.8%) HIV-infected MSM patients were newly infected with HCV during the study period, with an incidence of 9.35 per 1000 PY. The incidence of HCV infection showed statistically significant increase over the observation period, from 0 per 1000 PY in 2005–2006 to 24.9 per 1000 PY in 2011–2012. Most incident HCV cases were noninjection drug users, as 17 (81%) patients were not identified as using injection drugs. The incidence of new HCV infection was >3-folds higher in any illicit drug users than nonusers, with estimated incidence of 16.6 and 4.48 per 1000 PY, respectively, and illicit drug use was identified as a significant risk factor for incident HCV infection in multivariate analysis.

To our knowledge, this is the first study in Japan and is the second in Asia to report the incidence and risk factors for incident HCV infection among HIV-infected MSM.[23] The incidence of 9.35 per 1000 PY was similar to that reported in North America and Western Europe (8.7–16.3/1000 PY) and was also very close to that of Taiwan (9.25/1000 PY).[23–26] The results of this study demonstrated the emergence of noninjection drug-related HCV infection in HIV-1-infected MSM in resource-rich settings in Asia and support routine rescreening for HCVAb among HIV-infected MSM, in particular among drugs users, both injectors and noninjectors.

The reason for the increasing trend of incident HCV infection in HIV-infected MSM noninjection drug users remains to be elucidated. Both baseline positive HBsAg and TPHA, markers for sexual activity, were not associated with incident HCV in this study. This is probably because these variables were collected at the baseline and do not necessarily reflect the ongoing sexual activity during observation period. However, based on reports from Western Europe and North America, it is reasonable to assume that such change in the incidence is mainly because of increased practice of high-risk sexual behaviors, such as unprotected anal intercourse, group sex, and fisting (often practiced in the context of illicit drug use that can lower the pain threshold, leading to increased bleeding) among MSM with HIV-1 infection in Japan.[10] Sharing of drug paraphernalia and possible intranasal/intrarectal transmission can also contribute.[27,28] Because of the introduction of effective and tolerable antiretroviral therapy, the life expectancy and quality of life of people with HIV infection has improved substantially, which could associate with increased high-risk behavior in MSM with HIV-1 infection.[29,30]

Several limitations need to be acknowledged. First, because this study did not count the number of HCVAb tests for each patient during each observation period, it is possible that the increasing trend of incident HCV infection is because of increasing number of HCV testing in recent years. However, most patients with incident HCV infection were tested for HCVAb in clinical practice, because they were suspected to have acute HCV infection based on elevated liver enzymes. Thus, it is highly likely that the increasing trend of HCV infection shown in this study is true for this cohort. Second, we were not able to rescreen for HCVAb for 28% of the patients with negative baseline HCVAb result. Third, the structured interview designed for data collection and chart review do not necessarily prevent underreporting of illicit drug use and injection drug use. However, underreporting to a certain degree is unavoidable with regard to issues such as illicit drugs.[31]

In conclusion, this study showed that incident HCV is increasing among mostly noninjection drug use MSM with HIV-1 infection at resource-rich setting in Asia. Illicit drug use was identified as an independent risk factor for incident HCV infection. It is important to raise awareness of noninjection drug-related HCV infection and to take appropriate measures to prevent HCV infection in this population.

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PLOS ONE

Traditional but Not HIV-Related Factors Are Associated with Nonalcoholic Fatty Liver Disease in Asian Patients with HIV-1 Infection

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Abstract

Background: The prevalence and factors associated with nonalcoholic fatty liver disease (NAFLD) are largely unknown in HIV-1 monoinfected patients.

Methods: The present study elucidated the prevalence and factors associated with NAFLD among Asian patients with HIV-1 infection who underwent abdominal ultrasonography between January 2004 and March 2013. Diagnosis of NAFLD was based on the liver to kidney contrast and diffusion in hepatic echogenicity. Uni- and multi-variate logistic regression analyses were applied to estimate factors associated with NAFLD.

Results: 435 Asian patients free of chronic hepatitis B or C virus infection and without excessive alcohol intake were analyzed. NAFLD was diagnosed in 135 (31%) patients. Obesity (BMI >30 kg/m^2) was evident in 18 (4.1%) patients, and BMI was >25 kg/m^2 in 103 (24%). Multivariate analysis identified higher BMI (per 1 kg/m^2 increment, adjusted OR = 1.198; 95% CI, 1.112–1.290; $p<0.001$), dyslipidemia (adjusted OR = 2.045; 95% CI, 1.183–3.538; $p = 0.010$), and higher ALT to AST ratio (per 1 increment, adjusted OR = 3.557; 95% CI, 2.129–5.941; $p<0.001$) as significant factors associated with NAFLD. No HIV-specific variables, including treatment with dideoxynucleoside analogues (didanosine, stavudine, and zalcitabine) and cumulative duration of antiretroviral therapy (ART), were associated with NAFLD.

Conclusions: The incidence of NALFD among Asian patients with HIV-1 infection is similar to that in Western countries. NAFLD was associated with high BMI, dyslipidemia, and high ALT/AST ratio, but not with HIV-related factors. The results highlight the importance of early recognition and management of NAFLD and traditional factors associated with NAFLD for Asian patients with HIV-1 infection.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by the presence of fat infiltration in the liver in the absence of excessive alcohol consumption or other causes of liver disease, such as viral hepatitis, and is considered the most common cause of fatty liver [1]. NAFLD is a major health issue since it can lead to fibrosis, cirrhosis, liver cancer, and mortality [2]. Although the prevalence of NAFLD seems increasing in parallel with the current epidemic of obesity, it varies among the general population according to the geographical area; for example, the prevalence of NAFLD in the US ranges from 10 to 46% [3,4], whereas in Asia it is 5–30% [5].

In the general population, obesity, type 2 diabetes mellitus, dyslipidemia, and metabolic syndrome are established conditions associated with NAFLD [6]. In addition to abovementioned environmental risk factors, genetic factors are also associated with the incidence of NAFLD [7]. However, only two studies (one from Italian metabolic clinic and the other from American naval hospital) have previously examined the prevalence and associated factors with NAFLD in patients infected with HIV-1 only (those without chronic hepatitis C virus (HCV) infection) [8,9]. At this stage, it is unknown whether variables specific to HIV-1 infection, such as HIV-1 viral load and cumulative years of antiretroviral therapy (ART) are associated with NAFLD. Although the use of so called "D drugs": dideoxynucleoside analogues [didanosine (ddI), stavudine (d4T), and zalcitabine (ddC)], a subgroup of antiretroviral agents nucleoside reverse transcriptase inhibitors (NRTI), is

reported to be associated with NAFLD, others have argued against such relation [8,9].

Liver diseases are important causes of morbidity and mortality among patients with HIV-1 infection [10–12], especially following the wide availability of ART and substantial improvement in prognosis of such patients [13]. Currently, there is no information on the prevalence and associated factors related to NAFLD among patients with HIV-1 infection in Asia, the region with the second largest number of patients with HIV-1 infection. The present study was designed to elucidate the prevalence and associated factors, including D drug use, with NAFLD in Asian patients with HIV-1 infection.

Methods

Ethics statement

This study was approved by the Human Research Ethics Committee of the National Center for Global Health and Medicine, Tokyo. Each participant provided a written informed consent for the clinical and laboratory data to be used and published for research purposes. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Study design

We performed a single-center cross-sectional study of HIV-1-infected patients using the abdominal ultrasonography data and medical records at the National Center for Global Health and Medicine, Tokyo, Japan. Our facility is one of the largest clinics for patients with HIV infection in Japan with approximately 3,500 registered patients [14]. The study population was HIV-infected patients, aged >17 years, who underwent routine abdominal ultrasonography conducted by certified medical technologists at the Physiological Examination Unit of the hospital, between January 1, 2004 and March 31, 2013. The following exclusion criteria were employed in this study; 1) HCV or hepatitis B virus (HBV) infection defined by positive hepatitis C antibody or positive hepatitis B surface antigen, respectively, 2) use of injection drugs, 3) hemophilia, because all HIV-infected hemophiliacs in Japan were exposed to HCV through contaminated blood products [15], and 4) alcohol consumption >20 g of ethanol per day for males and >10 g/day for females. Fatty liver was diagnosed based on hyperechogenicity of the liver compared to renal cortex and diffusion in hepatic echogenicity [8,16,17]. The ultrasonographic images and diagnosis were double-checked and confirmed by radiologists, hepatologists, or gastroenterologists. If abdominal ultrasonography was conducted more than once during the study period, the latest data were used for the study.

Measurements

The potential risk factors for NAFLD were selected according to previous studies and collected from the medical records [18,19], together with the basic demographic data. These factors included age, sex, race, body weight, body mass index (BMI) = {bodyweight (kg)/[(height (m)]^2}, and presence or absence of other medical conditions [diabetes mellitus, defined by use of glucose-lowering agents or fasting plasma glucose ≥126 mg/dl or plasma glucose ≥ 200 mg/dl on two different days, dyslipidemia, defined by current treatment with lipid-lowering agents or two successive measurements of either low-density lipoprotein cholesterol (LDL-C) > 140 mg/dl, high-density lipoprotein cholesterol (HDL-C) < 40 mg/dl, total cholesterol (TC) >240 mg/dl, triglyceride (TG) >500 mg/dl, and hypertension defined by current treatment with antihypertensive agents or two successive measurements of systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥ 90 mmHg at the clinic]. Data on smoking status and alcohol consumption were collected through a structured interview conducted at the first visit as part of routine clinical practice by the nurses specializing at the HIV outpatient care. Patients were divided into three groups according to the smoking status: non-smokers, low (<20 cigarettes/day) and heavy smokers (≥20 cigarettes/day). They were also divided according to alcohol consumption into two groups: non-drinkers and light drinkers (< 20 g ethanol/day for men and <10 g ethanol/day for women). The values of alanine aminotransferase (ALT), aspartate aminotransferase (AST), TC, LDL-C, HDL-C, and TG within three months and closest to the day ultrasonography was conducted were collected. HIV-specific variables, such as CD4 cell count, HIV viral load, ART-experienced or ART-naïve, ART regimen at ultrasonography, history of AIDS, and duration of ART were also collected. The duration of D drugs use, as a possible risk factor for NAFLD, was collected, regardless of continuation of these drugs at the time of abdominal ultrasonography [9,20]. Patients were divided into four groups according to duration of treatment with D drugs; no D drugs use, <1 year exposure, 1–3 years of use, and >3 years of use. In our clinic, it is customary for the patient to visit the clinic once a month before the initiation of ART and until the suppression of HIV-1 viral load, but the visit interval is extended up to every three months after viral load suppression.

Statistical analysis

Baseline characteristics were compared between patients with and without NAFLD, using the Student's *t*-test or χ^2 test (Fisher's exact test) for continuous or categorical variables, respectively. Univariate logistic regression analysis was used to identify factors associated with NAFLD. Basic demographics, such as age and sex, and variables with p values <0.05 in univariate analysis were entered into multivariate logistic regression models. ALT, and TG and LDL-C were not added to the model, based on their multicollinearity with ALT to AST ratio and dyslipidemia, respectively. Statistical significance was defined as two-sided p value <0.05. We used the odds ratio (OR) and 95% confidence interval (95%CI) to estimate the association of each variable with NAFLD. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 20.0 (SPSS, Chicago, IL).

Results

Of the total of 895 patients with HIV-1 infection who underwent abdominal ultrasonography during the study period, 435 were included in the analysis (Figure 1). NAFLD was diagnosed by abdominal ultrasonography in 135 cases, with a prevalence of 31%. None of these patients had any ultrasonographic finding compatible with cirrhosis. Table 1 shows the characteristics of the study population, patients with NAFLD, and those without NAFLD. The study patients were mostly East Asian males with maintained CD4 count [median 349/μl, interquartile range (IQR) 203–512], and approximately half of the patients had suppressed viral load. Obesity (BMI >30 kg/m^2) was noted in 18 (4.1%) patients, and BMI was >25 kg/m^2 in 103 (24%). Body weight was significantly heavier in patients with NAFLD (median 71 kg, IQR 61–78 kg), compared with non-NAFLD (median 61 kg, IQR 55–68 kg, p<0.001), as was BMI (median 25, IQR 21.7–27.5 versus median 21.5, IQR 20–23.3, p<0.001). Dyslipidemia (p<0.001), hypertension (p = 0.019), high ALT (p = 0.017), high LDL-C (p = 0.041), hypertriglyceridemia (p = 0.008), and high CD4 count (p = 0.001) were significantly more common in

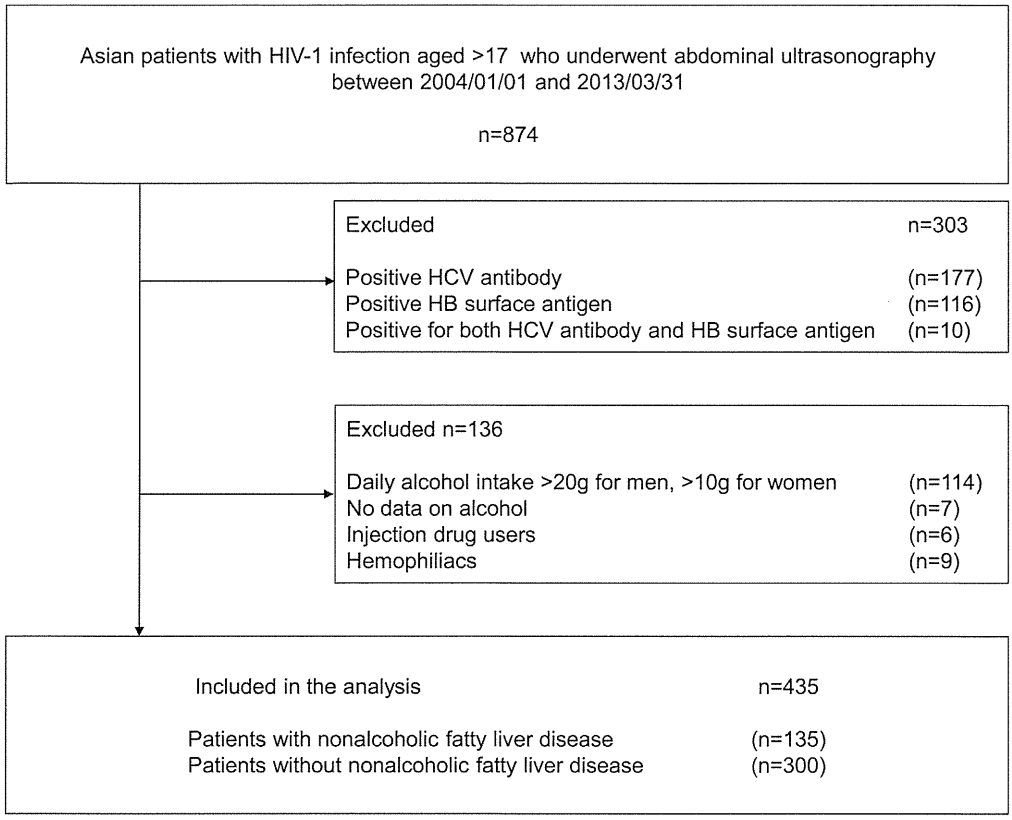

Figure 1. Patient enrollment process.
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patients with NAFLD than those without (Table 1). On the other hand, history of D drug use and cumulative years of ART were not significantly different between the two groups.

Univariate analysis showed a significant association between NAFLD and the following non-HIV specific variables (Table 2): higher BMI (per 1 kg/m^2 increment, OR = 1.282; 95% CI, 1.197–1.373; p<0.001), dyslipidemia (OR = 2.475; 95% CI, 1.594–3.842; p<0.001), hypertension (OR = 1.818; 95% CI, 1.117–2.961; p = 0.016), ALT to AST ratio (per 1 increment, OR = 4.831; 95% CI, 3.073–7.594; p<0.001), higher ALT (per 10 IU/l increment, OR = 1.027; 95% CI, 1.002–1.053; p = 0.034), higher triglyceride (per 10 mg/dl increment, OR = 1.021; 95% CI, 1.005–1.038; p = 0.010), and higher LDL-C (per 10 mg/dl increment, OR = 1.096; 95% CI, 1.003–1.196; p = 0.042). Among HIV-specific variables, only higher CD4 count was associated with NAFLD (per 1/μl increment, OR = 1.001; 95% CI, 1.001–1.002; p = 0.002)(Table 3). On the other hand, older age (per 1 year increment, OR = 0.996; 95% CI, 0.980–1.013; p = 0.668) and diabetes mellitus (OR = 1.577; 95% CI, 0.657–3.784; p = 0.308) were not associated with NAFLD. Compared to no D drug use, history of D drug use was not associated with NAFLD (Any to <1 year of D drug use, n = 42, OR = 0.956; 95% CI, 0.476–1.919; p = 0.899)(1 to 3 years of D drug use, n = 46, OR = 1.137; 95% CI, 0.592–2.184; p = 0.699)(> 3 years of D drug use, n = 40, OR = 0.533; 95% CI, 0.237–1.200; p = 0.129)(Table 3).

Among patients treated with D drugs (n = 128), the median time period since withdrawal was 3.46 years (IQR 1.03–6.29). Compared to treatment-naïve patients, ART use was not associated with NAFLD as well (<2 year of ART exposure, n = 80, OR = 1.110; 95% CI, 0.620–1.985; p = 0.726) (2 to 6 years of ART exposure, n = 100, OR = 0.941; 95% CI, 0.541–1.637; p = 0.830) (>6 year of ART exposure, n = 103, OR = 1.135; 95% CI, 0.664–1.943; p = 0.643)(Table 3).

Multivariate analyses identified the following variables as independently associated with NAFLD: BMI (per 1 kg/m^2 increment, adjusted OR = 1.198; 95% CI, 1.112–1.290; p< 0.001), dyslipidemia (adjusted OR = 2.045; 95% CI, 1.183–3.538; p = 0.010), ALT to AST ratio (per 1 increment, adjusted OR = 3.557; 95% CI, 2.129–5.941; p<0.001)(Table 4).

Discussion

To our knowledge, this is the first study that investigated the prevalence and associated factors of NAFLD in Asian patients with HIV-1 infection, and is the largest study that focused on NAFLD in patients with HIV-1 monoinfection (without chronic hepatitis C infection). The prevalence of NAFLD in this study was 31%, which is comparable to 31% at the Naval hospital in San Diego, US, and 36.9% at the metabolic clinic in Modena, Italy [8,9]. Multivariate analysis indicated that traditional predictors for NAFLD in the general population, such as higher BMI, dyslipidemia, and ALT to AST ratio [6], were significantly associated with NAFLD, whereas HIV-specific variables, including history of D drug use and cumulative years of ART, were not associated with NAFLD.

Our result of nearly one third of Asian patients with HIV-1 monoinfection have NAFLD highlights the importance of screening for NAFLD among this patient population, due to the potential progression of NAFLD to liver fibrosis, cirrhosis, and liver cancer [2,21]. In addition, the finding that higher BMI,