



Acute Hepatitis C in HIV-1 Infected Japanese Cohort: Single Center Retrospective Cohort Study

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Abstract

Objectives: HCV co-infection is a poor prognostic factor in HIV-1-infected patients. Although the number of newly reported patients who show seroconversion is increasing, the clinical features are still unclear, especially in Asian countries.

Design: A single-center retrospective cohort study of patients diagnosed between 2001–2012.

Methods: Acute hepatitis C (AHC) was diagnosed upon detection of high serum ALT (>100 IU) followed by anti-HCV seroconversion. Clinical characteristics, HIV-1-related immunological status and IL-28B genotypes (rs12979860, rs8099917) were collected. We compared these variables between patients with and without spontaneous clearance of HCV and between responders and non-responders to treatment with pegylated interferon (PEG-IFN) plus ribavirin.

Results: Thirty-five patients were diagnosed with AHC during the study period. The majority (96.9%) were MSM. Three were lost to follow-up. Seventy-five percent of patients with AHC (24/32) were asymptomatic and found incidentally to have high serum ALT. Compared to those who did not show spontaneous clearance, patients with spontaneous HCV viral clearance showed more symptoms and more severe abnormalities related to acute hepatitis. Spontaneous clearance was seen in 4 out of 28 patients with CC+TT genotype, but not in 6 patients with IL-28B CT+TG genotype. PEG-IFN plus ribavirin treatment was initiated in 12 out of 28 cases without spontaneous clearance. The sustained virological response rate was high (81.8%, 9/11), even in cases with CT+TG genotype infected with HCV genotype 1b (SVR 2/2).

Conclusions: Careful attention to AHC is needed in HIV-1-infected MSM. Early diagnosis and PEG-IFN plus ribavirin treatment should be considered for AHC cases.

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All data supporting our conclusions are included within the manuscript. Original data of our retrospective analyses are available in medical records of our hospital.

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Introduction

The estimated worldwide prevalence of hepatitis C virus (HCV) infection is 2–3% [1]. HCV co-infection increases morbidity rate in HIV infected individuals, and previous meta-analysis reported mortality among patients co-infected with HCV was 1.35 times higher than that among patients with HIV-infection alone even in the highly active antiretroviral therapy (HAART) era [2]. In HIV-1/HCV co-infected patients, progression to liver cirrhosis and hepatocellular carcinoma (HCC) is faster than that in patients without HIV-1 infection [3]. Furthermore, the response to treatment with pegylated interferon (PEG-IFN) plus ribavirin (RBV) in HIV-positive patients with chronic HCV infection is

poor (sustained virological response: SVR 19–40%), compared with patients infected with HCV alone (SVR 54–61%) [4–9].

The risk of HCV acquisition via heterosexual intercourse is estimated to be very low [10]. Recently, however, a high incidence of HCV seroconversion has been reported in HIV-1 infected men who have sex with men (MSM) [11–13]. These results suggest that new HCV infection can be a potential future problem in the clinical management of HIV-1 infected patients. On the other hand, a favorable response to treatment with PEG-IFN plus RBV for acute hepatitis C (AHC) relative to that for chronic one has been reported in HCV-infected (SVR 85–98%) [14,15] and HIV/HCV co-infected patients (SVR 60–80%) [16,17]. In this regard, the recent guidelines recommend PEG-IFN plus RBV treatment

for AHC in HIV-1 co-infected patients [18–20]. However, data of AHC among HIV-1 infected patients is still limited, especially from Asian countries.

The response to treatment with PEG-IFN plus RBV is closely associated with the interleukin-28B (IL-28B) genotype, which encodes interferon- λ 3 (IFN- λ 3), in chronic HCV hepatitis, even in HIV-1 co-infected cases [21–23]. Furthermore, HCV mono-infected individuals with favorable IL-28B genotype (CC at rs12979860, TT at rs8099917) seem to achieve spontaneous clearance of HCV compared to those with non-favorable genotypes [21,22,24,25]. To our knowledge, there are no studies on the effect of IL-28B genotype on the natural course and response to treatment of AHC in HIV-1 infected individuals in Asian population [24,26,27].

In the last 12 years, 35 patients with HIV-1 infection were diagnosed with AHC in our hospital. In the present retrospective study, we report the results of analysis of data of 32 of these cases, and discuss the factors associated with spontaneous HCV clearance and response to treatment with PEG-IFN plus RBV (Fig. 1).

Methods

Study Design

This single-center retrospective cohort study was conducted in accordance with the ethical principles of the Declaration of Helsinki and of Good Clinical Practice. The ethics committee of National Center for Global Health and Medicine approved the study. All patients provided written informed consent.

Study Participants

The medical records of HIV-1 infected patients in our institution, the largest HIV clinic in Japan, admitted and treated between January 2001 and December 2012, were retrospectively reviewed. AHC was defined according to the following criteria; elevation of alanine transferase (ALT) >100 IU/L accompanied by seroconversion of anti-HCV antibody, and exclusion of other causes (e.g., acute hepatitis B and drug-induced hepatitis). Patients who were lost to follow-up within 1 year from the diagnosis of AHC were excluded from the analysis since they could not be assessed for clinical presentation including spontaneous clearance. Spontaneous clearance was defined as a decrease in HCV RNA to undetectable level without treatment within one year from the diagnosis and remaining as such thereafter. For patients receiving PEG-IFN plus RBV treatment, we assessed the SVR rate. SVR was defined as continued undetectable HCV RNA at 24 weeks

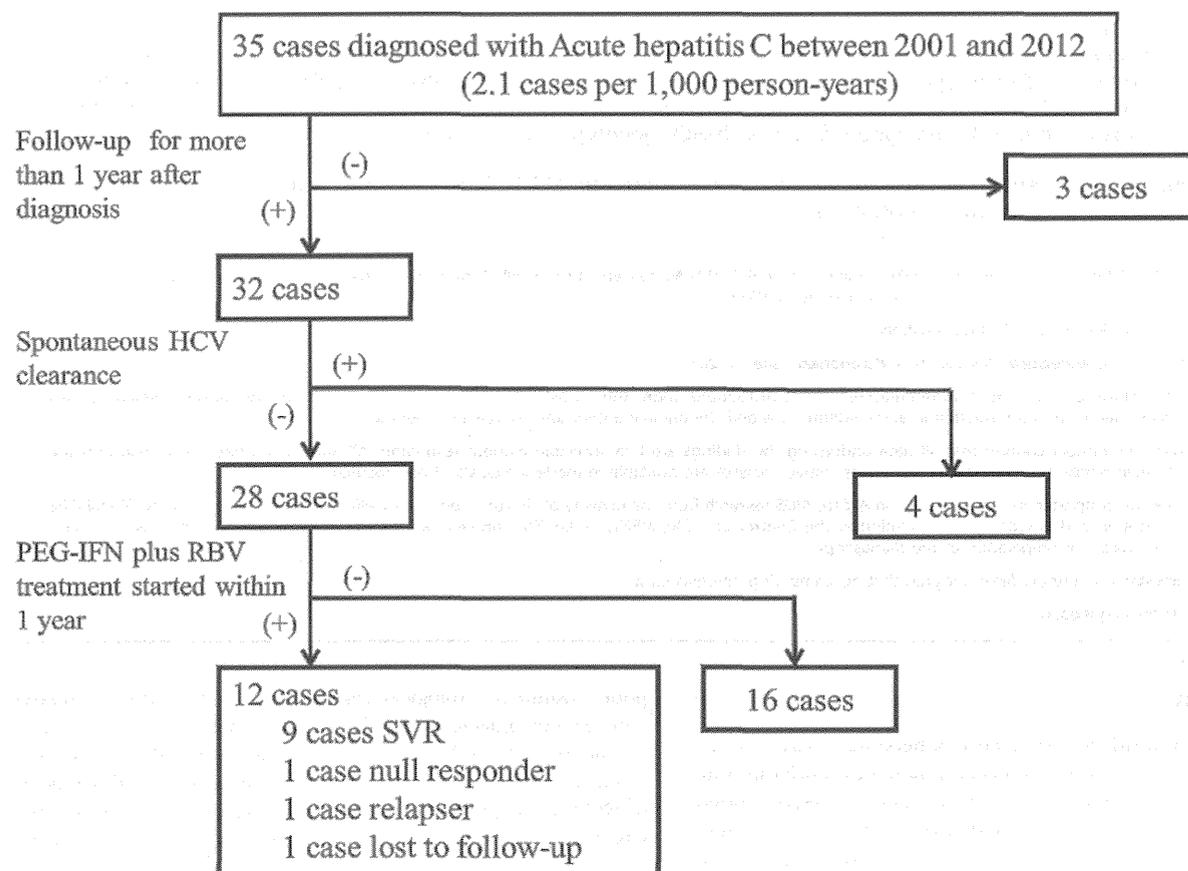


Figure 1. Patient enrollment process. Acute hepatitis C (AHC) was defined as elevation of alanine transaminase (ALT) >100 IU/L accompanied by seroconversion of anti-hepatitis C virus (HCV) antibody. Three patients could not be followed up for 1 year after diagnosis and were excluded from further analysis. HCV cleared spontaneously in 4 cases. PEG-IFN plus RBV treatment was initiated within 1 year of diagnosis of AHC in 12 out of 28 patients who did not show spontaneous clearance. One patient with missing treatment data following transfer to another clinic about two weeks after initiation of IFN plus RBV, was excluded from analysis related to the effect of PEG-IFN plus RBV. PEG-IFN: pegylated interferon, RBV: ribavirin. doi:10.1371/journal.pone.0100517.g001

Table 1. Characteristics of AHC patients (n = 32).

	All patients (n = 32)	Spontaneous clearance (n = 4)	Non-spontaneous clearance (n = 28)	P-value
Age (years)	40 [30–58]	44 [37–56]	40 [30–58]	0.361
Male sex	32 (100)	4 (100)	28 (100)	-
Men who have sex with men	31(96.9)	4 (100)	27 (96.4)	1.000
IL-28B genotypes (rs12979860+rs8099917)				
CC+TT genotype	26 (81.2)	4 (100)	22 (78.6)	0.416
CT+TG genotype	6 (18.8)	none	6 (21.4)	-
TT+GG genotype	none	none	none	-
Injecting drug users	4 (12.5)	none	4 (14.3)	1.000
Received ART at diagnosis	29 (90.6)	4 (100)	25 (89.3)	1.000
CD4 count at diagnosis (cells/ μ L)	420 [167–824]	317 [184–616]	424 [167–824]	0.424
HIV-RNA at diagnosis (copies/mL)	UD [UD– 9.4×10^4]	50 [UD-50]	42.5 [UD- 9.4×10^4]	0.737

Data are number (%) of patients or median [range].

ART, antiretroviral therapy; UD, undetectable.

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after completion of therapy. Baseline characteristics, status of HIV-1 infection, history of injecting drug usage (IDU), symptoms related to AHC (fatigue and jaundice), laboratory data abnormalities from AHC (ALT, T-bil), treatment of HCV infection and histological findings of liver biopsy, where available, and were collected from the medical records. We compared these variables between patients with and without spontaneous clearance of HCV and between responders and non-responders to treatment with PEG-IFN plus RBV.

HCV Analysis

For each patient, titers of anti-HCV antibody were measured by a third-generation Latex aggregation assay (Ortho HCV Ab LPIA test III, Ortho Clinical Diagnostics, NJ) at first visit to our hospital and at diagnosis of AHC. Serum HCV RNA at each time point was extracted automatically (Cobas Ampliprep, Roche In Vitro Diagnostics, Switzerland). Thereafter, cDNA was prepared and its titer was measured by quantitative polymerase chain reaction (Cobas TaqMan 48, Roche In Vitro Diagnostics). Direct sequencing was performed using DNA probe assay by ABI PRISM 3100 (Applied Biosystems, Foster City, CA). Finally, the

Table 2. Clinical presentation of AHC patients (n = 32).

	All patients (n = 32)	Spontaneous clearance (n = 4)	Non-spontaneous clearance (n = 28)	P-value
No symptoms	24 (75)	1 (25)	23 (82.1)	-
Symptoms	8 (25)	3 (75)	5 (17.9)	0.039
Fatigue	8 (25)	3 (75)	5 (17.9)	-
Jaundice	2 (6.25)	1 (25)	1(3.6)	-
Peak Alanine transaminase level (IU/L)	661 [117–2194]	707 [1237–2126]	614 [117–2194]	0.072
Peak total bilirubin level (mg/dL)	1.9 [0.7–17.0]	9.8 [4.2–17.0]	1.6 [0.7–6.8]	0.002
HCV genotype				
1a	1/27 (3.7)	None	1/2 (4.3)	-
1b	19/27 (70.4)	3/4 (75)	16/23 (69.6)	-
2a	4/27 (14.8)	1/4 (25)	3/23 (13)	-
2b	3/27 (11.1)	None	3/23 (13)	-
Not available	5	None	5	-
HCV-RNA at diagnosis (Log IU/mL)	6.6 [1.9–7.8] [§]	6.6 [4.9–6.8] [†]	6.6 [1.9–7.8] [‡]	0.594
Latency to HCV clearance (wks) [*]	-	11 [7–31]	-	-

Data are number (%) of patients or median [range] values.

*Time between AHC diagnosis and HCV clearance (weeks).

[§]Data of 6 patients not available for analysis.

[†]Data of 5 patients not available for analysis.

[‡]Data of 1 patient not available for analysis.

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Table 3. Comparison of patients of the SVR and non-SVR groups.

	All patients (n = 11)	SVR (n = 9)	Non-SVR (n = 2)
Age (years)	38 [30–58]	38 [30–48]	52 [47–58]
Male sex	11 (100)	9 (100)	2 (100)
Men who have sex with men	11 (100)	9 (100)	2 (100)
IL-28B genotype			
CC+TT genotype	9 (81.8)	7 (77.8)	2 (100)
CT+TG genotype	2 (18.2)	2 (22.2)	None
Injecting drug users	1 (9.1)	None	1 (50)
Received ART before treatment	10 (90.9)	8 (88.9)	2 (100)
CD4 count before treatment (cells/ μ L)	382 [230–655]	440 [272–655]	238 [254–278]
HIV-RNA before treatment (copies/mL)	UD [UD-3.3 \times 10 ⁴]	UD [UD-3.3 \times 10 ⁴]	UD [305–610]
HCV genotype			
1b	10 (90.9)	8 (88.9)	2 (100)
2a	1 (9.1)	1 (11.1)	None
HCV-RNA before treatment (Log IU/mL)	6.3 [3.3–7.8]	6.3 [5–7.8]	5.7 [3.6–8.0]
Latency to AHC diagnosis (months)*	3.2 [0.9–6.9]	3.2 [0.9–6.9]	4.4 [3.7–5.1]
Duration of PEG-IFN+RBV therapy (wks)	43 [11–72]	43 [11–72]	36 [11–60]
Latency to HCV clearance (wks) [†]	-	8 [3–16]	-
RVR	3 (27.2)	3 (33.3)	None
EVR	7 (63.6)	6 (66.7)	1 (50)
Histopathology positive for liver fibrosis			
F0	3/6	3	0
F1	2/6	1	1
F2	1/6	0	1
F3	0	0	0

Data are number (%) of patients or median [range] values.

*Time between AHC diagnosis and initiation of therapy (months).

[†]Time between initiation of therapy and HCV clearance (weeks).

ART, antiretroviral therapy; UD, undetectable; PEG-IFN+RBV, pegylated interferon plus ribavirin; SVR, sustained viral response; EVR, early viral response; RVR, rapid viral response.

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genotype was determined from the amino acid sequences of 5' untranslated region [28].

Genotyping of IL-28b Alleles

Genomic DNA was isolated from peripheral blood mononuclear cells, using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). SNPs, rs12979860, and rs 8099917 were genotyped, using the TaqMan Drug Metabolism Assays by ABI PRISM 7900 HT sequence detection system (Applied Biosystems) according to the instructions provided by the manufacturer. The researchers responsible for genotyping were blinded to clinical data of the patients.

Statistical Analysis

The patients' characteristics and results of differences in viral clearance and virological response were compared using chi-square test (for qualitative variables) or Mann-Whitney U-test (for quantitative variables). Statistical significance was defined at two-sided *p* value of <0.05. All statistical analyses were performed with The Statistical Package for Social Sciences Version 21 (SPSS Inc, Chicago, IL).

Results

Patient Enrollment

A total of 35 patients were diagnosed with AHC during the study period. The incidence of AHC was 2.1 cases per 1,000 person-years. Three patients who were lost to follow-up within 1 year after diagnosis of AHC were excluded from the analysis. No deaths or fulminant hepatitis were recorded during the study period. Spontaneous HCV clearance was achieved by 4 patients, including 2 patients in whom HCV clearance was achieved within 3 months of diagnosis of AHC. The median time between diagnosis of AHC and HCV clearance was 11 weeks (range, 7–31 weeks). Among the 28 patients who did not show spontaneous HCV clearance, treatment with PEG-IFN plus RBV was initiated within 6 months of diagnosis in 9 patients and between 6 and 12 months of diagnosis of AHC in 3 patients (6.1, 6.4 and 6.9 months, respectively), whereas treatment was not initiated in the remaining 16 patients due to cost (*n* = 7) or other comorbidity (depression, history of epilepsy) (Fig. 1).

Patients' Characteristics and Clinical Presentations of AHC

The characteristics and clinical presentation of AHC patients are listed in Tables 1 and 2, respectively. All patients were

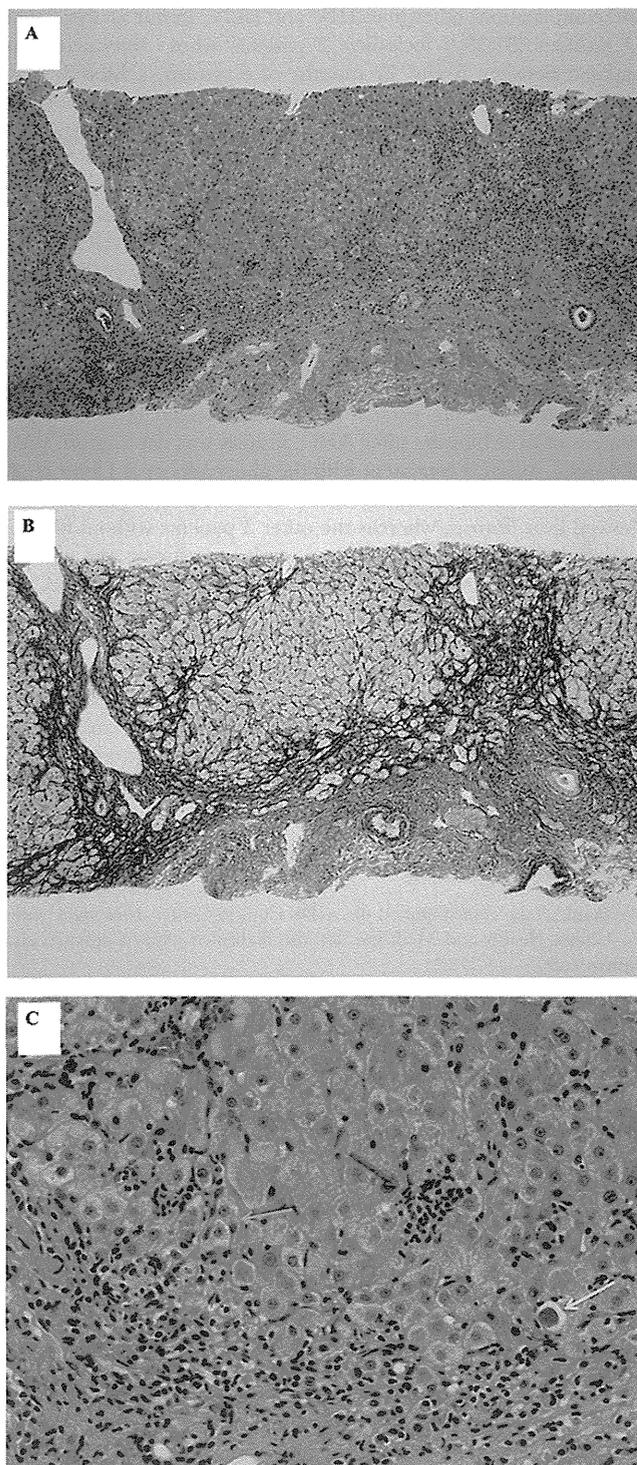


Figure 2. Histological findings in needle liver biopsy specimen from the patient who showed null-response (Table 3). The pre-treatment biopsy specimen obtained at 13 weeks after AHC diagnosis showed stage 2 fibrosis (F2) according to the classification of chronic hepatitis C (New Inuyama Classification). (A and B) Formation of bridging fibrosis by fibrous and cellular expansion in the portal tract. (C) Magnified view showing centrilobular piece-meal necrosis (green arrow), acid folic body (yellow arrow) and spotty necrosis (red arrow). (A) Hematoxylin-eosin stain, x100, (B) Silver impregnation stain, x100, (C) Hematoxylin-eosin stain, x400. PEG-IFN: pegylated interferon, RBV: ribavirin, AHC: acute C hepatitis.
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Japanese men, including 31 (96.9%) MSM. Twenty nine patients (90.6%) received antiretroviral therapy (ART) and HIV-RNA was well suppressed in these patients. Four patients (12.5%) had a history of IDU, whereas none had a history of occupational exposure to HCV or blood transfusion. Although there was no significant difference between patients with and without spontaneous HCV clearance, the IL-28B CC+TT genotype was rs12979860 and rs8099917 in all 4 patients who showed spontaneous clearance and none of the patients with IL-28B CT+TG genotype showed spontaneous clearance (Table 1).

The majority of patients with AHC (24/32, 75%) were asymptomatic at the onset of AHC. High ALT was identified incidentally at routine visit for HIV-1 infection, with subsequent tests confirming the diagnosis of AHC. Compared to patients who did not show spontaneous clearance, patients with spontaneous clearance showed more severe clinical presentation of hepatitis (symptomatic, with higher serum total bilirubin and ALT value at diagnosis). The most frequent HCV genotype was 1b (70.4%). At diagnosis, HCV RNA was higher than 5.0 LC/mL in 24 out of 26 patients (Table 2).

Response to Treatment with PEG-IFN Plus RBV

Treatment with PEG-IFN plus RBV was initiated in 12 patients within 1 year of diagnosis of AHC (median interval from AHC diagnosis, 3.2 months). We assessed the response to treatment in only 11 patients; the other patient was lost to follow-up within two weeks of treatment initiation (Fig. 1, Table 3). SVR was achieved in 9 of 11 patients (81.8%) despite the high incidence of HCV genotype 1b and high viral load.

Two patients did not achieve SVR. Both patients were infected by genotype 1b with high viral load, and treatment was initiated within 6 months of diagnosis. One achieved viral clearance within 12 weeks (early virological response: EVR) but showed viral rebound at 15 weeks after completion of the treatment (relapser), whereas viral clearance was not achieved during treatment in the other patient (null-responder). Both patients were relatively older and their CD4 counts were lower, compared to those with SVR, although statistical analysis was not performed due to the small number of cases. In patients with SVR, the median time between initiation of therapy and clearance of HCV was 8 weeks (range, 3–16 weeks). Surprisingly, both patients with IL-28B CT+TG alleles achieved SVR despite genotype 1b and high viral load, although we could not compare the SVR rate among different genotypes since only one patient was infected with genotype 2a in this study.

Histological Findings of AHC in HIV-1 Co-infected Patients

HBs antigen was negative and ALT was within the normal range in the year preceding AHC in all 6 patients, whereas HBs Ab and/or HBc Ab was positive in 5 patients. No pre-existing factors of liver fibrosis other than HIV infection were evident before AHC. Liver biopsy was performed in 6 patients before treatment with PEG-IFN plus RBV. The median interval between diagnosis of AHC and biopsy was 4.3 months (range, 3.3–6.1 months). Fibrotic changes were confirmed in 3 cases by hematoxylin-eosin staining and silver impregnation staining (Fig. 2, Table 3). These lesions were paler-staining by Victoria Blue stain, indicating that the fibrotic areas did not reflect chronic changes.

Discussion

In the present study, we identified 35 cases of AHC during the study period and nearly all such patients (34/35) were MSM, and

the most frequent HCV genotype was 1b (19/27). These findings are consistent with previous reports from other countries [11–13]. In this regard, a high incidence of HCV seroconversion in HIV-1 infected MSM was reported recently by two separate groups [11–13]. The same studies also reported that genotype 1b was the major genotype among their patients [11–13], and that HCV infection was frequently not detected during the acute phase and diagnosed only at the chronic stage mainly due to the lack of symptoms.

Similar to the previous reports on AHC, 75% of our cases were asymptomatic, and only 6.3% of the study population showed mild elevation of serum ALT (100 IU/L < ALT < 150 IU/L). In this regard, ALT elevation during acute HCV infection is often relatively transient, and therefore could be easily missed during routine clinical care. The need of regular screening for anti-HCV antibody in HIV-1 infected MSM is controversial, and the recommendations are different in guidelines from different developed countries [29,30]. Our results emphasize the importance of regular ALT monitoring and HCV re-screening at the time of mild ALT elevation during follow-up, especially in high-risk populations such as sexually active MSM.

There are few reports on the relationship between IL-28B CC+TT genotype and spontaneous clearance of HCV [21,31]. In the present study, spontaneous HCV clearance was seen in 4 out of 26 patients with IL-28B CC+TT genotype, whereas no spontaneous HCV clearance was seen in all 6 patients with IL-28B CT+TG genotype. Although this difference could not be confirmed to be statistically significant due to the small number of patients (4 patients), this is, to our knowledge, the first report on the relation between IL-28B and spontaneous HCV clearance during AHC in HIV-1 co-infected patients in Asian population. Our study also showed that the severity of clinical symptoms was an important factor related to spontaneous HCV clearance. Further investigation is needed for a better understanding of the pathogenesis of AHC, especially factors involved in spontaneous clearance.

The use of PEG-IFN plus RBV treatment for AHC within 6 months of diagnosis is now recommended for HIV-1 co-infected cases [17–19] although data on the response of HIV-1 infected individuals with AHC to the PEG-IFN plus RBV remain limited. One study reported spontaneous clearance of HCV between 6 and 12 months of diagnosis [32]. In this regard, it is sometimes difficult in the clinical setting to start PEG-IFN plus RBV treatment within 6 months of diagnosis because some patients have comorbidities

and complications other than HIV and HCV. In our analysis, 9 of 11 patients (81.8%), including 2 patients whose treatment was initiated between 6 and 12 months of diagnosis, achieved SVR despite high rate of genotype 1b infection (SVR 90.0% among those with genotype 1b virus). Furthermore, HCV genotype 1b-infected patients carrying the IL-28B CT+TG genotype (n = 2), which is a predictor of poor response to the treatment of chronic HCV infection, achieved SVR. These results emphasize the advantage of the PEG-IFN plus RBV treatment for AHC.

Little is known about the progression of AHC to liver fibrosis in patients with HIV/HCV co-infection [33], although rapid progression of liver fibrosis during the chronic phase is well recognized [3]. Fierer et al. [34] reported that the development of fibrosis occurs even in the acute phase of HCV infection in HIV-Infected men. In the present study with limited cross-sectional analysis of liver biopsies after AHC, fibrosis was detected in 3 out of 6 cases, which is consistent with the above report of Fierer et al. [34]. Moreover, SVR was not achieved in 2 out of 3 patients who showed liver fibrosis, whereas the other 3 patients without fibrosis achieved SVR (**Table 3**). These results emphasize the clinical importance of early diagnosis and early treatment for AHC in HIV-1 infected individuals.

In conclusion, the potential of AHC should always be considered in HIV-1 infected MSM, even in asymptomatic case, who present with mild ALT elevation. Favorable response can be expected if anti-HCV treatment is initiated during the early phase. Further investigation is needed to determine the predictor(s) of spontaneous HCV clearance, appropriate timing of treatment initiation, and duration of treatment.

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Author Contributions

Conceived and designed the experiments: MI KW TK YK SO HG. Performed the experiments: MI KW TK YN MY TI NM. Analyzed the data: MI KW TK HG. Contributed reagents/materials/analysis tools: MI KW TK YN MY TI NM YK SO HG. Contributed to the writing of the manuscript: MI KW KT HG.

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Long-term exposure to tenofovir continuously decrease renal function in HIV-1-infected patients with low body weight: results from 10 years of follow-up

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Objectives: To investigate the effect of long-term tenofovir disoproxil fumarate (TDF) use on renal function, especially in patients with low body weight who are vulnerable to TDF nephrotoxicity.

Design: A single-center, observational study in Tokyo, Japan.

Methods: We performed a 10 years cohort study of 792 HIV-1-infected patients. The effect of long-term TDF use on estimated glomerular filtration rate (eGFR) was investigated on treatment-naïve patients who started TDF-containing antiretroviral therapy ($n = 422$) and those who started abacavir-containing antiretroviral therapy as control ($n = 370$). Three renal endpoints were examined by the logistic regression model: decrement in eGFR of higher than 10 ml/min per 1.73 m² relative to the baseline, more than 25% decrement in eGFR, and eGFR lower than 60 ml/min per 1.73 m² at least 3 months apart. The loss in eGFR was estimated using linear mixed models for repeated measures.

Results: The median weight at baseline was 63 kg. TDF use increased the risk of all three renal outcomes compared with the control group: higher than 10 ml/min per 1.73 m² decrement in eGFR [adjusted odds ratio (OR) = 2.1, 95% confidence interval (CI) 1.45–3.14, $P < 0.001$], more than 25% decrement (adjusted OR = 2.1, 95% CI 1.50–2.90, $P < 0.001$), and eGFR lower than 60 ml/min per 1.73 m² at least 3 months apart (adjusted OR = 3.9, 95% CI 1.62–9.36, $P = 0.002$). The cumulative mean loss relative to the control after 1, 2, 3, 4, and 5 years of TDF exposure was –3.8, –3.6, –5.5, –6.6, and –10.3 ml/min per 1.73 m², respectively, indicating that the loss in eGFR increased over time ($P < 0.001$).

Conclusion: In this cohort of patients with low body weight, TDF exposure increased the risk of renal dysfunction. Furthermore, the loss in eGFR relative to the control increased continuously up to 5 years.

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Introduction

Tenofovir disoproxil fumarate (TDF) is one of the most widely used nucleotide reverse transcriptase inhibitors (NRTIs) for the treatment of HIV-1 infection in both resource-rich and resource-limited settings [1,2], and also for the treatment of hepatitis B infection [3,4]. Furthermore, TDF, at a fixed dose with emtricitabine, has been approved in the United States for the prevention of sexual transmission of HIV-1 in uninfected adults as preexposure prophylaxis [5,6].

TDF is known to cause renal proximal tubular dysfunction [7–10] and also reduces estimated glomerular filtration rate (eGFR) more than other NRTIs [11–13]. To date, the extent of TDF-induced renal dysfunction is regarded as mild and tolerable [14,15], and one meta-analysis recommended that TDF use should not be restricted even when regular monitoring of renal function and serum phosphate levels is impractical [16]. Furthermore, although evidence is limited, most of the TDF-induced loss in renal function is considered to occur during the first year of exposure [12,15].

However, a large proportion of studies that investigated TDF nephrotoxicity were based on an analysis of a relatively short observation period, typically a few years, and little information is available on the effect of long-term TDF use on the prognosis of renal function. This is important as HIV-1 infection requires lifelong antiretroviral therapy (ART). In this regard, although small body weight is a well established risk factor for TDF nephrotoxicity [16,17], the TDF-related renal dysfunction has hardly been evaluated in patients with small body weight, who are potentially at higher risk for larger drug exposure and, thus, more severe toxicity [17–20].

Based on the above background, the current study was designed to investigate the effects of long-term TDF use on renal function in HIV-1-infected patients with low body weight, using 10 years data from our observational cohort study.

Methods

Study design and patients

We performed a single-center cohort study of HIV-1-infected patients using the medical records at AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo. The effect of long-term TDF use on renal function was investigated on treatment-naïve patients who started TDF-containing ART, and those who started abacavir (ABC)-containing ART as the control. ABC was chosen as the control because this NRTI is not known to be nephrotoxic and is not renally eliminated [21] and because the Japanese guidelines for

the treatment of HIV-1 infection placed both TDF and ABC as the preferred NRTIs throughout the observational period [22]. The inclusion criteria were treatment-naïvety, Japanese, age older than 17 years, and treatment with either the recommended 300 mg/day dose of TDF or 600 mg/day dose of ABC-containing standard ART (consisting of one nonnucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI) or integrase strand transfer inhibitor (INSTI), and two NRTIs) at our clinic between 1 January 2004 and 31 December 2011. Furthermore, the following exclusion criteria were applied: start of ART at other facilities, baseline eGFR of lower than 60 ml/min per 1.73 m², discontinuation of TDF or ABC within 90 days after initiation of ART, or start of ART with both TDF and ABC. Of the 1334 patients who started ART at our clinic during the study period, 792 patients fulfilled these criteria and constituted the study patients (see Figure, Supplemental Digital Content 1, <http://links.lww.com/QAD/A537>, which shows patient enrollment process). The study patients were followed up until 31 December 2013. Censoring occurred at discontinuation of TDF or ABC, referral to other hospitals, loss to follow-up, death, or end of the observation period. The inclusion of Japanese patients only served to examine a population with relatively small body stature, compared with whites and African Americans [17]. The selection of TDF or ABC at baseline was left to the discretion of the attending physician, because both drugs were the preferred NRTIs during the study period in the Japanese guidelines [22]. The attending physician also selected the key drug (NNRTI, PI, or INSTI). In Japan, TDF became available from April 2004 and ABC from September 1999.

The study was approved by the human research ethics committee of National Center for Global Health and Medicine. All patients included in this study provided written informed consent for their 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.

Measurements

eGFR was calculated using the Japanese equation based on standardized serum creatinine, sex, and age, which was developed by the Japanese Society of Nephrology (JSN): $eGFR = 194 \times [\text{serum creatinine}]^{-1.094} \times [\text{age}]^{-0.287} \times [0.739 \text{ if woman}]$ [23]. This equation was used because the Japanese equation performs better than The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [24] for patients with small body stature, such as Japanese, especially in individuals with GFR lower than 60 ml/min per 1.73 m² [25]. The 2013 practice guidelines for patients with CKD published by JSN also recommend the use of this equation for the Japanese, rather than CKD-EPI, which was derived mostly from whites and African Americans [25,26].

The baseline eGFR was estimated for each patient from age, sex, and serum creatinine measurements made closest to and preceding the commencement of ART by no more than 90 days. Patients visited our clinic at least every 3 months for monitoring CD4⁺ cell count, HIV-1 viral load, and eGFR as the prescription period under the Japanese healthcare system is limited to 3 months. Thus, for calculation of follow-up eGFR value, we collected serum creatinine values measured closest to every 90 day within a range of 45 days from initiation of ART.

The potential risk factors for renal dysfunction were determined according to previous studies and collected together with the basic demographics from the medical records [16,19,27,28]. They included age, sex, body weight, BMI = {body weight (kg)/[height (m)]²}, history of AIDS, route of HIV-1 transmission, baseline laboratory data (CD4⁺ cell count, HIV viral load, and serum creatinine), and presence or absence of other medical conditions (concurrent use of ritonavir-boosted PIs (PI/r), concurrent nephrotoxic drugs such as ganciclovir and sulfamethoxazole/trimethoprim, diabetes mellitus defined by using antidiabetic agents or fasting plasma glucose higher than 126 mg/dl or plasma glucose higher than 200 mg/dl on two different days, hypertension defined by current treatment with antihypertensive agents or two successive measurements of SBP higher than 140 mmHg or DBP higher than 90 mmHg at the clinic, dyslipidemia defined by current treatment with lipid-lowering agents, coinfection with hepatitis B defined by positive hepatitis B surface antigen, coinfection with hepatitis C defined by positive HCV viral load, and current smoking). At our clinic, body weight and blood pressure were 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.

Statistical analysis

The primary exposure variable was TDF use over the control (ABC) as part of the initial ART. Three renal endpoints were applied in this study; we primarily focused on decrement in eGFR of higher than 10 ml/min per 1.73 m² relative to the baseline [29], because this endpoint is considered appropriate for patients with well maintained renal function, such as the study population; more than 25% decrement in eGFR relative to the baseline [17,18]; and two consecutive measurements of eGFR lower than 60 ml/min per 1.73 m² at least 90 days apart [30]. Changes in eGFR were plotted from the baseline measurement until occurrence of each of the three renal endpoints, and the logistic regression model was used to estimate the effect of TDF use over control on the occurrence of these renal endpoints. The model was adjusted for baseline eGFR, baseline body weight, nephrotoxic drug use, PI/r use, CD4⁺ cell count, hypertension, dyslipidemia, and diabetes mellitus, which

are established risk factors for TDF nephrotoxicity [13,16,27,28]. Baseline age was not added to the model to avoid over adjustment because the equation for eGFR calculation already includes age, and the baseline age was not associated with TDF use, indicating that age is not a confounding factor for the association between TDF use and eGFR. Furthermore, older age at baseline was shown to be a predictive variable for lower baseline eGFR (linear regression, $P < 0.0001$). In this case, adding predictive covariates to the logistic regression model will have detrimental effects on precision [31].

To investigate the effect of body weight on TDF-related nephrotoxicity, we did subgroup analysis for baseline weight categories: at least 70 kg and lower than 70 kg. Then, the multivariate logistic analysis for the renal endpoint of the occurrence of higher than 10 ml/min per 1.73 m² decrement in eGFR was conducted for each subgroup.

To further investigate the effect of TDF on renal function, we estimated the decrement in eGFR in the TDF group relative to the control group by calculating the difference in eGFR loss between the TDF and control group from baseline to 5 years after initiation of ART by 90 days intervals with a linear mixed models for repeated measures. We constructed the model with a random effect for patients. This model also included fixed effects for assigned treatment, baseline eGFR, baseline body weight, nephrotoxic drug use, PI/r use, CD4⁺ cell count, hypertension, dyslipidemia, and diabetes mellitus. Interaction terms for time by treatment were included.

As additional analyses, the statistical analyses using eGFR calculated with CKD-EPI equation adjusted with the Japanese coefficient were also performed: $eGFR = 0.813$ (a Japanese coefficient) $\times 141 \times \min(SCr/\kappa, 1)^\alpha \times \max(SCr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ (if female), where SCr is serum creatinine, κ is 0.7 for women and 0.9 for men, α is -0.329 for women and -0.411 for men, min indicates the minimum of SCr/ κ or 1, and max indicates the maximum of SCr/ κ or 1 [32].

Statistical significance was defined at two-sided $P < 0.05$. We used odds ratios (ORs) with 95% confidence intervals (95% CIs) as a measure of the effect of TDF use on renal endpoints. All statistical analyses were performed with SAS Software, version 9.3 (SAS Institute, Cary, North Carolina, USA).

Results

Of the 792 study patients, 422 patients started TDF-containing ART (TDF group) whereas the remaining 370 patients formed the control group who started ABC-containing ART (see Figure, Supplemental Digital

Table 1. Baseline characteristics of patients who started tenofovir disoproxil fumarate-containing antiretroviral therapy and controls (abacavir-containing antiretroviral therapy).

	Study patients (n = 792)	TDF (n = 422)	Control (ABC) (n = 370)	P
Sex (male), n (%)	769 (97)	412 (98)	357 (97)	0.40
Age ^a	36 (31–43)	36 (31–43)	36 (31–44)	0.23
Weight (kg) ^a	63 (57.8–70.4)	62.9 (57.2–69.8)	63.8 (58.0–71.4)	0.25
BMI (kg/m ²) ^a	22 (20.1–24.1)	21.9 (20.1–23.8)	22.2 (20.3–24.6)	0.23
eGFR (ml/min per 1.73 m ²) ^a	95.7 (84–110)	96.5 (84.7–111.5)	95.4 (83.7–108.6)	0.32
Serum creatinine (mg/dl) ^a	0.74 (0.66–0.82)	0.73 (0.66–0.82)	0.74 (0.67–0.83)	0.27
CD4 ⁺ cell count (/μl) ^a	189 (78–266)	199 (85–281)	183 (73–241)	0.002
HIV RNA viral load (log ₁₀ /ml) ^a	4.76 (4.26–5.23)	4.76 (4.26–5.23)	4.76 (4.27–5.26)	0.93
Ritonavir-boosted protease inhibitors, n (%)	673 (85)	368 (87)	305 (82)	0.073
Protease inhibitors (unboosted), n (%)	28 (4)	8 (2)	20 (5)	0.011
NNRTIs, n (%)	48 (6)	20 (5)	28 (8)	0.10
INSTIs, n (%)	45 (6)	28 (7)	17 (5)	0.22
Hypertension, n (%)	118 (15)	41 (10)	77 (21)	0.001
Dyslipidemia, n (%)	9 (1)	5 (1)	4 (1)	1.00
Diabetes mellitus, n (%)	29 (4)	9 (2)	20 (5)	0.021
Concurrent use of nephrotoxic drugs, n (%)	218 (28)	88 (21)	130 (35)	<0.001
Hepatitis B, n (%)	62 (8)	57 (14)	5 (1)	<0.001
Hepatitis C, n (%)	37 (5)	20 (5)	17 (5)	1.00
History of AIDS, n (%)	183 (23)	89 (21)	94 (25)	0.15
Homosexual contact, n (%)	689 (87)	364 (86)	325 (88)	0.94
Current smoker, n (%)	369 (47)	193 (46)	176 (48)	0.57
ART duration (years) ^a	3.52 (2.29–5.18)	3.19 (2.20–4.67)	4.59 (2.48–5.18)	<0.001

ABC, abacavir; ART, antiretroviral therapy; eGFR, estimated glomerular filtration rate; INSTI, integrase strand transfer inhibitor; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitor; TDF, tenofovir disoproxil fumarate.

^aMedian (interquartile range).

Content 1, <http://links.lww.com/QAD/A537>, which shows patient enrollment process). Table 1 shows the characteristics of the study population at baseline. The majority of the study population was men, comparatively young, and had a small body stature [median weight, 63 kg (interquartile range [IQR] 57.8–70.4 kg), median BMI 22.0 kg/m² (IQR 20.1–24.1)]. There was no difference in baseline eGFR between the two groups ($P=0.32$). More than 80% of the patients of the two groups used PI/r. Patients of the TDF group had higher CD4⁺ cell count ($P=0.002$) and were less likely to have hypertension ($P=0.001$), diabetes mellitus ($P=0.021$), and on concurrent nephrotoxic drugs ($P<0.001$), than the control. The median duration of ART was longer in the control group [median, 1675 days, interquartile range (IQR), 904–1890 days] than in the TDF group [median, 1164 days, IQR, 802–1705 days] ($P<0.001$). The total observation period was 1347.5 patient-years for the TDF group and 1379.3 patient-years for the controls.

During the observation period, an eGFR decline from baseline of higher than 10 ml/min per 1.73 m² occurred in 348 (82.5%) of the TDF group and 265 (71.6%) of the control group (TDF use over control: adjusted OR 2.1, 95% CI 1.45–3.14, $P<0.001$) (Table 2). Furthermore, higher baseline eGFR, higher CD4⁺ cell count also increased the risk of higher than 10 ml/min per 1.73 m² decrement in eGFR.

More than 25% decrement in eGFR occurred in 172 (40.8%) patients of the TDF group and 97 (26.2%) of the

control (adjusted OR = 2.1, 95% CI 1.50–2.90, $P<0.001$) (Table 3), and two consecutive measurements of eGFR lower than 60 ml/min per 1.73 m² were encountered in 26 (6.2%) patients of the TDF group and in 14 (3.8%) of the control (adjusted OR = 3.9, 95% CI 1.62–9.36, $P=0.002$) (Table 4).

Subgroup analysis by baseline body weight above and below 70 kg showed that among patients with body weight at least 70 kg, TDF use relative to the control marginally increased the risk of higher than 10 ml/min per 1.73 m² decrement in eGFR (adjusted OR = 1.7, 95% CI 0.83–3.29, $P=0.15$), whereas among patients weighing lower than 70 kg, the effect of TDF use was more evident (adjusted OR = 2.5, 95% CI 1.55–4.00, $P<0.001$) than that among the entire study population (see Table 1, Supplemental Digital Content 2, <http://links.lww.com/QAD/A537>, which shows effects of initiating TDF-containing ART over control on higher than 10 ml/min per 1.73 m² decrement in eGFR according to baseline body weight).

Figure 1 shows the results of the linear mixed models for repeated measures up to 5 years. The adjusted cumulative mean loss increased continuously over the years in both the TDF and control groups: in TDF group, from –11.8 ml/min per 1.73 m² at 1 year of TDF to –23.7 ml/min per 1.73 m² at 5 years of TDF exposure, and in the control, from –8.0 ml/min per 1.73 m² at 1 year to –13.5 ml/min per 1.73 m² at 5 year of ART exposure. The adjusted cumulative mean loss in the TDF group

Table 2. Effects of initiating tenofovir disoproxil fumarate-containing antiretroviral therapy over control on >10 ml/min per 1.73 m² decrement in estimated glomerular filtration rate: multivariate logistic regression analysis.

	Adjusted OR	95% CI	P
TDF use relative to the control	2.1	1.45–3.14	<0.001
Baseline eGFR per 1 ml/min per 1.73 m ² increment	1.1	1.05–1.08	<0.001
Weight per 1 kg increment	1.0	0.99–1.01	0.92
Use of nephrotoxic drugs	0.8	0.50–1.25	0.31
Use of ritonavir-boosted protease inhibitors	1.3	0.78–2.16	0.32
CD4 ⁺ cell count per 1/μl increment	1.0	1.00–1.00	<0.001
Hypertension	2.1	1.17–3.64	0.013
Dyslipidemia	1.0	0.21–4.60	0.98
Diabetes mellitus	1.9	0.63–5.86	0.25

ART, antiretroviral therapy; eGFR, estimated glomerular filtration rate; OR, odds ratio; TDF, tenofovir disoproxil fumarate.

relative to the control continuously increased over time: at 1 year of exposure –3.8 ml/min per 1.73 m², at 2 years –3.6 ml/min per 1.73 m², at 3 years –5.5 ml/min per 1.73 m², at 4 years –6.6 ml/min per 1.73 m², and at 5 years –10.3 ml/min per 1.73 m² (see Table 2, Supplemental Digital Content 3, <http://links.lww.com/QAD/A537>, which shows adjusted loss in eGFR in the TDF group relative to the control estimated with mixed model for repeated measures). There was significant interaction between time and TDF use ($P < 0.001$), suggesting that the adjusted mean loss in eGFR relative to the control increased significantly over time.

Additional analyses of renal function calculated with CKD-EPI equation also showed that TDF use doubled the risk of higher than 10 ml/min per 1.73 m² decrement (adjusted OR = 2.1, 95% CI 1.57–2.86, $P < 0.001$) and more than 25% decrement (adjusted OR = 1.8, 95% CI

Table 3. Effects of initiating tenofovir disoproxil fumarate-containing antiretroviral therapy over control on >25% decrement in estimated glomerular filtration rate relative to baseline: multivariate logistic regression analysis.

	Adjusted OR	95% CI	P
TDF use over control	2.1	1.50–2.90	<0.001
Baseline eGFR per 1 ml/min per 1.73 m ²	1.0	1.03–1.04	<0.001
Weight per 1 kg increment	1.0	0.98–1.01	0.37
Nephrotoxic drug use	0.7	0.47–1.03	0.073
Ritonavir-boosted protease inhibitor use	0.9	0.58–1.44	0.69
CD4 ⁺ cell count per 1/μl increment	1.0	1.00–1.00	0.007
Hypertension	1.5	0.96–2.49	0.074
Dyslipidemia	0.7	0.13–3.69	0.67
Diabetes mellitus	1.8	0.77–4.30	0.17

ART, antiretroviral therapy; CI, confidence interval; eGFR, estimated glomerular filtration rate; OR, odds ratio; TDF, tenofovir disoproxil fumarate.

1.12–2.99, $P = 0.017$). The effect of TDF use on the renal endpoint of lower than 60 ml/min per 1.73 m² was also marginally significant (adjusted OR = 2.7, 95% CI 0.71–10.5, $P = 0.15$). The adjusted cumulative mean loss increased continuously in both the TDF and control groups: in TDF group, from –6.3 ml/min per 1.73 m² at 1 year to 15.0 ml/min per 1.73 m² at 5 years of TDF exposure, and in the control, from –4.1 ml/min per 1.73 m² at 1 year to –8.3 ml/min per 1.73 m² at 5 year of ART exposure. The cumulative mean loss in the TDF group relative to the control after 1, 2, 3, 4, and 5 years of TDF exposure was –2.2, –2.3, –3.2, –4.4, and –6.7 ml/min per 1.73 m², respectively, which indicated that the loss in eGFR relative to control increased over time ($P < 0.001$).

Discussion

In this 10 years observational cohort of treatment-naïve patients with low median body weight of 63 kg, initiation of TDF-containing ART doubled the risk of higher than 10 ml/min per 1.73 m² decrement or more than 25% decrement in eGFR relative to baseline, compared with the control patients who started ABC-containing ART, and also increased four-fold the risk of deterioration of eGFR to lower than 60 ml/min per 1.73 m². The effect of TDF on the decrement in eGFR was more evident in patients with body weight of lower than 70 kg (TDF use over control: adjusted OR = 2.5, 95% CI 1.55–4.00, $P < 0.001$) compared with the entire study population (adjusted OR = 2.1, 95% CI 1.45–3.14, $P < 0.001$), whereas the effect of TDF on renal dysfunction was only marginally significant among patients with body weight of at least 70 kg (adjusted OR = 1.7, 95% CI 0.83–3.29, $P = 0.15$).

More importantly, eGFR of the patients who started TDF-containing ART decreased continuously during the 5-year observation compared with the controls who started ABC-containing ART. The adjusted mean loss relative to the control increased from –3.8 ml/min per 1.73 m² at 1 year of TDF exposure to –5.5 ml/min per 1.73 m² at 3 years, and to –10.3 ml/min per 1.73 m² at 5 years of TDF exposure. This decrement in eGFR associated with TDF use is alarming considering that the aging-related decrement in normal renal function is only 0.4 ml/min per year [33]. The findings of the present study warrant long-term monitoring of renal function in HIV-1-infected patients with low body weight who start TDF-containing ART.

The present study has three main strengths. First, to our knowledge, this is the first study that elucidated the effect of long-term TDF use on the prognosis of renal function among HIV-1-infected patients with low body weight. Low body weight has been identified as a risk for TDF

Table 4. Effects of initiating tenofovir disoproxil fumarate-containing antiretroviral therapy over the control on estimated glomerular filtration rate <60 ml/min per 1.73 m²; multivariate logistic regression analysis.

	Adjusted OR	95% CI	P
TDF use over control	3.9	1.62–9.36	0.002
Baseline eGFR per 1 ml/min per 1.73 m ²	0.9	0.83–0.90	<0.001
Weight per 1 kg increment	1.0	0.93–1.00	0.069
Use of nephrotoxic drugs	0.6	0.22–1.52	0.27
Use of ritonavir-boosted protease inhibitors	1.4	0.47–3.89	0.57
CD4 ⁺ cell count per 1/μl increment	1.0	1.00–1.00	0.94
Hypertension	1.9	0.73–5.13	0.18
Dyslipidemia	2.1	0.23–18.7	0.52
Diabetes mellitus	3.7	0.85–16.2	0.083

ART, antiretroviral therapy; CI, confidence interval; eGFR, estimated glomerular filtration rate; OR, odds ratio; TDF, tenofovir disoproxil fumarate.

nephrotoxicity [16,17], and it is noteworthy that many patients with HIV-1 infection are of small body stature.

Of 35.3 million estimated to be infected with HIV-1 at the end of 2012, most were from sub-Saharan Africa (25 million) and south and south-east Asia (3.9 million) [34], and studies from these regions report that HIV-1-infected patients were of low body weight (mean weight of 57.6 kg in treatment-naive patients in Zimbabwe and Uganda [35], median 60 kg in west India [36], median 56.5 kg in Thailand [18], and mean 55 kg in Vietnam [37]). Considering that body weight of these patients are even lower than that in the present study of 63 kg, the effect of long-term TDF use on renal function might be more severe among patients in these regions.

Second, the study enrolled only treatment-naive patients and followed their renal function up to 5 years after initiation of standard ART with one key drug and two NRTIs (including either TDF or ABC as control). This study design, together with its observational setting, allowed examination of the effect of long-term TDF use on the prognosis of renal function after the start of ART under ‘real-world’ setting, making the results of the present study more generalizable.

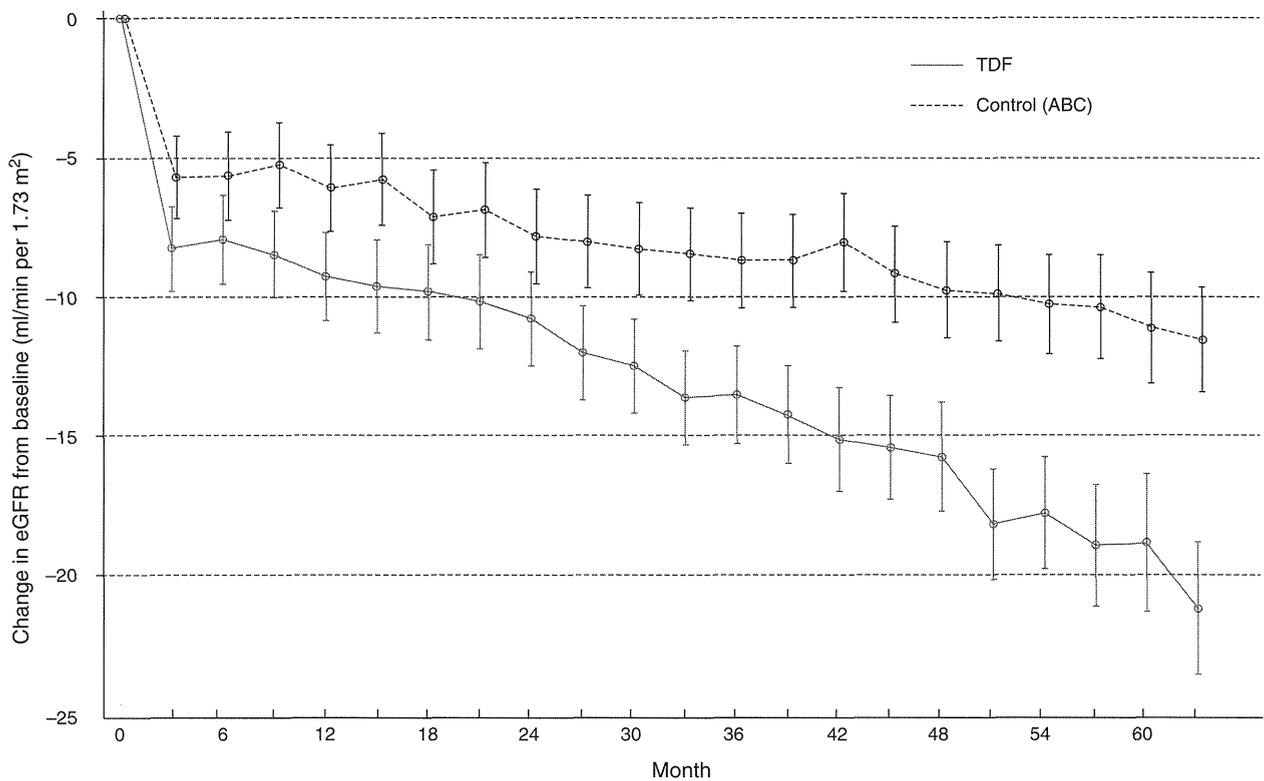


Fig. 1. Adjusted mean change in estimated glomerular filtration rate from baseline to 5 years in treatment-naive patients treated with tenofovir disoproxil fumarate-containing antiretroviral therapy (red line) and controls (patients treated with abacavir-containing ART) (black line). Least-square means and their 95% confidence intervals were estimated by the linear mixed model. The x-axis is labeled ‘Months’ to make the figure visually understandable; however, 30 days is labeled here as 1 month. Thus, 3 months equals to 90 days and so on. The model included five fixed effects (assigned treatment, baseline eGFR, baseline body weight, nephrotoxic drug use and ritonavir-boosted protease use) in this figure. ABC, abacavir; ART, antiretroviral therapy; gGFR, estimated glomerular filtration rate; TDF, tenofovir disoproxil fumarate.

Third, the study employed the Japanese equation developed by the JSN for the calculation of eGFR [23,26]. Because commonly used methods, such as MDRD and CKD-EPI equations, were validated mostly in whites and African Americans, they are probably not appropriate for people of other ethnicity or of different body stature [23,38,39]. With regard to body stature, CKD-EPI was derived from datasets of people with mean weight of 79–82 kg [24], whereas the Japanese equation was derived from the set of people with mean weight of 60.4–61 kg [23]. Accordingly, clinicians are usually encouraged to validate their own equation or use MDRD or CKD-EPI equation with ethnic coefficient [25,38]. In the present study, using the Japanese equation for eGFR for Japanese patients probably yielded a better estimate of the effect of long-term TDF use on renal function [25]. Furthermore, additional analyses with use of CKD-EPI equation adjusted with the Japanese coefficient again showed that TDF exposure increased the risk of renal dysfunction and the loss in eGFR relative to the control increased continuously up to 5 years.

Apart from the above strengths, the present study has several limitations. First, because of its observational nature, there is a potential for channeling bias by indication for TDF use. Indeed, control patients were more likely to have risks for renal dysfunction, such as diabetes mellitus, hypertension, concurrent nephrotoxic drugs, and lower CD4⁺ cell count [16,27], than patients who started TDF-containing ART. Thus, the incidence of TDF nephrotoxicity might have been underestimated in the present study. The median observation period of the control group was longer than that of the TDF group, and this might as well contribute to underestimation of TDF nephrotoxicity. Second, a high percentage of our study population used PI/r, which is considered a risk for TDF nephrotoxicity [28]. Although it is difficult to completely exclude the effect of concurrent PI/r, it should be noted that PI/r use itself (even without concurrent TDF) has been considered a risk for CKD [30,40], and the percentage of PI/r use was similarly high in both the TDF and control group, suggesting that PI/r affected renal function of the control patients to some extent as well. Furthermore, the use of PI/rs did not correlate with any of the three renal outcomes in this study (Tables 2–4). Third, all study participants were Japanese and we had a small number of women. Further studies are needed to determine whether the findings of this study are also applicable to women and patients of different racial background.

In conclusion, this long-term observational study of HIV-1-infected patients with predominantly low body weight demonstrated that initiation of TDF-containing ART doubled the risk of higher than 10 ml/min per 1.73 m² decrement and more than 25% decrement in eGFR, and also four-fold increased the risk of deterioration of eGFR to lower than 60 ml/min per 1.73 m², compared with the controls who started ABC-containing ART. The loss in

eGFR in the TDF group relative to the control increased continuously over time and reached –10 ml/min per 1.73 m² at 5 years of TDF exposure. The results of the study certainly warrant regular and long-term monitoring of renal function in patients with low body weight who start TDF-containing ART. Further larger studies are needed to confirm the long-term renal prognosis with TDF use in patients with low body weight.

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Conflicts of interest

S.O. has received honoraria and research grants from MSD K.K., Abbott Japan, Co., Janssen Pharmaceutical K.K., Pfizer, Co., and Roche Diagnostics K.K.; has received honoraria from Astellas Pharmaceutical K.K., Bristol-Myers K.K., Daiichisankyo, Co., Dainippon Sumitomo Pharma, Co., GlaxoSmithKline, K.K., Taisho Toyama Pharmaceutical, Co., Torii Pharmaceutical, Co., and ViiV Healthcare. H.G. has received honoraria from MSD K.K., Abbott Japan, Co., Janssen Pharmaceutical K.K., Torii Pharmaceutical, Co., Roche Diagnostics K.K., and ViiV Healthcare, Co.

The remaining authors declare no conflict of interest.

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Single-nucleotide polymorphisms in the UDP-glucuronosyltransferase 1A-3' untranslated region are associated with atazanavir-induced nephrolithiasis in patients with HIV-1 infection: a pharmacogenetic study

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Objectives: Ritonavir-boosted atazanavir (atazanavir/ritonavir) is a widely used antiretroviral drug, though it can potentially cause nephrolithiasis. The aim of this study was to determine the relationship between polymorphisms in genes encoding proteins involved in metabolism and transportation of atazanavir, and atazanavir/ritonavir-induced nephrolithiasis in HIV-1-infected patients treated with atazanavir/ritonavir.

Methods: Nineteen SNPs in the *ABCB1*, *NR1I2*, *UGT1A1*, *SLCO1B1* and *CYP3A5* genes were examined in case patients with atazanavir/ritonavir-induced nephrolithiasis ($n=31$) and controls ($n=47$). Case patients were those with a clinical diagnosis of nephrolithiasis while on atazanavir/ritonavir, based on new-onset acute flank pain plus one of the following: (i) new-onset haematuria; (ii) documented presence of stones by either abdominal ultrasonography or CT; or (iii) confirmed stone passage. Control patients were consecutively enrolled among those with >2 years of atazanavir/ritonavir exposure free of nephrolithiasis. Genotyping was performed by allelic discrimination using TaqMan 5'-nuclease assays with standard protocols. Associations between alleles and atazanavir/ritonavir-induced nephrolithiasis were tested by univariate and multivariate logistic regression analyses.

Results: Multivariate analysis showed a significant association between atazanavir/ritonavir-induced nephrolithiasis and genotype T/C versus C/C at position c.211 (adjusted OR=3.7; 95% CI, 1.13–11.9; $P=0.030$), genotype G/C versus C/C at 339 (adjusted OR=5.8; 95% CI, 1.56–21.3; $P=0.009$) and genotype G/G or G/C versus C/C at 440 (adjusted OR=5.8; 95% CI, 1.56–21.3; $P=0.009$) of the *UGT1A*-3' untranslated region (UTR).

Conclusions: This is the first known study to identify the association between SNPs in the *UGT1A*-3'-UTR and atazanavir-induced nephrolithiasis. Further studies are warranted to confirm this association and to elucidate how these SNPs might influence atazanavir exposure.

Keywords: atazanavir sulphate, renal stones, SNPs

Introduction

Ritonavir-boosted atazanavir (atazanavir/ritonavir) is a widely used protease inhibitor for the treatment of HIV-1 infection.¹ However, some case reports/series have documented nephrolithiasis containing atazanavir,^{2–5} and cohort studies demonstrated that the incidence of nephrolithiasis is substantially higher in patients on atazanavir/ritonavir-containing ART than

patients on other protease inhibitor- or efavirenz-containing ART.^{6–8} The development of renal stones, even a single episode, is a risk factor for significant decrement in renal function, which could affect the prognosis of patients.^{9–11}

The mechanism of atazanavir-induced nephrolithiasis is not fully understood. However, unchanged atazanavir is reported to be excreted in urine at 7% of the administered dose, and strong acidity (e.g. pH 1.9) is required to achieve optimal dissolution of

atazanavir,¹² whereas urine is usually mildly acidic.⁶ These characteristics of atazanavir are similar to those of indinavir, an old protease inhibitor well known for its precipitation and renal stone formation, and could explain the high incidence of nephrolithiasis in patients treated with atazanavir/ritonavir.^{7,13}

A number of proteins are considered to take part in the metabolism or transportation of atazanavir, and thus can affect atazanavir exposure. Atazanavir is mainly metabolized by cytochrome P450 (CYP3A), including CYP3A4 and CYP3A5, and their variants can affect the concentration and clearance of atazanavir.^{14,15} Minor biotransformation pathways for atazanavir or its metabolites include glucuronidation, suggesting that UDP-glucuronosyltransferase 1A1 (*UGT1A1*), known for its association with atazanavir-induced unconjugated hyperbilirubinaemia, is also involved in the metabolism of atazanavir.¹² SNPs in the *NR1I2* gene, which encodes the nuclear receptor pregnane X receptor (PXR), regulate the expression of *CYP3A4*¹⁶ and *ABCB1*,¹⁷ and also influence atazanavir concentration.¹⁸ With regard to atazanavir transportation, P-glycoprotein is a membrane protein expressed on the cells of the intestine, hepatocytes and renal proximal tubules. Encoded by the *ABCB1* gene, P-glycoprotein regulates atazanavir intestinal absorption, and thus affects exposure to atazanavir.^{19–22} The organic anion-transporting polypeptide 1B1 (*OATP1B1*), encoded by the gene *SLCO1B1*, is another protein involved in influx transportation of protease inhibitors and unconjugated bilirubin. SNPs in *SLCO1B1* also modify atazanavir concentration.²³

To our knowledge, there are no published studies that investigated the association between genetic variants in the genes that encode proteins involved in the metabolism or transport of atazanavir and atazanavir-induced nephrolithiasis. Based on the above background, the present study was designed to elucidate the association between polymorphisms in genes encoding the abovementioned proteins and atazanavir-induced nephrolithiasis.

Methods

Ethics statement

The study was approved by the Human Genetics Research Ethics Committee of the National Center for Global Health and Medicine, Tokyo, Japan. Each patient included in this study provided written informed consent for genetic testing and publication of the clinical data. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Study design

We performed a case–control study to elucidate the association between SNPs in genes encoding proteins that take part in the metabolism of atazanavir and drug transporters and atazanavir-induced nephrolithiasis in a single-centre cohort.

Study subjects

The eligible subjects were HIV-1-infected Japanese patients, aged >17 years, who commenced treatment with atazanavir/ritonavir-containing ART between 1 January 2004 and 30 June 2012,⁷ including both treatment-naïve and treatment-experienced patients. Patients were excluded if they had (i) commenced atazanavir/ritonavir-containing ART during the study period at other facilities or (ii) been prescribed

unboosted atazanavir. Case patients were those in whom nephrolithiasis occurred while on atazanavir/ritonavir-containing ART. Nephrolithiasis was defined as described in previous studies:^{6,7} cases with a clinical diagnosis by the attending physician based on new onset of acute flank pain plus one of the following: (i) new-onset haematuria confirmed by urine dipstick test; (ii) 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 CT; or (iii) stone passage confirmed by either the patient or the attending physician. Control patients were consecutively enrolled HIV-1-infected patients with >2 years of atazanavir/ritonavir experience who were free of nephrolithiasis based on the chart review. Enrolment took place from September 2012 to February 2013.

Measurements

The potential risk factors for nephrolithiasis were determined according to previous studies and collected from the medical records, together with basic demographics.^{4,5,24–27} They included age, sex, body weight, BMI (body weight (kg)/[height (m)]²), baseline laboratory data [CD4 cell count, HIV viral load, estimated glomerular filtration rate (eGFR) and serum uric acid] and the presence or absence of other medical conditions (concurrent use of tenofovir, past history of nephrolithiasis, previous exposure to indinavir, diabetes mellitus defined by using antidiabetic agents or fasting plasma glucose >126 mg/dL or plasma glucose >200 mg/dL on two different days, 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, infection with hepatitis B virus defined by positive hepatitis B surface antigen, and infection with hepatitis C virus defined by positive hepatitis C viral load). eGFR was calculated using the equation of the four-variable Modification of Diet in Renal Diseases (MDRD) study.²⁸ We used the data on or closest to and preceding the day of starting atazanavir/ritonavir-containing 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.⁷ The value of serum total bilirubin was collected in two ways: for patients who continued atazanavir/ritonavir for >2 years, the value of total bilirubin closest to 2 years after initiation of atazanavir/ritonavir was collected. For patients who discontinued atazanavir/ritonavir within 2 years, the value closest to and preceding the day of discontinuation was used. At our clinic, body weight and blood pressure were measured on every visit.

Genetic polymorphisms

SNPs in genes encoding proteins that take part in the metabolism of atazanavir and drug transport were selected based on their functional significance, findings of previously published reports and/or reported minor-allele frequencies >5% in the Japanese.^{15,18,20,23,29–32} The allele frequency data for the Japanese were obtained from the Japanese SNP (JSNP) database.³³ The 19 selected SNPs were: (i) *ABCB1* (encodes P-glycoprotein) 2677T→A/G (A:Ser893Thr, G:Ser893Ala; rs2032582); 1236T→C (Gly412Gly; rs1128503); 3435C→T (Ile1145Ile; rs1045642); 193A→G [in the 3' untranslated region (UTR); (rs3842)]; 365T→C (5'-UTR; rs3213619); (ii) *NR1I2* (encodes PXR) 370G→A (3'-UTR; rs3732359); 522C→T (3'-UTR; rs3732360); 131C→A (5'-UTR; rs1523127); 1232T→C (3'-UTR; rs3814058); 1195A→C (3'-UTR; rs3814057); 63396T→C (intron; rs2472677); 44477T→C (5'-UTR; rs1523130); (iii) *UGT1A1* 211G→A (Gly71Arg; rs4148323); c.211T→C (3'-UTR; rs10929303), 339G→C (3'-UTR; rs1042640); 440G→C (3'-UTR; rs8330); (iv) *SLCO1B1* (encodes OATP1B1) 521T→C (Val174Ala; rs4149056); 388A→G (Asn130Asp; rs2306283); and (v) *CYP3A5* (encodes cytochrome P450 3A5) 14T→C (3'-UTR; rs15524). We did not find appropriate SNPs in *CYP3A4* to examine. The *UGT1A1* variant that contains seven thymine adenine (TA) nucleotide

repeats, A (TA)₇TAA (UGT1A1*28), which is known to be less transcriptionally active than the common promoter with six TA repeats (UGT1A1*1),³⁴ was also examined.

Pharmacogenetic analyses

Genomic DNA was extracted from peripheral blood leucocytes using the QIAamp DNA MiniKit and the protocol provided by the manufacturer (Qiagen, Valencia, CA, USA). All genotyping was performed by allelic discrimination using TaqMan 5'-nuclease assays with standard protocols (TaqMan SNP Genotyping Assays; Applied Biosystems, Foster City, CA, USA). The primer and probe sequences are available on request. Primer sequences for PCR amplification of the TATA box of the UGT1A1 promoter were 5'-GTCACGTGACACAGTCAAAC-3' and 3'-TTTGTCCTGCCAGAGGT T-5';³⁵ the PCR conditions were as follows: 95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 58°C for 30 s and 72°C for 30 s, and 72°C for 7 min.

Statistical analysis

Baseline characteristics were compared between case patients and control patients by Student's *t*-test for continuous variables and by either the χ^2 test or Fisher's exact test for categorical variables. Differences in genotype frequencies and allele frequencies between the two groups were assessed by Fisher's exact test using a 2x3 table (2x6 table for rs2032582) and the χ^2 test, respectively. Associations between genotypes and atazanavir-induced nephrolithiasis were tested by univariate and multivariate logistic regression analyses. The risk of atazanavir-induced nephrolithiasis of other variables was estimated with univariate analysis and the variables with *P*<0.10 were incorporated into multivariate analysis as covariates, in addition to the basic demographics, such as age and sex. Statistical significance was defined as a two-sided *P* value of <0.05. We used ORs and 95% CIs to estimate the strength of association between nephrolithiasis and each variable. Haploview software was used to test for Hardy-Weinberg equilibrium and to estimate the linkage

disequilibrium measure *D'*. All other statistical analyses were performed with the Statistical Package for Social Sciences version 21.0 (SPSS, Chicago, IL, USA).

Results

Of 37 patients diagnosed with nephrolithiasis while on atazanavir/ritonavir-containing ART,⁷ 31 provided written informed consent, and thereby constituted the case patients. Furthermore, 47 consecutive control patients who continued atazanavir/ritonavir for >2 years were enrolled in the study. The baseline characteristics and laboratory data of patients in the two groups are listed in Table 1. The basic demographics (sex and age) and established risk factors for nephrolithiasis (weight, BMI, serum uric acid, hypertension, diabetes mellitus, history of nephrolithiasis and history of indinavir use) were not different between the two groups, except for hepatitis C infection, which was more common among case patients (*P*=0.034). Serum total bilirubin was higher in case patients than the controls (*P*<0.001).

Table 2 summarizes the distribution of genotypes and allele frequencies at the *ABCB1*, *NR1I2*, *UGT1A1* (including *UGT1A1*28*), *SLCO1B1* and *CYP3A5* genes in the two groups. The genotype distributions for all polymorphisms were in Hardy-Weinberg equilibrium with a cut-off *P* value of 0.001. In single SNP analysis, a higher percentage of patients with nephrolithiasis had genotype T/C versus C/C at position c.211 (*P*=0.025), genotype G/C versus C/C at position 339 (*P*=0.007) and genotype G/G and G/C versus C/C at position 440 (*P*=0.009) of the UGT1A-3'-UTR. These results were consistent with allele frequency analysis, as case patients were more likely to possess allele T versus C at c.211 (*P*=0.033), allele G versus C at position 339 (*P*=0.012) and allele G versus C at 440 (*P*=0.006) of the UGT1A-3'-UTR, respectively. These three SNPs were in linkage disequilibrium with each other (*D'*>0.9). Figure 1

Table 1. Characteristics of patients with and without nephrolithiasis

	Total patients	Patients with nephrolithiasis (n=31)	No nephrolithiasis (n=47)	<i>P</i> value
Male, n (%)	71 (91)	29 (94)	42 (89)	0.70
Age (years), median (IQR)	40 (35-47)	39 (35-45)	40 (35-49)	0.40
Weight (kg), median (IQR)	65.6 (58.2-72.3)	64.8 (57.6-71.9)	66.5 (58.2-73.2)	0.60
BMI (kg/m ²), median (IQR)	22.7 (20.3-24.6)	22.7 (19.9-24.9)	22.7 (20.4-24.6)	0.58
eGFR (mL/min/1.73 m ²), median (IQR)	88.3 (76.8-98.4)	85.8 (69.7-97.9)	88.5 (78.4-98.6)	0.81
Serum creatinine (mg/dL), median (IQR)	0.75 (0.68-0.89)	0.75 (0.72-0.93)	0.75 (0.66-0.85)	0.25
CD4 cell count (cells/mm ³), median (IQR)	230 (187-302)	232 (194-356)	229 (187-290)	0.63
HIV-1 load (log ₁₀ /mL), median (IQR)	4.19 (3.38-4.77)	3.96 (2.25-4.79)	4.22 (3.41-4.77)	0.65
Serum uric acid (mg/dL), median (IQR)	6.1 (5.1-7.0)	6.3 (5.5-7.7)	5.9 (4.9-6.7)	0.15
History of nephrolithiasis, n (%)	6 (8)	4 (13)	2 (4)	0.21
Hypertension, n (%)	9 (12)	3 (10)	6 (13)	1.00
Diabetes mellitus, n (%)	3 (4)	1 (3)	2 (4)	1.00
Hepatitis C infection, n (%)	6 (8)	5 (16)	1 (2)	0.034
Hepatitis B infection, n (%)	2 (3)	1 (3)	1 (2)	1.00
Treatment-naive, n (%)	50 (64)	18 (58)	32 (68)	0.47
Co-administration of TDF, n (%)	26 (33)	9 (29)	17 (36)	0.63
History of indinavir use, n (%)	6 (8)	3 (10)	3 (6)	0.68
Serum total bilirubin (mg/dL)	1.9 (1.4-2.4)	2.3 (1.8-3.4)	1.7 (1.3-1.9)	<0.001

TDF, tenofovir disoproxil fumarate.

Table 2. Genotype/allele frequencies for *ABCB1*, *NR1I2*, *UGT1A1*, *SLCO1B1* and *CYP3A5* in patients with and without nephrolithiasis

	Amino acid	Genotype frequency			Allele frequency		
		nephrolithiasis (n=31)	no nephrolithiasis (n=47)	P value ^a	nephrolithiasis (n=31)	no nephrolithiasis (n=47)	P value ^a
<i>ABCB1</i> (P-glycoprotein)				<i>ABCB1</i> (P-glycoprotein)			
193 A→G, rs3842				193 A→G, rs3842			
	A/A	16 (52)	27 (57)	0.24	A	46 (74)	0.81
	A/G	14 (45)	14 (30)		G	16 (26)	
	G/G	1 (3)	6 (13)				
365 T→C, rs3213619				365 T→C, rs3213619			
	T/T	28 (90)	37 (79)	0.23	T	59 (95)	0.33
	T/C	3 (10)	10 (21)		C	3 (5)	
	C/C	0	0				
1236 T→C, rs1128503				1236 T→C, rs1128503			
	T/T	7 (23)	15 (32)	0.48	T	33 (53)	0.80
	T/C	19 (61)	22 (47)		C	29 (47)	
	C/C	5 (16)	10 (21)				
2677 T→A/G, rs2032582				2677T→A/G, rs2032582			
	T/T	5 (16)	5 (11)	0.45	T	26 (42)	0.20
	T/A	6 (19)	5 (11)		A	14 (23)	
	G/G	3 (10)	10 (21)		G	22 (35)	
	G/T	10 (32)	15 (32)				
	G/A	6 (19)	12 (26)				
	A/A	1 (3)	0				
3435 C→T, rs1045642				3435 C→T, rs1045642			
	C/C	10 (32)	22 (47)	0.35	C	36 (58)	0.16
	C/T	16 (52)	21 (45)		T	26 (42)	
	T/T	5 (16)	4 (9)				
<i>NR1I2</i> (PXR)				<i>NR1I2</i> (PXR)			
131 A→C, rs1523127				131 A→C, rs1523127			
	C/C	1 (3)	6 (13)	0.38	C	20 (32)	0.53
	C/A	18 (58)	23 (49)		A	42 (68)	
	A/A	12 (39)	18 (38)				
370 G→A, rs3732359				370 G→A, rs3732359			
	G/G	8 (26)	16 (34)	0.37	G	35 (56)	1.00
	G/A	19 (61)	21 (45)		A	27 (44)	
	A/A	4 (13)	10 (21)				
522 C→T, rs3732360				522 C→T, rs3732360			
	C/C	8 (26)	15 (32)	0.60	C	35 (56)	1.00
	C/T	19 (61)	23 (49)		T	27 (44)	
	T/T	4 (13)	9 (19)				
1195 A→C, rs3814057				1195 A→C, rs3814057			
	A/A	4 (13)	11 (23)	0.39	A	27 (44)	0.69
	A/C	19 (61)	22 (47)		C	35 (56)	
	C/C	8 (26)	14 (30)				

1232 T→C, rs3814058				1232 T→C, rs3814058			
T/T	4 (13)	10 (21)		T	27 (44)	41 (44)	1.00
T/C	19 (61)	21 (45)	0.37	C	35 (56)	53 (56)	
C/C	8 (26)	16 (34)					
44477 T→C, rs1523130				44477 T→C, rs1523130			
T/T	1 (3)	6 (13)		T	19 (31)	34 (36)	0.48
T/C	17 (55)	22 (47)	0.38	C	43 (69)	60 (64)	
C/C	13 (42)	19 (40)					
63396 T→C, rs2472677				63396 T→C, rs2472677			
T/T	18 (58)	24 (51)		T	45 (73)	68 (72)	0.98
T/C	9 (29)	20 (43)	0.38	C	17 (27)	26 (28)	
C/C	4 (13)	3 (6)					
<i>UGT1A1</i>				<i>UGT1A1</i>			
*28 ^b				*28 ^b			
*1/*1	27 (87)	44 (94)		*1	57 (92)	91 (97)	0.21
*1/*28	3 (10)	3 (6)	0.50	*28	5 (8)	3 (3)	
*28/*28	1 (3)	0					
211 G→A, rs4148323	Gly71Arg			211 G→A, rs4148323			
G/G	19 (61)	30 (64)		G	49 (79)	73 (78)	0.85
G/A	11 (36)	13 (28)	0.61	A	13 (21)	21 (22)	
A/A	1 (3)	4 (9)					
c.211 T→C, rs10929303				c.211 T→C, rs10929303			
T/T	0	0		T	11 (18)	6 (6)	0.033
T/C	11 (35)	6 (13)	0.025	C	51 (82)	88 (94)	
C/C	20 (65)	41 (87)					
339 G→C, rs1042640				339 G→C, rs1042640			
G/G	0	0		G	11 (18)	4 (4)	0.012
G/C	11 (35)	4 (9)	0.007	C	51 (82)	90 (96)	
C/C	20 (65)	43 (91)					
440 G→C, rs8330				440 G→C, rs8330			
G/G	1 (3)	0		G	12 (19)	4 (4)	0.006
G/C	10 (32)	4 (9)	0.007	C	50 (81)	90 (96)	
C/C	20 (65)	43 (91)					
<i>SLCO1B1</i>				<i>SLCO1B1</i>			
388 A→G, rs2306283	Asn130Asp			388 A→G, rs2306283			
A/A	14 (45)	24 (51)		A	42 (68)	68 (72)	0.54
A/G	14 (45)	20 (43)	0.83	G	20 (32)	26 (28)	
G/G	3 (10)	3 (6)					
521 T→C, rs4149056	Val174Ala			521 T→C, rs4149056			
T/T	20 (65)	31 (66)		T	51 (82)	75 (80)	0.71
T/C	11 (36)	13 (28)	0.34	C	11 (18)	19 (20)	
C/C	0	3 (6)					
<i>CYP3A5</i>				<i>CYP3A5</i>			
14 T→C, rs15524				14 T→C, rs15524			
T/T	16 (52)	25 (53)		T	46 (74)	67 (71)	0.70
T/C	14 (45)	17 (36)	0.50	C	16 (26)	27 (29)	
C/C	1 (3)	5 (11)					

^aBy Fisher's exact test.

^b*1, reference sequence A(TA)₆TAA; *28, A(TA)₇TAA.