All authors contributed to the concept, design, and writing of this submission.

Conflicts of interest

No financial support was received for this article. The authors declare that there are no conflicts of interest.

Takeshi Nishijima[a,c], Kunihisa Tsukada[a], Naoyoshi Nagata[b], Koji Watanabe[a,c], Katsuji Teruya[a], Hiroyuki Gatanaga[a,c], Yoshimi Kikuchi[a] and Shinichi Oka[a,c],
[a]AIDS Clinical Center, [b]Department of Gastroenterology, National Center for Global Health and Medicine, Tokyo, and [c]Center for AIDS Research, Kumamoto University, Kumamoto, Japan.

Correspondence to Takeshi Nishijima, MD, AIDS Clinical Center, National Center for Global Health and Medicine, 1–21–1 Toyama, Shinjuku-ku, Tokyo 162–0052, Japan.
Tel: +81 3 3202 7181; fax: +81 3 5273 6483;
e-mail: tnishiji@acc.ncgm.go.jp

Received: 8 June 2011; accepted: 10 June 2011.

References

1. Bonacini M, Young T, Laine L. **The causes of esophageal symptoms in human immunodeficiency virus infection. A prospective study of 110 patients.** *Arch Intern Med* 1991; **151**:1567–1572.
2. Wilcox CM, Schwartz DA, Clark WS. **Esophageal ulceration in human immunodeficiency virus infection. Causes, response to therapy, and long-term outcome.** *Ann Intern Med* 1995; **123**: 143–149.
3. Rabeneck L, Popovic M, Gartner S, McLean DM, McLeod WA, Read E, *et al.* **Acute HIV infection presenting with painful swallowing and esophageal ulcers.** *JAMA* 1990; **263**:2318–2322.
4. Vietri NJ, Skidmore PJ, Dooley DP. **Esophageal ulceration due to cytomegalovirus infection in a patient with acute retroviral syndrome.** *Clin Infect Dis* 2002; **34**:E14–15.
5. Siegmund B, Moos V, Loddenkemper C, Wahnschaffe U, Engelmann E, Zeitz M, *et al.* **Esophageal giant ulcer in primary human immunodeficiency virus infection is associated with an infiltration of activated T cells.** *Scand J Gastroenterol* 2007; **42**:890–895.
6. Wilcox CM, Schwartz DA. **Comparison of two corticosteroid regimens for the treatment of HIV-associated idiopathic esophageal ulcer.** *Am J Gastroenterol* 1994; **89**:2163–2167.
7. Houghton JM, Korah RM, Kim KH, Small MB. **A role for apoptosis in the pathogenesis of AIDS-related idiopathic esophageal ulcers.** *J Infect Dis* 1997; **175**:1216–1219.
8. Wilcox CM, Rodgers W, Lazenby A. **Prospective comparison of brush cytology, viral culture, and histology for the diagnosis of ulcerative esophagitis in AIDS.** *Clin Gastroenterol Hepatol* 2004; **2**:564–567.
9. Miendje Deyi Y, Goubau P, Bodeus M. **False-positive IgM antibody tests for cytomegalovirus in patients with acute Epstein-Barr virus infection.** *Eur J Clin Microbiol Infect Dis* 2000; **19**:557–560.
10. Bronze MS, Warr AG, Spigel D, Smith VD, Smalley D. **False-positive enzyme immunoassay for human immunodeficiency virus due to acute cytomegalovirus infection.** *Clin Infect Dis* 1998; **27**:221–222.

DOI:10.1097/QAD.0b013e328349c6cc

Impact of Small Body Weight on Tenofovir-Associated Renal Dysfunction in HIV-Infected Patients: A Retrospective Cohort Study of Japanese Patients

Takeshi Nishijima[1,3]*, Hirokazu Komatsu[2], Hiroyuki Gatanaga[1,3], Takahiro Aoki[1], Koji Watanabe[1,3], Ei Kinai[1], Haruhito Honda[1], Junko Tanuma[1], Hirohisa Yazaki[1], Kunihisa Tsukada[1], Miwako Honda[1], Katsuji Teruya[1], Yoshimi Kikuchi[1], Shinichi Oka[1,3]

1 AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan, **2** Department of Community Care, Saku Central Hospital, Nagano, Japan, **3** Center for AIDS Research, Kumamoto University, Kumamoto, Japan

Abstract

Background: Treatment with tenofovir is sometimes associated with renal dysfunction. Limited information is available on this side effect in patients with small body weight, although the use of tenofovir will spread rapidly in Asia and Africa, where patients are likely to be of smaller body weight.

Methods: In a single-center cohort, Japanese patients with HIV infection who started tenofovir-containing antiretroviral therapy were retrospectively analyzed. The incidence of tenofovir-associated renal dysfunction, defined as more than 25% decrement of estimated glomerular filtration rate (eGFR) from the baseline, was determined. The effects of small body weight and body mass index (BMI) on tenofovir-associated renal dysfunction, respectively, were estimated in univariate and multivariate Cox hazards models as the primary exposure. Other possible risk factors were evaluated by univariate analysis and those found significant were entered into the multivariate analysis.

Results: The median weight of 495 patients was 63 kg. Tenofovir-related renal dysfunction occurred in 97 (19.6%) patients (incidence: 10.5 per 100 person-years). Univariate analysis showed that the incidence of tenofovir-related renal dysfunction was significantly associated with smaller body weight and BMI, respectively (per 5 kg decrement, HR = 1.23; 95% CI, 1.10–1.37; $p<0.001$)(per 1 kg/m^2 decrement, HR = 1.14; 95% CI, 1.05–1.23; $p = 0.001$). Old age, high baseline eGFR, low serum creatinine, low CD4 count, high HIV viral load, concurrent nephrotoxic drugs, hepatitis C infection, and current smoking were also associated with tenofovir-related renal dysfunction. Multivariate analysis identified small body weight as a significant risk (adjusted HR = 1.13; 95% CI, 1.01–1.27; $p = 0.039$), while small BMI had marginal significance (adjusted HR = 1.07; 95% CI 1.00–1.16; $p = 0.058$).

Conclusion: The incidence of tenofovir-associated renal dysfunction in Japanese patients was high. Small body weight was identified as an independent risk factor for tenofovir-associated renal dysfunction. Close monitoring of renal function is advocated for patients with small body weight treated with tenofovir.

Citation: Nishijima T, Komatsu H, Gatanaga H, Aoki T, Watanabe K, et al. (2011) Impact of Small Body Weight on Tenofovir-Associated Renal Dysfunction in HIV-Infected Patients: A Retrospective Cohort Study of Japanese Patients. PLoS ONE 6(7): e22661. doi:10.1371/journal.pone.0022661

Editor: Gary Maartens, University of Cape Town, South Africa

Received February 24, 2011; **Accepted** June 28, 2011; **Published** July 25, 2011

Funding: This work was supported by a Grant-in-Aid for AIDS Research from the Ministry of Health, Labor, and Welfare of Japan (H20-AIDS-002). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: tnishiji@acc.ncgm.go.jp

Introduction

Tenofovir disoproxil fumarate (TDF) is one of the most widely used nucleotide reverse transcriptase inhibitors (NRTI) for patients with HIV infection, with proven efficacy and safety [1–6]. However, TDF is known to cause renal proximal tubular dysfunction, and several case reports have been published with TDF-related Fanconi syndrome, diabetes insipidus, and acute tubular necrosis, which sometimes lead to acute renal failure [7–10]. Long-term TDF use also reduces glomerular filtration rate more than other NRTIs [11–14]. To date, the nephrotoxic effect of TDF is regarded as mild and tolerable. A recently published meta-analysis has reported that the use of TDF is associated with a statistically significant but only modest renal dysfunction, and recommended that TDF use should not be restricted even when regular monitoring of renal function and serum phosphate levels is impractical [15]. However, 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 [16–19].

The 2010 WHO guideline on antiretroviral therapy for HIV infection in adults and adolescents, usually applied to resource-constrained settings, recommends TDF as one of the components of first line therapies (URL:http://whqlibdoc.who.int/publications/

2010/9789241599764_eng.pdf). It is expected that the use of TDF will spread rapidly in Asia and Africa in the near future, where patients are more likely to be of small body weight. Thus, at this stage, it is important to establish the relationship between TDF-associated renal dysfunction and body weight. A small body weight is considered a risk factor for TDF-associated renal dysfunction, in addition to old age, high baseline serum creatinine level, low CD4 count, concurrent use of ritonavir-boosted protease inhibitor, and concurrent use of nephrotoxic drugs [4,17,19–21]. To our knowledge, there is almost no report that primarily analyzed the influence of body weight on TDF-associated renal dysfunction. Since Japanese are generally of smaller stature and have a lower median body weight than Whites and African Americans, who mostly comprise the cohorts of studies published to date, it is important to investigate the impact of TDF-associated renal dysfunction in Japanese patients.

Based on the above background, the present study was designed to determine the incidence of TDF-associated renal dysfunction in Japanese patients and analyze the impact of small body weight on TDF-associated renal dysfunction.

Methods

Ethics Statement

This study was approved by the Human Research Ethics Committee of National Center for Global Health and Medicine (Text S1). All patients included in this study provided a written informed consent for their clinical and laboratory data to be used and be published for research purposes. This study has been conducted according to the principles expressed in the Declaration of Helsinki.

Study Design and Settings

We performed a single-center, retrospective cohort study of HIV-infected Japanese patients using 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 more than 2,700 registered patients.

Study Subjects

The study population were patients >17 years of age who commenced treatment with standard 300 mg/day of TDF-containing antiretroviral regimen at our clinic between January 1, 2002 to March 31, 2009. Both treatment-naive and patients with experience in antiretroviral treatment but not TDF, with an estimated glomerular filtration rate (eGFR) of >60 ml/min/1.73 m^2 were enrolled. Patients were followed up until September 31, 2009. Patients were excluded if their follow-up period at our facility was less than 24 weeks after commencement of TDF-based therapy, if they had started TDF at other facilities, or if there was evidence of prior TDF use. We only included Japanese patients in order to examine a population with comparatively homogenous basic demographics and background.

Measurements

Outcome measure: TDF-associated renal dysfunction. We defined TDF-associated renal dysfunction as more than 25% decrease in eGFR relative to the baseline [17,22,23]. Baseline eGFR was estimated for each patient from the average of two successive serum creatinine measurements made closest to and preceding the commencement of TDF by no more than 90 days. Changes in eGFR were plotted from the baseline measurement until the value diminished to less than 75% of the baseline or at the end of the follow-up period. The eGFR values at occurrence of TDF-associated renal dysfunction, at censoring, and closest to and preceding 24, 48, and 96 weeks to the diagnosis were collected. Patients generally visited our clinic between every month to every 3 months, and measurement of eGFR was usually conducted on every visit. eGFR was calculated using the equation from the 4-variable Modification of Diet in Renal Disease (MDRD) study [24].

Primary exposure variable. Our primary exposure variables were body weight and body mass index (BMI) at the time of commencement of TDF-containing antiretroviral therapy (ART). BMI was calculated by the equation: BMI = [body weight (kg)/height $(m)^2$].

Other variables: potential risk factors. Potential risk factors for TDF-associated renal dysfunction were determined according to previous studies and collected together with the basic demographics from the medical charts [4,19,20,25]. They included sex, age, baseline laboratory data: CD4 cell count, HIV viral load, and serum creatinine, and other medical conditions (antiretroviral treatment-naïve or experienced, concurrent ritonavir-boosted protease inhibitors, concurrent nephrotoxic drugs such as ganciclovir, sulfamethoxazole/trimethoprim, ciprofloxacin, and NSAIDs, diabetes mellitus, co-infection with hepatitis B defined by positive hepatitis B surface antigen, co-infection with hepatitis C defined by positive HCV viral load, hypertension defined by current treatment with antihypertensive agents, dyslipidemia defined by current treatment with lipid-lowering agents, and current smoking) [26]. We used the data on or closest to and preceding the day of starting TDF-containing ART by no more than 90 days. The data on weight change from the baseline to the end of follow-up period and the frequency of eGFR monitoring for each patient were collected.

Statistical analysis

The time to 25% decline in eGFR from the baseline was calculated from the date of treatment initiation to the date of occurrence of TDF-associated renal dysfunction. Censored cases represented those who discontinued TDF, dropped out, referred to other facilities, or at the end of follow-up period. The time from TDF initiation to 25% decrease in eGFR was analyzed by the Kaplan Meier method for the whole cohort. To estimate the impact of body weight on the incidence of TDF-associated renal dysfunction, we calculated the impact of every 5 kg decrement from the median weight using Cox proportional hazards regression analysis. The impact of every 1 kg/m^2 decrement in BMI on the incidence of TDF-associated renal dysfunction was estimated by the same method. The impact of each basic demographics, 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 weight on TDF-associated renal dysfunction, we conducted three models using multivariate Cox proportional hazards regression analysis. Model 1 was the aforementioned univariate analysis for every 5 kg decrement. Model 2 included sex, age plus model 1 in order to adjust for basic characteristics. In model 3, we added variables with P values<0.05 in univariate analysis for adjustment (these included age per 10 years, serum creatinine >0.8 mg/dl, CD4 count <200/μl, HIV viral load per log10/ml, concurrent nephrotoxic drugs, co-infection with hepatitis C, and current smoking). Concurrent ritonavir-boosted protease inhibitors were also added in Model 3 although their p value was 0.116 in the univariate analysis. This was based on the results of several studies suggesting that concurrent use of ritonavir-boosted protease inhibitors is a risk factor for TDF-associated renal dysfunction

[19,20]. The eGFR was excluded from multivariate analysis because of its multicollinearity with sex, age, and serum creatinine, since eGFR was gained by the equation of those variables. The impact of every 1 kg/m^2 decrement in BMI on the incidence of TDF-associated renal dysfunction was estimated by the same method with Model 1 to Model 3.

Four other analyses were conducted to further examine the relationship between low body weight and TDF-associated renal dysfunction. First, the time from initiation of TDF therapy to 25% decrease in eGFR was analyzed by the Kaplan Meier method for intertertile baseline body weight categories: <59, 59–67, and >67 kg. The log-rank test was used to determine statistical significance. Second, to investigate the impact of changes in muscle mass on changes in the eGFR as calculated by MDRD, we compared weight changes with one-way ANOVA among intertertile baseline weight categories. We also conducted the sensitivity analysis by adding the variable "weight change" in multivariate analysis. Third, the median and interquartile value for the actual fall in eGFR from the baseline to 24, 48, and 96 weeks for the whole cohort and three baseline weight categories, respectively, were calculated. The eGFR value at 24, 48, and 96 weeks included those that were censored before reaching 24, 48, and 96 weeks, respectively, so that we could interpret the data for actual fall in eGFR, including not only survived cases but also censored cases. Fourth, we counted the number of patients whose eGFR decreased to <60 and <10 ml/min/1.73 m^2, and who discontinued TDF with the clinical diagnosis of renal dysfunction due to TDF. Chi-square test was used to determine whether the difference among the weight categories was statistically significant.

Statistical significance was defined at two-sided p values< 0.05. We used hazard ratios (HRs) and 95% confidence intervals (CIs) to estimate the impact of each variable on TDF-associated renal dysfunction. All statistical analyses were performed with The Statistical Package for Social Sciences ver. 17.0 (SPSS, Chicago, IL).

Results

Between January 1, 2002 to March 31, 2009, 599 patients started TDF-containing ART (Figure 1). Of these, 104 patients were excluded based on the abovementioned criteria. Thus 495 patients were included in the present study (Dataset S1). Table 1 shows the demographics, laboratory data, and medical conditions of the study population at baseline. Two patients received ART with 3 NRTIs, 3 patients received ART with one protease inhibitor (PI), one non-NRTI (NNRTI), and tenofovir/emtricitabine, and the remaining patients had a standard ART with 2 NRTIs and either PI, NNRTI, or integrase inhibitor (INI). The median body weight and BMI were 63 kg and 21.9 kg/m^2, respectively. The median age of the patients was 38 years and 95.2% were males. The eGFR was well maintained (median: 120.9 ml/min/1.73 m^2), and the median baseline CD4 count was 247/μl. Of the total, 208 patients (42%) were antiretroviral treatment naïve, while 287 were treatment-experienced patients. Viral load was suppressed to <50 copies/ml in 162 (32.7%) patients. 403 (81.4%) were on concurrent PIs as the key drug, 367 (74.1%) were on ritonavir-boosted PIs, and only 83 (16.8%) had NNRTIs as the key drug. Smoking was prevalent among the study population, as 240 (48.5%) were identified as a current smoker.

TDF-associated renal dysfunction defined by more than 25% decrease of eGFR from baseline occurred in 97 patients (19.6%), with an estimated incidence of 10.5 per 100 person-years. The median time from commencement of TDF to occurrence of TDF-associated renal dysfunction was 39 weeks (IQR 13.5–99.4 weeks) (range: 1–1,841 days). The total observation period was 924.7 patient-years (median 72 weeks, IQR 38.6–139.3 weeks). Figure 2 shows the Kaplan-Meier survival curve for the occurrence of TDF-associated renal dysfunction for the whole cohort.

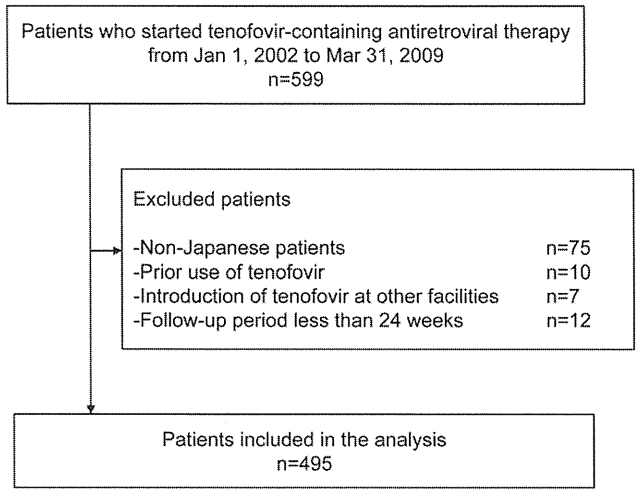

Figure 1. Flow diagram of patient selection.
doi:10.1371/journal.pone.0022661.g001

Univariate analysis showed a significant relationship between TDF-associated renal dysfunction and every 5 kg less than the median body weight (HR = 1.23; 95% CI, 1.10–1.37; p<0.001), and 1 kg/m^2 less BMI than the median BMI (HR = 1.14; 95% CI, 1.05–1.23; p = 0.001) (Table 2). Furthermore, old age, high eGFR, low serum creatinine, low CD4 counts, high HIV viral load, concurrent use of nephrotoxic drugs, presence of chronic hepatitis C, and smoking were associated with TDF-related renal dysfunction. On the other hand, concurrent use of PIs, ritonavir boosted PIs, and LPV/r tended to be associated with TDF-related renal dysfunction, albeit statistically insignificant. Treatment-naïve or Treatment-experienced was not associated with TDF-related renal dysfunction.

Multivariate analysis showed that every 5 kg less than the median body weight was a significant risk for TDF-associated renal dysfunction after adjustment for sex and age (adjusted HR = 1.21; 95% CI, 1.07–1.36; p = 0.002) (Table 3, Model 2), and also after adjustment for other risk factors (adjusted HR = 1.13; 95% CI, 1.01–1.27; p = 0.039) (Table 3, Model 3). Similarly, every 1 kg/m^2 less than the median BMI was also a significant risk factor for TDF-associated renal dysfunction even after adjustment for sex and age (adjusted HR = 1.13; 95% CI 1.05–1.22; p = 0.002) (Table 4, Model 2), and tended to be a significant factor after adjustment for other variables (adjusted HR = 1.07; 95% CI 1.00–1.16; p = 0.058) (Table 4, Model 3). Old age and current smoking were also independent risk factors in both multivariate analysis for body weight and BMI (Table 3, Model 3 and Table 4, Model 3).

In complementary analyses, First, Figure 3 shows the relation between probability of TDF-associated nephrotoxicity and time from initiation of TDF therapy to 25% decrease in eGFR analyzed by the Kaplan Meier method for intertertile baseline weight categories. Compared to patients with baseline body weight >67 kg, patients with baseline weight <59 kg were significantly more likely to develop >25% decline in eGFR (p = 0.002). On the other hand, the difference in this probability between patients with baseline weight 59–67 kg and those >67 kg was only marginally significant (p = 0.073, log-rank test). Secondly, one-way ANOVA

Table 1. Baseline demographics and laboratory data.

Characteristics		
Median (IQR) weight (kg)	63	(57–69)
Median (IQR) BMI (kg/m^2)	21.9	(20.3–23.8)
Male, n (%)	471	(95.2)
Median (IQR) age	38	(33–46)
Median (IQR) eGFR (ml/min/1.73 m^2)	120.9	(104.8–138.2)
Median (IQR) serum creatinine (mg/dl)	0.72	(0.64–0.81)
Median (IQR) CD4 count (/μl)	247	(159–371)
Median (IQR) HIV viral load (log10/ml)	3.73	(1.60–4.81)
HIV viral load <50 copies/ml, n (%)	162	(32.7)
Antiretroviral therapy naïve, n (%)	208	(42.0)
Key drugs, n (%)*		
PIs	403	(81.4)
Ritonavir-boosted PIs	367	(74.1)
LPV/r	175	(35.4)
ATV/r	131	(26.5)
FPV/r	52	(10.5)
DRV/r	9	(1.8)
FPV	14	(2.8)
ATV	4	(0.8)
NFV	15	(3)
SQV	2	(0.4)
IDV	1	(0.2)
NNRTIs	83	(16.8)
EFV	65	(13.1)
NVP	17	(3.4)
ETR	1	(0.2)
INI		
RAL	10	(2.0)
Concurrent use of nephrotoxic drug, n (%)	131	(26.5)
Diabetes mellitus, n (%)	30	(6.1)
Hepatitis B, n (%)	75	(15.2)
Hepatitis C, n (%)	52	(10.5)
Hypertension, n (%)	28	(5.7)
Dyslipidemia, n (%)	40	(8.1)
Smoking, n (%)	240	(48.5)
Median (IQR) weight change (kg)	0.0	(−2.0–2.25)
Median (IQR) frequency of eGFR monitoring	16	(9.0–27)

(n = 495).
*Two patients did not take any key drugs. Three patients took both PI and NNRTI.
IQR: interquartile range, BMI: body mass index, eGFR: estimated glomerular filtration rate, PI: protease inhibitor, LPV/r: lopinavir/ritonavir, ATV: atazanavir, FPV: fosamprenavir, DRV: darunavir, NFV: nelfinavir, SQV: saquinavir, IDV: indinavir, NNRTI: non-nucleos(t)ide reverse transcriptase inhibitor, EFV: efavirenz, NVP: nevirapine, ETR: etravirine, INI: integrase inhibitor, RAL: raltegravir.
doi:10.1371/journal.pone.0022661.t001

showed that weight changes among the three baseline weight categories were not significantly different (p = 0.206). Sensitivity analysis after adding the variable "weight change" in Model 3 multivariate analysis (Table 3) showed that adjusted hazard ratio for weight per 5 kg decrement hardly changed (adjusted HR 1.131; 95% CI, 1.007–1.271; p = 0.038). Thirdly, Table 5 shows

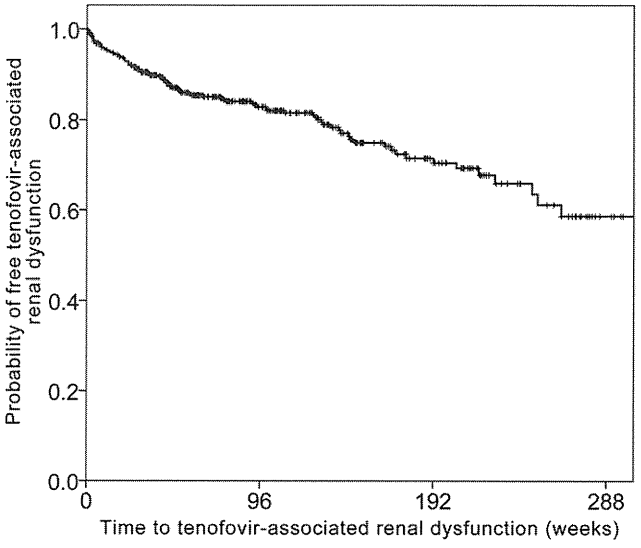

Figure 2. Kaplan-Meier curve showing the time to 25% reduction in eGFR for the whole cohort. eGFR: estimated glomerular filtration rate.
doi:10.1371/journal.pone.0022661.g002

the median and interquartile values for the actual falls in eGFR from the baseline to 24, 48, and 96 weeks. The eGFR decreased gradually in all categories, except for patients with baseline weight

Table 2. Univariate analysis for TDF-associated renal dysfunction.

	HR	95%CI	P value
Weight per 5 kg decrement	1.23	1.10–1.37	<0.001
BMI per 1 kg/m^2 decrement	1.14	1.05–1.23	0.001
Male gender	0.54	0.26–1.11	0.094
Age per 10 years	1.22	1.02–1.45	0.027
eGFR per 10 ml/min/1.73 m^2	1.10	1.05–1.15	<0.001
Serum creatinine >0.8 mg/dl	0.51	0.30–0.88	0.014
CD4 count <200/μl	1.97	1.32–2.93	0.001
HIV viral load per log10/ml	1.15	1.01–1.30	0.037
Antiretroviral therapy naïve	0.98	0.63–1.52	0.927
Concurrent key drugs			
Any PIs	1.52	0.89–2.59	0.124
Ritonavir boosted PIs	1.46	0.91–2.33	0.116
LPV/r	1.45	0.97–2.17	0.072
ATV/r	1.05	0.66–1.68	0.826
Concurrent nephrotoxic drug	1.59	1.04–2.42	0.031
Diabetes mellitus	1.57	0.76–3.24	0.220
Hepatitis B	1.36	0.82–2.24	0.231
Hepatitis C	1.80	1.07–3.04	0.028
Hypertension	1.18	0.51–2.69	0.702
Dyslipidemia	0.97	0.47–2.00	0.932
Smoking	1.57	1.05–2.36	0.028

TDF: tenofovir, HR: hazard ratio, CI: confidence interval, BMI: body mass index, eGFR: estimated glomerular filtration rate, PI: protease inhibitor, LPV/r: lopinavir/ritonavir, ATV: atazanavir.
doi:10.1371/journal.pone.0022661.t002

Table 3. Multivariate analysis to estimate the effect of lower body weight on TDF-associated renal dysfunction.

	Model 1 Crude		Model 2 Adjusted		Model 3 Adjusted	
	HR	95%CI	HR	95%CI	HR	95%CI
Weight per 5 kg decrement¶	1.23	1.10–1.37	1.21	1.07–1.36	1.13	1.01–1.27
Male gender			0.88	0.41–1.89	0.57	0.26–1.26
Age per 10 years¶			1.16	0.98–1.38	1.24	1.04–1.49
Serum creatinine >0.8 mg/dl					0.62	0.35–1.07
CD4 count <200/μl					1.65	0.97–2.79
HIV viral load per log10/ml					1.05	0.90–1.23
Boosted PIs					1.54	0.93–2.54
Concurrent use of nephrotoxic drug					1.23	0.77–1.97
Hepatitis C					1.57	0.92–2.69
Smoking¶					1.65	1.09–2.48

¶P<0.05 in Model 3.
TDF: tenofovir, HR: hazard ratio, CI: confidence interval, PI: protease inhibitor.
doi:10.1371/journal.pone.0022661.t003

>67 kg. Fourthly, the number (percentage) of patients whose eGFR decreased to <60 ml/min/1.73 m^2 was not different among the baseline weight categories (p = 0.229), whereas the number of patients who discontinued TDF with a clinical diagnosis of renal dysfunction due to TDF varied significantly according to body weight (p = 0.001, chi-square test, Table 6). None of the patients showed reduction of eGFR to <10 ml/min/1.73 m^2.

Discussion

In this Japanese cohort, 19.6% of the patients experienced eGFR decline of more than 25% from the baseline after commencement of TDF. The incidence of TDF-associated renal dysfunction was 10.5 per 100 person-years. Multivariate analysis identified smaller body weight and smaller body mass index as significant and almost significant factors, respectively, for TDF-associated renal dysfunction.

The incidence of TDF-associated renal dysfunction in patients with small body weight might be higher than previously reported in studies of patients with larger statures. Studies from North America, Europe, and Australia reported an incidence of <1% to 4.3% for TDF-related renal dysfunction, although the definition used for the diagnosis of renal impairment was different among the studies and varied from an increase in serum creatinine from >0.5 to >2 mg/dL from baseline [1–3,5,13]. Several studies conducted in these regions indicated that the range of patients' mean body weight was 69–74 kg, indicating that their patients were heavier than those of the present study with a median weight of 63 kg [2,6,12,14]. The impact of the comparatively lower body weight seems stronger in our patients probably because they do not appear to have many of the other established risk factors for TDF-associated renal dysfunction despite the high incidence of 10.5 per 100 person-years. For example, they were comparatively young with a median age of 38 years, CD4 count was relatively maintained, and approximately 30% had suppressed HIV viral load at baseline (Table 1). Furthermore, they were less likely to have hypertension, dyslipidemia, and diabetes mellitus.

Table 4. Multivariate analysis to estimate the impact of BMI decrement on TDF-associated renal dysfunction.

	Model 1 Crude		Model 2 Adjusted		Model 3 Adjusted	
	HR	95%CI	HR	95%CI	HR	95%CI
BMI per 1 kg/m^2 decrement	1.14	1.05–1.23	1.13	1.05–1.22	1.07	1.00–1.16
Male gender			0.67	0.32–1.38	0.48	0.23–1.03
Age per 10 years¶			1.20	1.01–1.43	1.27	1.06–1.52
Serum creatinine >0.8 mg/dl					0.60	0.35–1.04
CD4 count <200/μl					1.64	0.97–2.79
HIV viral load per log10/ml					1.05	0.90–1.23
Boosted PIs					1.49	0.90–2.45
Concurrent use of nephrotoxic drugs					1.22	0.76–1.94
Hepatitis C					1.62	0.94–2.76
Smoking¶					1.63	1.08–2.46

¶P<0.05 in Model 3.
BMI: body mass index, TDF: tenofovir, HR: hazard ratio, CI: confidence interval, PI: protease inhibitor.
doi:10.1371/journal.pone.0022661.t004

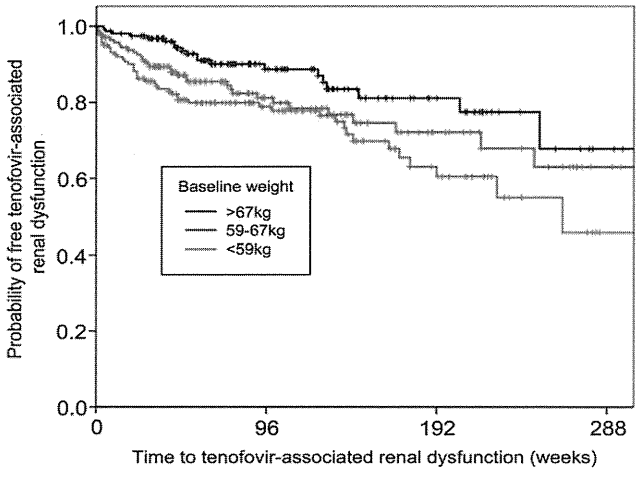

Figure 3. Kaplan-Meier curve showing the time to 25% reduction in eGFR according to baseline weight categories. Compared to patients with body weight >67 kg, those with weight <59 kg were more likely to develop >25% decline in eGFR (P = 0.002), whereas those with weight 59–67 kg showed only a marginal significance (P = 0.073, log-rank test). eGFR: estimated glomerular filtration rate.
doi:10.1371/journal.pone.0022661.g003

The results of multivariate analysis that each 5 kg decrement in body weight was significantly associated with TDF-associated renal dysfunction but not each 1 kg/m^2 decrement in BMI suggests that weight might be more useful and handy information to estimate the risk for TDF-associated renal dysfunction than BMI. Thus, patient's body weight is an important risk factor to consider at the time of TDF prescription.

Our study is one of a few that have examined the impact of TDF-associated renal dysfunction in patients with small body weight, but is the first to examine the impact of small body weight as a primary exposure by creating the model used for multivariate analysis [16–18]. One study from Thailand that included patients with a median weight of 56.5 kg reported a similar incidence of 16.2 per 100 person-years for developing TDF-associated renal dysfunction [17]. They concluded that the small body weight of their patients was probably associated with the high incidence of TDF-associated renal dysfunction. Our study confirmed that conclusion and provided statistically-backed evidence that small body weight is a significant risk factor of TDF-associated renal dysfunction by using a multivariate model with least multi-collinearity to evaluate the impact of small body weight. The results of the present study could be applied to many countries in Asia and Africa, where stature and body weight of the population are comparatively smaller.

This study adopted a decrease in eGFR of >25% as a definition for TDF-associated renal dysfunction. This criterion is one of common methods in evaluating renal function [22,23]. Using this definition, however, does not mean that all patients with >25% fall in eGFR have severe renal dysfunction. However, the definition of renal dysfunction based on a fall in eGFR of >25% is probably more sensitive than that based on eGFR <60 ml/min/1.73 m^2, in patients with comparatively good baseline renal function, such as patients of our study. Adopting this definition could be useful in detecting early renal dysfunction and in the clinical decision making regarding the need for certain interventions, for example, discontinuation of TDF. Early detection of renal dysfunction is particularly important in patients with HIV infection, because kidney disease may be associated with AIDS and death, and TDF-associated renal dysfunction might be irreversible [27,28].

Since the calculation of eGFR using the MDRD formula is based on serum creatinine, age, race and gender, any fall in eGFR is influenced by hypercreatininemia caused by increased muscle mass [29]. It is possible that the muscle mass increases in patients on ART, especially those with low weight at baseline compared to those with higher weight, reflecting reversal of wasting in those patients who were most malnourished. Such increase in muscle mass could then result in a fall in eGFR despite no change in actual renal elimination of creatinine. However, complementary analysis showed that weight change throughout the follow-up period was not significantly different among patients with different baseline weight, and the sensitivity analysis demonstrated that weight change did not alter the significance of every 5 kg decrement.

In the present study, high eGFR and low serum creatinine levels at baseline were identified as risk factors for falls in eGFR of more than 25%, in contrast to several previous studies that showed high serum creatinine and low eGFR were risk factors [4,10,25]. While the exact reason for this discrepancy is unknown at present, it could be related to differences in the definition of TDF-associated renal dysfunction. The aforementioned Thai study used the same definition applied in the present study and a Canadian study that used the definition of 1.5-fold increase in serum creatinine from baseline also reported high eGFR and low serum creatinine level at baseline as risk factors [17,30]. Thus, it is plausible to observe a fall in eGFR when the baseline value is high, since Horberg et al. reported that patients with baseline eGFR of >80 ml/min/1.73 m^2 were likely to show a pronounced fall in eGFR with TDF use [31].

Multivariate analysis also suggested that old age and current smoking are significant risks for TDF-associated renal dysfunction

Table 5. Median and interquartile range of the actual fall in eGFR from the baseline to 24, 48, and 96 weeks, according to body weight.

	Total (n = 495)		<59 kg (n = 167)		59–67 kg (n = 168)		>67 kg (n = 160)	
	fall in eGFR(ml/min/1.73 m^2)		fall in eGFR		fall in eGFR		fall in eGFR	
	median	IQR	median	IQR	median	IQR	median	IQR
to 24 weeks	7.8	(−1.7–18.1)	9.8	(−3.6–22.6)	6.8	(−1.5–17.3)	7.3	(−1.8–15.4)
to 48 weeks	9.0	(−0.7–21.9)	13.0	(−0.2–29.3)	7.2	(−1.2–20.0)	8.1	(−0.6–18.6)
to 96 weeks	9.3	(−0.5–23.1)	13.4	(1.2–33.2)	8.6	(−0.2–21.7)	7.5	(−2.4–19.8)

eGFR: estimated glomerular filtration rate, IQR: interquartile range.
doi:10.1371/journal.pone.0022661.t005

Table 6. Number of patients whose eGFR decreased to <60 ml/min/1.73 m^2 and who discontinued tenofovir with clinical diagnosis of renal dysfunction due to tenofovir.

	<59 kg (n = 167)	59–67 kg (n = 168)	>67 kg (n = 160)	p value
eGFR <60 ml/min/1.73 m^2	4 (2.4%)	1 (0.6%)	1 (0.6%)	0.229
Discontinued tenofovir	16 (9.6%)	8 (4.8%)	1 (0.6%)	0.001
Reasons for discontinuation				
>25% eGFR decrement	8 (4.8%)	4 (2.4%)	0 (0%)	
Urine β2 microglobulin >5000 μg/l	11 (6.6%)	4 (2.4%)	1 (0.6%)	

Among the patients who discontinued tenofovir, both >25% fall in eGFR and urine β2 microglobulin >5000 μg/l were registered in six patients with body weight <59 kg, and in three patients with body weight 59–67 kg.
eGFR: estimated glomerular filtration rate.
doi:10.1371/journal.pone.0022661.t006

(Table 3, Model 3 and Table 4, Model 3). However, these results have to be interpreted with caution, because these multivariate analyses were formulated to primarily evaluate weight decrement, not age or smoking.

The mechanism of TDF-associated renal dysfunction is not fully understood. TDF-associated renal dysfunction probably develops as a result of complex interaction of pharmacological, environmental, and genetic factors, rather than small body weight only [32]. It should be noted, however, that small body weight has been identified as a risk factor for TDF-associated renal dysfunction not only in clinical trials, but also in *in vitro* and pharmacokinetic studies [33–36]. TDF is the prodrug of acyclic nucleotide analog tenofovir, which is excreted by both glomerular filtration and active tubular secretion. *In vitro* studies showed that tenofovir exhibits mitochondrial toxicity in renal proximal tubular cells, and animal studies demonstrated that renal tubular dysfunction was associated with the dose and plasma drug concentrations of TDF [34,35]. Furthermore, pharmacokinetic studies showed that small body weight is associated with reduced plasma TDF clearance and thus high plasma TDF concentrations, which could result in renal tubular dysfunction. [33,36].

There are several limitations to our study. First, because of the retrospective nature of the study, patients with possible risks for TDF-associated renal dysfunction could have not been prescribed TDF. Because of this selection bias, the incidence of TDF-associated renal dysfunction might be underestimated. Second, the study did not compare the incidence of renal dysfunction in a control group (TDF-free ART). Due to the small body weight in Japanese or other factors such as genetics, the use of ART without TDF might cause higher incidence of renal dysfunction as well. Third, as discussed above, the definition of TDF-associated renal dysfunction, especially the criteria used to evaluate proximal renal tubular damage, is not uniformly established in the field and is different in the published studies. Accordingly, we decided to adopt changes in eGFR, instead of parameters for proximal renal tubular damage. Using the eGFR as a marker for TDF-associated renal dysfunction, our results might have underestimated the incidence of TDF-associated renal dysfunction.

In conclusion, the present study demonstrated a high incidence of TDF-associated renal dysfunction among Japanese patients, a potentially high-risk group due to the low median body weight. The results also identified small body weight as a risk for TDF-associated renal dysfunction in a statistical model that included small body weight as a primary exposure. TDF is certainly a drug of choice for one of the components of the first line therapies for HIV infection. However, the importance of close monitoring for renal function in patients with small body weight should be emphasized for early detection of TDF-associated renal dysfunction.

Supporting Information

Text S1 Letter of Approval from Human Research Ethics Committee of National Center for Global Health and Medicine.
(PDF)

Dataset S1 Raw data of the target population.
(XLS)

Acknowledgments

The authors thank all the clinical staff at the AIDS Clinical Center for their help in completion of this study.

Author Contributions

Conceived and designed the experiments: AN TH KU TN MKS. Performed the experiments: AN H. Sakai. Analyzed the data: AN TH KU KO IK H. Sakai. TN MKS. Contributed reagents/materials/analysis tools: H. Suemori H. Sakai. Wrote the manuscript: AN TN MKS.

References

1. Gallant JE, DeJesus E, Arribas JR, Pozniak AL, Gazzard B, et al. (2006) Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. N Engl J Med 354: 251–260.
2. Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, et al. (2004) Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. JAMA 292: 191–201.
3. Izzedine H, Hulot JS, Vittecoq D, Gallant JE, Staszewski S, et al. (2005) Long-term renal safety of tenofovir disoproxil fumarate in antiretroviral-naive HIV-1-infected patients. Data from a double-blind randomized active-controlled multicentre study. Nephrol Dial Transplant 20: 743–746.
4. Nelson MR, Katlama C, Montaner JS, Cooper DA, Gazzard B, et al. (2007) The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. AIDS 21: 1273–1281.
5. Arribas JR, Pozniak AL, Gallant JE, Dejesus E, Gazzard B, et al. (2008) Tenofovir disoproxil fumarate, emtricitabine, and efavirenz compared with zidovudine/lamivudine and efavirenz in treatment-naive patients: 144-week analysis. J Acquir Immune Defic Syndr 47: 74–78.
6. Post FA, Moyle GJ, Stellbrink HJ, Domingo P, Podzamczer D, et al. (2010) Randomized comparison of renal effects, efficacy, and safety with once-daily abacavir/lamivudine versus tenofovir/emtricitabine, administered with efavir-

enz, in antiretroviral-naive, HIV-1-infected adults: 48-week results from the ASSERT study. J Acquir Immune Defic Syndr 55: 49–57.
7. Verhelst D, Monge M, Meynard JL, Fouqueray B, Mougenot B, et al. (2002) Fanconi syndrome and renal failure induced by tenofovir: a first case report. Am J Kidney Dis 40: 1331–1333.
8. Schaaf B, Aries SP, Kramme E, Steinhoff J, Dalhoff K (2003) Acute renal failure associated with tenofovir treatment in a patient with acquired immunodeficiency syndrome. Clin Infect Dis 37: e41–43.
9. Rollot F, Nazal EM, Chauvelot-Moachon L, Kelaidi C, Daniel N, et al. (2003) Tenofovir-related Fanconi syndrome with nephrogenic diabetes insipidus in a patient with acquired immunodeficiency syndrome: the role of lopinavir-ritonavir-didanosine. Clin Infect Dis 37: e174–176.
10. Peyriere H, Reynes J, Rouanet I, Daniel N, de Boever CM, et al. (2004) Renal tubular dysfunction associated with tenofovir therapy: report of 7 cases. J Acquir Immune Defic Syndr 35: 269–273.
11. Kinai E, Hanabusa H (2009) Progressive renal tubular dysfunction associated with long-term use of tenofovir DF. AIDS Res Hum Retroviruses 25: 387–394.
12. Winston A, Amin J, Mallon P, Marriott D, Carr A, et al. (2006) Minor changes in calculated creatinine clearance and anion-gap are associated with tenofovir disoproxil fumarate-containing highly active antiretroviral therapy. HIV Med 7: 105–111.
13. Gallant JE, Winston JA, DeJesus E, Pozniak AL, Chen SS, et al. (2008) The 3-year renal safety of a tenofovir disoproxil fumarate vs. a thymidine analogue-containing regimen in antiretroviral-naive patients. AIDS 22: 2155–2163.
14. Fux CA, Simcock M, Wolbers M, Bucher HC, Hirschel B, et al. (2007) Tenofovir use is associated with a reduction in calculated glomerular filtration rates in the Swiss HIV Cohort Study. Antivir Ther 12: 1165–1173.
15. Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, et al. (2010) Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. Clin Infect Dis 51: 496–505.
16. Gayet-Ageron A, Ananworanich J, Jupimai T, Chetchotisakd P, Prasithsirikul W, et al. (2007) No change in calculated creatinine clearance after tenofovir initiation among Thai patients. J Antimicrob Chemother 59: 1034–1037.
17. Chaisiri K, Bowonwatanuwong C, Kasettratat N, Kiertiburanakul S (2010) Incidence and risk factors for tenofovir-associated renal function decline among Thai HIV-infected patients with low-body weight. Curr HIV Res 8: 504–509.
18. Reid A, Stohr W, Walker AS, Williams IG, Kityo C, et al. (2008) Severe renal dysfunction and risk factors associated with renal impairment in HIV-infected adults in Africa initiating antiretroviral therapy. Clin Infect Dis 46: 1271–1281.
19. Gatanaga H, Tachikawa N, Kikuchi Y, Teruya K, Genka I, et al. (2006) Urinary beta2-microglobulin as a possible sensitive marker for renal injury caused by tenofovir disoproxil fumarate. AIDS Res Hum Retroviruses 22: 744–748.
20. Goicoechea M, Liu S, Best B, Sun S, Jain S, et al. (2008) Greater tenofovir-associated renal function decline with protease inhibitor-based versus nonnucleoside reverse-transcriptase inhibitor-based therapy. J Infect Dis 197: 102–108.
21. Rodriguez-Novoa S, Labarga P, Soriano V, Egan D, Albalater M, et al. (2009) Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir: a pharmacogenetic study. Clin Infect Dis 48: e108–116.
22. Bash LD, Coresh J, Kottgen A, Parekh RS, Fulop T, et al. (2009) Defining incident chronic kidney disease in the research setting: The ARIC Study. Am J Epidemiol 170: 414–424.
23. Chue CD, Edwards NC, Davis LJ, Steeds RP, Townend JN, et al. (2011) Serum phosphate but not pulse wave velocity predicts decline in renal function in patients with early chronic kidney disease. Nephrol Dial Transplant;doi: 10.1093/ndt/gfq78.
24. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, et al. (2006) Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med 145: 247–254.
25. Gupta SK, Eustace JA, Winston JA, Boydstun II, Ahuja TS, et al. (2005) Guidelines for the management of chronic kidney disease in HIV-infected patients: recommendations of the HIV Medicine Association of the Infectious Diseases Society of America. Clin Infect Dis 40: 1559–1585.
26. Lomaestro BM (2000) Fluoroquinolone-induced renal failure. Drug Saf 22: 479–485.
27. Wever K, van Agtmael MA, Carr A (2010) Incomplete reversibility of tenofovir-related renal toxicity in HIV-infected men. J Acquir Immune Defic Syndr 55: 78–81.
28. Szczech LA, Hoover DR, Feldman JG, Cohen MH, Gange SJ, et al. (2004) Association between renal disease and outcomes among HIV-infected women receiving or not receiving antiretroviral therapy. Clin Infect Dis 39: 1199–1206.
29. Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, et al. (2008) Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. Clin J Am Soc Nephrol 3: 348–354.
30. Antoniou T, Raboud J, Chirhin S, Yoong D, Govan V, et al. (2005) Incidence of and risk factors for tenofovir-induced nephrotoxicity: a retrospective cohort study. HIV Med 6: 284–290.
31. Horberg M, Tang B, Towner W, Silverberg M, Bersoff-Matcha S, et al. (2010) Impact of tenofovir on renal function in HIV-infected, antiretroviral-naive patients. J Acquir Immune Defic Syndr 53: 62–69.
32. Post FA, Holt SG (2009) Recent developments in HIV and the kidney. Curr Opin Infect Dis 22: 43–48.
33. Jullien V, Treluyer JM, Rey E, Jaffray P, Krivine A, et al. (2005) Population pharmacokinetics of tenofovir in human immunodeficiency virus-infected patients taking highly active antiretroviral therapy. Antimicrob Agents Chemother 49: 3361–3366.
34. Van Rompay KK, Durand-Gasselin L, Brignolo LL, Ray AS, Abel K, et al. (2008) Chronic administration of tenofovir to rhesus macaques from infancy through adulthood and pregnancy: summary of pharmacokinetics and biological and virological effects. Antimicrob Agents Chemother 52: 3144–3160.
35. Kohler JJ, Hosseini SH, Hoying-Brandt A, Green E, Johnson DM, et al. (2009) Tenofovir renal toxicity targets mitochondria of renal proximal tubules. Lab Invest 89: 513–519.
36. Kiser JJ, Fletcher CV, Flynn PM, Cunningham CK, Wilson CM, et al. (2008) Pharmacokinetics of antiretroviral regimens containing tenofovir disoproxil fumarate and atazanavir-ritonavir in adolescents and young adults with human immunodeficiency virus infection. Antimicrob Agents Chemother 52: 631–637.

Endocrine Journal 2011, **58** (6), 475-484

ORIGINAL

Prevalence of and risk factors for lipodystrophy among HIV-infected patients receiving combined antiretroviral treatment in the Asia-Pacific region: results from the TREAT Asia HIV Observational Database (TAHOD)

Sang Hoon Han[1], Jialun Zhou[2], Suneeta Saghayam[3], Sasheela Vanar[4], Nittaya Phanuphak[5], Yi-Ming A Chen[6], Thira Sirisanthana[7] Somnuek Sungkanuparph[8], Christopher KC Lee[9], Sanjay Pujari[10], Patrick CK Li[11], Shinichi Oka[12], Vonthanak Saphonn[13], Fujie Zhang[14], Tuti Parwati Merati[15], Matthew G Law[2] and Jun Yong Choi[1] on behalf of The TREAT Asia HIV Observational Database

[1] *Department of Internal Medicine and AIDS Research Institute, Yonsei University College of Medicine, Seoul, Korea*
[2] *National Centre in HIV Epidemiology and Clinical Research, The University of New South Wales, Sydney, Australia*
[3] *YRG Centre for AIDS Research and Education, Chennai, India*
[4] *Department of Medicine, University of Malaya, Lembah Pantai, Kuala Lumpur, Malaysia*
[5] *Thai Red Cross AIDS Research Centre, Bangkok, Thailand*
[6] *Taipei Veterans General Hospital and AIDS Prevention and Research Centre, National Yang-Ming University, Taipei, Taiwan*
[7] *Research Institute for Health Sciences, Chiang Mai, Thailand*
[8] *Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*
[9] *Hospital Sungai Buloh, Kuala Lumpur, Malaysia*
[10] *Institute of Infectious Diseases, Pune, India*
[11] *Queen Elizabeth Hospital, Hong Kong, China*
[12] *National Center for Global Health and Medicine, Tokyo, Japan*
[13] *National Center for HIV/AIDS, Dermatology & STDs, Phnom Penh, Cambodia*
[14] *Beijing Ditan Hospital, Beijing, China*
[15] *Faculty of Medicine Udayana University & Sanglah Hospital, Bali, Indonesia*

Abstract. The prevalence of and risk factors for lipodystrophy (LD) among patients receiving combined antiretroviral treatment (cART) in the Asia-Pacific region are largely unknown. LD diagnosis was based on the adverse event definition from the US NIH Division of AIDS (2004 version), and only cases with a severity grade of ≥ 3 were included. TAHOD patients who had recently commenced cART with ≥ 3 drugs after 1996 from sites which had ever reported LD were included in the analysis. Covariates for the forward multivariate logistic regression model included demographic variables, CDC disease classification, baseline CD4 and viral load, hepatitis B/C virus co-infection, and regimen and duration of cART. LD was diagnosed in 217 (10.5%) of 2072 patients. The median duration of cART was 3.8 (interquartile range, 2.2-5.3) years (stavudine, 2.0 (1.0-3.5) years; zidovudine, 1.8 (0.6-3.9) years; and protease inhibitors (PI), 2.6 (1.3-4.5) years). In the multivariate model, factors independently associated with LD included use of stavudine (≤ 2 years *vs.* no experience: OR 25.46, $p<0.001$, > 2 years *vs.* no experience: OR 14.92, $p<0.001$), use of PI (> 2.6 years *vs.* no experience: OR 0.26, $p<0.001$), and total duration of cART (> *vs.* ≤ 3.8 years: OR 4.84, $p<0.001$). The use of stavudine was strongly associated with LD in our cohort. Stavudine-sparing cART strategies are warranted to prevent the occurrence of LD in the Asia-Pacific region.

Key words: Lipodystrophy, HIV, Adverse effects, Combined antiretroviral treatment, Asia-Pacific

SINCE morphologic changes caused by fat redistribution was first reported in subjects receiving combined antiretroviral treatment (cART) in 1998 [1], lipodystrophy (LD) has become a recognized

Received Dec. 27, 2010; Accepted Mar. 18, 2011 as K10E-407
Released online in J-STAGE as advance publication Apr. 27, 2011

Correspondence to: Jun Yong Choi, Department of Internal Medicine, Yonsei University College of Medicine, 250 Seongsanno, Seodaemun-gu, Seoul 120-752, Republic of Korea.
E-mail: seran@yuhs.ac

The part of this data was presented at 49th Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC); SAN FRANCISCO, California, United States; September 12-15, 2009 (Abstract H-1576)

Statistical Abbreviations used: IQR, interquartile range; OR, odds ratio; CI, confidence interval.

complication in HIV-infected patients on cART around the world, with a prevalence ranging from 11% to 83% [2]. LD is acknowledged as an important adverse event in HIV-infected subjects in the era of cART, because it is associated with other metabolic abnormalities such as insulin resistance, dyslipidemia, and glucose intolerance, attributing to the development of cardiovascular disease [2]. In severe cases, LD can be disfiguring, which can cause stigma and discrimination against patients, leading to risks of medication refusal, poor adherence, and ultimately treatment failure [2-5].

Several characteristics, including older age, white race, lower body mass index (BMI), higher HIV-RNA levels, lower nadir CD4+ T-cell count, longer duration of cART and exposure of nucleoside reverse transcriptase inhibitors (NRTIs), especially stavudine (d4T), have been identified as risk factors for LD [2, 6]. Also, recent studies have reported that genetic variations can also influence the emergence of LD [7-9].

In East, South, and South-East Asia, the estimated number of people living with HIV and/or acquired immunodeficiency syndrome (AIDS) in 2007 was 5 million, the second largest regional epidemic in the world, after Sub-Saharan Africa [10]. Except for a small number of single-institution studies, there is little data on the prevalence and risk factors of LD among HIV-infected patients receiving cART in the Asia-Pacific region [11-14].

The Therapeutics Research, Education, and AIDS Training in Asia (TREAT Asia) HIV Observational Database (TAHOD) is a multicenter, observational cohort study that was initiated in 2003 to assess regional HIV treatment outcomes in the Asia-Pacific region [15]. Our objective was to examine the prevalence of and the risk factors for LD among HIV-infected patients receiving cART in TAHOD.

Materials and Methods

Study design and patient population

The structure of TAHOD and standardized mechanisms for data collection and follow-up has been previously described [15]. Data were combined *via* standardized formats and transferred electronically to the National Centre in HIV Epidemiology and Clinical Research (NCHECR), University of New South Wales, Sydney, Australia for central aggregation, quality control, and analyses. Ethics approval was obtained from the University of New South Wales and the local ethics committee for each site. Because all data transferred to the NCHECR were collected in an anonymous fashion and entirely observational, informed consent was not obtained, unless specifically requested by the local ethics committee.

Observational TAHOD data aggregated to NCHECR by April 2008, involving 17 institutions in 12 countries (Appendix), were included in this study. Patients who had recently commenced cART with ≥ 3 antiretroviral drugs after 1996 in any TAHOD participating sites which had ever reported LD were eligible for the analysis. Patients who had started treatment with < 3 antiretroviral drugs before 1996 were not included in this study.

Because TAHOD is a multicenter observational database, not all patients included in this analysis were receiving cART according to standardized guidelines. The timing of antiretroviral treatment and the regimens of combined antiretroviral drugs were decided upon by individual physicians depending on unique clinical circumstances.

Data collection and definitions

The following were included as covariates: age, gender, race, country income category, reported mode of transmission, hepatitis B and C virus (HBV/HCV) co-infection status, and baseline and monitoring values at and after start of cART (e.g., age, US Centers for Disease Control and Prevention (CDC) disease classification [16], CD4+ T-cell and HIV-RNA viral load, BMI, and cART regimen and duration). Country income category was divided into two groups based on the 2007 gross national income per capita, according to the World Bank criteria for classifying economies: low income country ($3,705 or less), and high income country ($3,706 or more) [17].

In TAHOD, LD data was collected as 1) fat accumulation according to a clinical spectrum of central fat accumulation in the abdomen, breasts, and over the dorsocervical spine, or localized lipomas and/or 2) lipoatrophy according to a clinical spectrum of peripheral fat loss in the face, limbs, or buttocks. LD was diagnosed based on the clinical definition of the US Division of AIDS table for grading the severity of adult and pediatric adverse events (2004 version) [18]. Patients with a severity of grade 3, defined as disfiguring or obvious body shape changes on casual visual inspection, or higher were included in this analysis [18].

Statistical analysis

Continuous data were represented using the median value (IQR) and categorical variables were reported by number (percent). cART-related covariates were analysed as 1) never treated, 2) below the median duration (MD) of treatment, and 3) above the MD of treatment. Associations of treatment duration between other antiretroviral drugs and d4T were evaluated using Spearman's correlation coefficient ρ. The difference in the number of patients who had an exposure history to both zidovudine (AZT) and d4T among the three groups according to time on AZT treatment was evaluated using a one-way ANOVA test.

Predictors associated with diagnosis of LD were assessed by forward, stepwise multivariate conditional logistic regression models. To control for different clinical practices in LD diagnosis, the final model was stratified by TAHOD sites. All variables with p-value of less than 0.10 in univariate analyses were included in the multivariate logistic models. All statistical analyses were performed using the STATA package (version 8.2, StataCorp, College Station, TX, USA). All p-values were two-tailed, and $p<0.05$ was considered to be statistically significant.

Results

Demographic and clinical characteristics of all study participants and in patients with LD

12 of the 17 participating TAHOD sites had previously reported LD according to our criteria and were included in the analysis. LD was diagnosed in 217 (10.5%) patients among a total of 2,072 study participants. Upon univariate analysis, the prevalence of LD did not differ significantly according to age, gender, race, HBV co-infection status, CD4+ T-cell count or HIV-RNA levels, or BMI at cART initiation. Patients with HCV co-infection had a significantly lower prevalence of LD than those without (5% *vs.* 12%; OR, 0.37; p=0.019). The rate of LD in patients living in high-income countries was lower than that of patients living in low-income countries, though the difference was not statistically significant (5% *vs.* 13%; OR, 0.32; p=0.095) (Table 1).

LD prevalence by cART exposure

The majority (72%) of patients received non-nucleoside reverse transcriptase inhibitors (NNRTI)-based cART as their first-line regimen; among these,

Table 1 Demographic and clinical characteristics of all study participants and patients with lipodystrophy

	Total (n=2072)	Lipodystrophy (n=217)	Univariate OR	*p*-value
Age (years) at cART initiation				
Median (IQR)	35 (30-41)	36 (31-41)		
≤ 30*	549 (27)	45 (8)	–	–
31~40	954 (46)	110 (12)	1.32	0.170
≥ 41	569 (27)	62 (11)	1.41	0.129
Gender				
Male*	1485 (72)	127 (9)	–	–
Female	587 (28)	90 (15)	1.20	0.330
Race				
Chinese	871 (42)	50 (6)	–	–
Thai	434 (21)	128 (29)	1.74	0.481
Indian	207 (10)	31 (15)	1.02	0.980
Other	560 (27)	8 (1)	0.89	0.849
Hepatitis B surface antigen				
Negative*	1257 (61)	144 (11)	–	–
Positive	164 (8)	15 (9)	0.76	0.378
Not tested	651 (31)	58 (9)	0.60	0.025
Hepatitis C antibody				
Negative*	1236 (60)	145 (12)	–	–
Positive	138 (7)	7 (5)	0.37	0.019
Not tested	698 (33)	65 (9)	0.68	0.066
CDC classification at cART initiation				
Category A*	1000 (48)	93 (9)	–	–
Category B	200 (10)	23 (12)	1.94	0.030
Category C	872 (42)	101 (12)	1.07	0.669
CD4 count (cells/μL) at cART initiation				
Median (IQR)	103 (33-198)	70 (25-152)		
≤ 100*	764 (37)	96 (13)	–	–
101~200	414 (20)	36 (9)	0.96	0.842
201~300	235 (11)	23 (10)	1.50	0.141
≥ 301	135 (7)	7 (5)	1.14	0.760
Not tested	524 (25)	55 (11)	1.74	0.025
HIV viral load (copies/mL) at cART initiation				
Median (IQR) – Log_{10}	4.9 (4.3-5.5)	4.9 (4.3-5.5)		
≥ 400*	690 (33)	56 (8)	–	–
< 400	56 (3)	3 (5)	0.79	0.713
Not tested	1326 (64)	158 (12)	0.97	0.891
BMI (kg/m^2) at cART initiation				
Median (IQR)	20.3 (18.3-22.7)	20.4 (18.6-22.5)		
≤ 18.5*	202 (10)	23 (11)	–	–
18.5~25	437 (21)	63 (14)	1.44	0.218
> 25	73 (4)	9 (12)	1.70	0.277
Not available	1360 (65)	122 (9)	1.79	0.097
Country income category				
Low-income*	1452 (70)	189 (13)	–	–
High-income	620 (30)	28 (5)	0.32	0.095

Data are expressed as median (interquartile range) or number (percent). *Reference category. Abbreviations used: OR, odds ratio; cART, combined antiretroviral treatment; IQR, interquartile range; BMI, body mass index.

41% received efavirenz (EFV) and 59% nevirapine. There was no difference in the prevalence of LD between patients who used NNRTI or protease inhibitor (PI) as their first antiretroviral regimen (12% *vs.* 8%; OR, 0.66; p=0.499). The median duration of cART in all the patients was 3.8 years overall, 1.8 years on AZT, 2.0 years on d4T, 1.9 years on didanosine (ddI), 2.9 years on any NNRTI, and 2.6 years on any PI. The patients receiving cART for > 3.8 years had a significantly higher prevalence of LD than those receiving cART for ≤ 3.8 years (16% *vs.* 5%; OR, 4.01; p=0.001). In addition, patients who had received d4T had a significantly higher prevalence of LD than those who had never received d4T (> 2 years *vs.* no experience [NE], 19% *vs.* 1%; OR, 41.01; p<0.001, and ≤ 2 years *vs.* NE, 12% *vs.* 1%; OR, 23.55; p<0.001]. However, patients who received AZT for > 1.8 years had a significantly lower prevalence of LD than those who had never received AZT (3% *vs.* 8%; OR, 0.34; p=0.003). Patients who received any PI for > 2.6 years had a significantly lower prevalence of LD than those who had never received any PI (3% *vs.* 13%; OR, 0.20; p=0.010) (Table 2).

In the group receiving d4T for > 2 years, patients with NNRTI-based cART had a higher, but not statistically significant, rate of LD than those with PI-based cART (20.7% *vs.* 14.4%; p=0.098). There were no significant differences in the rates of LD between NNRTI- and PI-based cART by d4T exposure (Fig. 1). The duration of AZT or PI use had a significantly negative correlation with duration of d4T treatment (r= -0.43; p<0.001, r= -0.06; p=0.006, respectively) (Table 3). The group of those who had received AZT for ≤ 1.8 years (514 of 690, 74.5%) had a significantly larger proportion of patients who had a history of both AZT and d4T use than did the groups who had never received AZT (0 of 692, 0%, p<0.001) or who had received AZT for > 1.8 years (285 of 690, 41.3%, p<0.001).

Independent predictive factors associated with the diagnosis of LD

In the multivariate logistic regression model, factors independently associated with LD included the use of d4T (≤ 2.0 years *vs.* NE, OR 25.46, 95% CI 9.01-71.97, p<0.001; and > 2.0 years *vs.* NE, OR 14.92, 95% CI 5.29-42.06, p<0.001), use of PI (> 2.6 years *vs.* NE, OR 0.26, 95% CI 0.12-0.56, p<0.001), and the total duration of cART (> *vs.* ≤ 3.8 years, OR 4.84, 95% CI 3.11-7.55, p<0.001) (Table 4).

Discussion

In general, the prevalence rates of LD were reported with ranges from 7 to 84% (average of 42%) in patients receiving PI-based cART, and from 0 to 38% (average of 13%) in those receiving NNRTI-based cART from various populations and countries with different clinical and metabolic characteristics [19]. This study, based on data from a multicenter observational database in the Asia-Pacific region, showed a lower prevalence of LD than the rates of 43 to 53% seen earlier in the cART era in large cohorts in Europe, Australia, and the United States [20-22]. However, in a Spanish cohort reported more recently, the prevalence of LD was 17.8% (420 of 2358) [23] and, according to data from a Swiss HIV study, patients starting cART in 2003-2006 were significantly less likely to experience LD than those starting between 2000 to 2002 [24]. As treatment patterns change with a decrease in thymidine analogue (d4T/AZT/ddI) use and an increase in tenofovir disoproxil fumarate (TDF) use, it is likely that LD rates will also decline [24].

To our knowledge, our analysis is the first regional cohort study of LD in the Asia-Pacific. Previous single-institution studies in Thailand, Singapore, and South Korea reported the prevalence of LD as ranging from 3.5% to 66.1% [11-14]. Because LD was identified clinically using a high threshold (i.e., severity grade ≥ 3) and without the use of quantitative tools such as dual-energy X-ray absorptiometry (DEXA) or computerized tomography (CT) scans, the true prevalence of LD may be higher than what we have reported. Also, the lower prevalence of LD in our sites might have been caused by different host factors such as unknown genetic background and insufficient concern for LD in several resource-limited settings. Actually, a few previous studies showed that LD was infrequent in non-white races as compared to caucasians [22, 25, 26], and that genetic variations can also influence the emergence of LD [7-9]. Further studies are warranted to confirm a more objective prevalence of LD through universalized and validated case definitions in the Asia-Pacific region.

We confirmed that the use of d4T is a strong risk factor for the development of LD in the Asia-Pacific region. Our results are consistent with other studies that show that treatment with NRTIs, and particularly with d4T, which has the greatest mitochondrial toxicity in the class, and longer duration of cART are key risk factors for the development of LD [20, 24, 26, 27]. The

Table 2. Prevalence of lipodystrophy according to combined antiretroviral treatment

	Total (n=2072)	Lipodystrophy (n=217)	Univariate	
			OR	*p*-value
Total duration on cART (years)	3.8 (2.2-5.3)	4.9 (4.0-5.9)		
≤ 3.8 years*	1036 (50)	48 (5)	-	-
> 3.8 years	1036 (50)	169 (16)	4.01	0.001
First-line combination				
NNRTI-based cART*	1497 (72)	175 (12)	-	-
PI-based cART	522 (25)	42 (8)	0.66	0.499
Other	53 (3)	0 (0)	-	-
Time on NRTI treatment				
Time on zidovudine (years)	1.8 (0.6-3.9)	0 (0-0.2)		
Never received*	692 (34)	53 (8)	-	-
≤ 1.8 years	690 (33)	145 (21)	3.21	0.168
> 1.8 years	690 (33)	19 (3)	0.34	0.003
Time on stavudine (years)	2.0 (1.0-3.5)	2.4 (1.6-3.3)		
Never received*	699 (34)	4 (1)	-	-
≤ 2.0 years	687 (33)	82 (12)	23.55	<0.001
> 2.0 years	686 (33)	131 (19)	41.01	<0.001
Time on didanosine (years)	1.9 (0.9-3.4)	1.8 (1.0-2.6)		
Never received*	1519 (74)	166 (11)	-	-
≤ 1.9 years	277 (13)	29 (10)	0.95	0.938
> 1.9 years	276 (13)	22 (8)	0.71	0.544
Time on NNRTI treatment (years)	2.9 (1.7-4.4)	2.7 (1.7-3.6)		
Never received*	355 (17)	25 (7)	-	-
≤ 2.9 years	859 (42)	115 (13)	2.04	0.399
> 2.9 years	858 (41)	77 (9)	1.30	0.759
Time on PI treatment (years)	2.6 (1.3-4.5)	1.6 (0.7-2.5)		
Never received*	1347 (65)	169 (13)	-	-
≤ 2.6 years	363 (18)	38 (10)	0.82	0.764
> 2.6 years	362 (17)	10 (3)	0.20	0.010
Time on atanazavir (years)	1.4 (0.7-1.6)	0 (0-0)		
Never received*	1879 (90)	216 (12)	-	-
≤ 1.4 years	97 (5)	1 (1)	0.08	0.005
> 1.4 years	96 (5)	0 (0)	-	-
Time on indinavir (years)	2.0 (0.9-3.6)	1.6 (0.6-2.3)		
Never received*	1714 (82)	175 (10)	-	-
≤ 2.0 years	179 (9)	30 (17)	1.77	0.366
> 2.0 years	179 (9)	12 (7)	0.37	0.434
Time on nelfinavir (years)	2.1 (0.7-4.0)	0.6 (0.1-1.1)		
Never received*	1951 (94)	205 (11)	-	-
≤ 2.1 years	61 (3)	12 (20)	2.09	0.216
> 2.1 years	60 (3)	0 (0)	-	-
Time on lopinavir (years)	1.8 (0.6-3.3)	1.2 (1.0-1.3)		
Never received*	1798 (86)	213 (12)	-	-
≤ 1.8 years	138 (7)	4 (3)	0.22	0.007
> 1.8 years	136 (7)	0 (0)	-	-

Data are expressed as median (interquartile range) or number (percent). NNRTI-based cART indicates antiretroviral treatment composed of a combination of more than two NRTI and one NNRTI antiretroviral drugs, but without PI; and PI-based cART indicates the antiretroviral treatment which is composed of the combination of more than two NRTI and PI antiretroviral drugs, but without NNRTI. *Reference category. Abbreviations used: OR, odds ratio; cART, combined antiretroviral treatment; IQR, interquartile range; NRTI, nucleoside analogue reverse transcriptase inhibitor; NNRTI, non-nucleoside analogue reverse transcriptase inhibitor; PI, protease inhibitor.

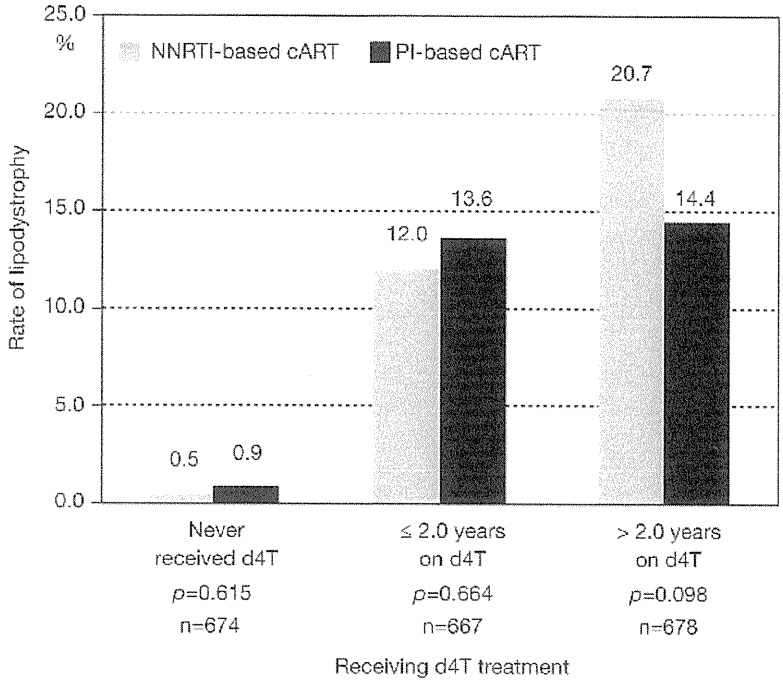

Fig. 1 The prevalence of lipodystrophy between NNRTI-based and PI-based combined antiretroviral treatment in each group, according to time on stavudine treatment
NNRTI-based cART indicates antiretroviral treatment composed of a combination of more than two NRTI and one NNRTI antiretroviral drugs, but without PI; and PI-based cART indicates the antiretroviral treatment which is composed of the combination of more than two NRTI and PI antiretroviral drugs, but without NNRTI. Abbreviations used: NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; cART, combined active antiretroviral treatment; d4T, stavudine; NRTI, nucleoside reverse transcriptase inhibitor.

Table 3 Association with other antiretroviral drugs treatment duration and exposure duration of stavudine in total patients (n=2072)

	Exposure duration of stavudine (months)		
	Median (IQR)	Spearman's ρ	*p*-value
Time on zidovudine treatment		-0.43	<0.001
Never received	23.6 (5.9-44.0)		
≤ 1.8 years	14.6 (0-34.3)		
> 1.8 years	0 (0-13.7)		
Time on didanosine treatment		0.14	<0.001
Never received	10.0 (0-31.3)		
≤ 1.9 years	13.7 (4.1-30.1)		
> 1.9 years	25.4 (0-44.9)		
Time on NNRTI treatment		0.31	<0.001
Never received	0 (0-18.6)		
≤ 2.9 years	9.4 (0-22.8)		
> 2.9 years	28.8 (0-47.4)		
Time on PI treatment		-0.06	0.006
Never received	13.1 (0-33.9)		
≤ 2.6 years	12.2 (0-24.3)		
> 2.6 years	10.4 (0-35.1)		

Abbreviations used: NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Table 4 Forward multivariate logistic regression analysis to identify the risk factors for lipodystrophy

Covariate	Odds ratio	95% CI	*p*-value
Experience on stavudine			
Never received*	1.00	-	-
≤ 2.0 years	25.46	9.01-71.97	<0.001
> 2.0 years	14.92	5.29-42.06	<0.001
Experience on PI			
Never received*	1.00	-	-
≤ 2.6 years	1.42	0.86-2.34	0.165
> 2.6 years	0.26	0.12-0.56	<0.001
Total duration on cART			
≤ 3.8 years*	1.00	-	-
> 3.8 years	4.84	3.11-7.55	<0.001

*Reference category. Abbreviations used: CI, confidence interval; PI, protease inhibitor; cART, combined active antiretroviral treatment.

finding that patients receiving AZT for longer than the MD of treatment were less likely to have LD may have been due to their relatively shorter exposure to d4T.

Our finding that patients living in high-income countries had a tendency toward a lower prevalence of LD than those in low-income countries might have been related to the wider range of available antiretroviral drugs and lower reliance on d4T in high-income countries.

It was unexpected that patients with longer PI treatment would have lower risk of developing LD, regardless of the duration of d4T treatment. Treatment with PIs is known to be an important attributable factor for the development of LD, and NNRTIs were not traditionally considered to be associated with the development of LD [24, 28, 29]. However, recent clinical trials have revealed that limb fat gain in patients receiving EFV was lower than in treatment with ritonavir-boosted lopinavir (LPV/r) [29-32]. In addition, a recent AIDS Clinical Trials Group (ACTG) A5142 study showed that lipoatrophy was more frequent with EFV than with LPV/r when combined with d4T or AZT [27, 30]. These findings suggest a protective role of ritonavir-boosted PIs for LD. The mechanisms for the protective role of ritonavir-boosted PIs are not well understood, but a potential explanation is that ritonavir may mitigate the mitochondrial damage caused by thymidine analogues [27]. Although PIs, especially ritonavir-boosted, may have potential protective roles for LD, other metabolic complications, such as dyslipidemia and insulin resistance, and the tolerances of drugs associated with PIs should be prudently considered when choosing an antiretroviral drug [27].

Our study had several limitations. The lack of a uniform, objective, diagnostic method for identifying LD could have led to a selection bias. In this study, we tried to capture the rate of LD in the Asia-Pacific sites that are capable of diagnosing LD. However, the sites themselves that have ever reported LD may add confounding factors such as antiretroviral regimens, race, and HCV infection. Although we limited the inclusion criteria to higher-grade LD to reduce this risk, cases of LD could have been missed, due to local variations in diagnosis and reporting. As this is an observational cohort across centers with varying levels of clinical and monitoring capacity, not all patients had baseline levels for all possible variables. We excluded pre-ART lipid and glucose tests from our final analysis for this reason, but acknowledge that other missing data could have impacted our findings.

Stavudine is one of the most commonly used NRTIs in the world and in the Asia-Pacific region [5, 33]. TDF is unavailable at many sites in the Asia-Pacific region, so d4T or AZT in combination with 3TC is usually the only NRTIs available for standard first-line regimens [5, 33]. Our findings emphasize the importance of phasing out d4T use and increasing access to TDF to minimize the risk of developing LD.

Acknowledgements

The TREAT Asia HIV Observational Database is part of the Asia Pacific HIV Observational Database and is an initiative of TREAT Asia, a program of amfAR, The Foundation for AIDS Research, with support from the

National Institute of Allergy and Infectious Diseases (NIAID) of the U.S. National Institutes of Health (NIH) as part of the International Epidemiologic Databases to Evaluate AIDS (IeDEA) (grant no. U01AI069907), and from the Dutch Ministry of Foreign Affairs through a partnership with Stichting Aids Fonds. The National Centre in HIV Epidemiology and Clinical Research is funded by the Australian Government Department of Health and Ageing, and is affiliated with the Faculty of Medicine, University of New South Wales. The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of any of the institutions mentioned above.

Author Disclosure Statement

All authors declare that they have no conflicts of interest associated with this manuscript.

References

1. Carr A, Cooper DA (1998) Images in clinical medicine. Lipodystrophy associated with an HIV-protease inhibitor. *N Engl J Med* 339: 1296.
2. Grinspoon S, Carr A (2005) Cardiovascular risk and body-fat abnormalities in HIV-infected adults. *N Engl J Med* 352: 48-62.
3. Spire B, Carrieri P, Sopha P, Protopopescu C, Prak N, Quillet C, Ngeth C, Ferradini L, Delfraissy JF, Laureillard D (2008) Adherence to antiretroviral therapy in patients enrolled in a comprehensive care program in Cambodia: a 24-month follow-up assessment. *Antivir Ther* 13: 697-703.
4. Fernandes AP, Sanches RS, Mill J, Lucy D, Palha PF, Dalri MC (2007) Lipodystrophy syndrome associated with antiretroviral therapy in HIV patients: considerations for psychosocial aspects. *Rev Lat Am Enfermagem* 15: 1041-1045.
5. Zhou J, Paton NI, Ditangco R, Chen YM, Kamarulzaman A, Kumarasamy N, Lee CK, Li PC, Merati TP, Phanuphak P, Pujari S, Vibhagool A, Zhang F, Chuah J, Frost KR, Cooper DA, Law MG (2007) Experience with the use of a first-line regimen of stavudine, lamivudine and nevirapine in patients in the TREAT Asia HIV Observational Database. *HIV Med* 8: 8-16.
6. Wohl DA, Brown TT (2008) Management of morphologic changes associated with antiretroviral use in HIV-infected patients. *J Acquir Immune Defic Syndr* 49 Suppl 2: S93-S100.
7. Asensi V, Rego C, Montes AH, Collazos J, Carton JA, Castro MG, Alvarez V, Fernandez C, Maradona JA, Valle-Garay E (2008) IL-1beta (+3954C/T) polymorphism could protect human immunodeficiency virus (HIV)-infected patients on highly active antiretroviral treatment (HAART) against lipodystrophic syndrome. *Genet Med* 10: 215-223.
8. Bonnet E, Bernard J, Fauvel J, Massip P, Ruidavets JB, Perret B (2008) Association of APOC3 polymorphisms with both dyslipidemia and lipoatrophy in HAART-receiving patients. *AIDS Res Hum Retroviruses* 24: 169-171.
9. Guallar JP, Gallego-Escuredo JM, Domingo JC, Alegre M, Fontdevila J, Martinez E, Hammond EL, Domingo P, Giralt M, Villarroya F (2008) Differential gene expression indicates that 'buffalo hump' is a distinct adipose tissue disturbance in HIV-1-associated lipodystrophy. *AIDS* 22: 575-584.
10. UNAIDS/WHO: 2008 Report on the global HIV/AIDS epidemic. The Joint United Nations Programme on HIV/AIDS page. Available at: http://www.unaids.org/en/KnowledgeCentre/HIVData/GlobalReport/2008/2008_Global_report.asp Geneva Switzerland 2008.
11. Chuapai Y, Kiertiburanakul S, Malathum K, Sungkanuparph S (2007) Lipodystrophy and dyslipidemia in human immunodeficiency virus-infected Thai patients receiving antiretroviral therapy. *J Med Assoc Thai* 90: 452-458.
12. Puttawong S, Prasithsirikul W, Vadcharavivad S (2004) Prevalence of lipodystrophy in Thai-HIV infected patients. *J Med Assoc Thai* 87: 605-611.
13. Paton NI, Earnest A, Ng YM, Karim F, Aboulhab J (2002) Lipodystrophy in a cohort of human immunodeficiency virus-infected Asian patients: prevalence, associated factors, and psychological impact. *Clin Infect Dis* 35: 1244-1249.
14. Chang KH, Kim JM, Song YG, Hong SK, Lee HC, Lim SK (2002) Does race protect an oriental population from developing lipodystrophy in HIV-infected individuals on HAART? *J Infect* 44: 33-38.
15. Zhou J, Kumarasamy N, Ditangco R, Kamarulzaman A, Lee CK, Li PC, Paton NI, Phanuphak P, Pujari S, Vibhagool A, Wong WW, Zhang F, Chuah J, Frost KR, Cooper DA, Law MG (2005) The TREAT Asia HIV Observational Database: baseline and retrospective data. *J Acquir Immune Defic Syndr* 38: 174-179.
16. Centers for Disease Control and Prevention (1992) 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR Recomm. Rep., 41, 1-19.
17. The World Bank Group: Country Classification. 2006

Available at: http://web.worldbank.org/WBSITE/EXTERNAL/DATASTATISTICS/0,,contentMDK:20420458~menuPK:64133156~pagePK:64133150~piPK:64133175~theSitePK:239419,00.html.
18. US National Institutes of Health DAIDS HIV Vaccines and Research Program. National Institutes of Health. Division of AIDS (DAIDS) revised toxicity tables for grading the severity of adult and pediatric adverse events experiences, Version 1.0, December 2004, Washington. DC. Available at http://rsc.tech- res.com/Document/safetyandpharmacovigilance/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Events.doc.
19. Chen D, Misra A, Garg A (2002) Clinical review 153: Lipodystrophy in human immunodeficiency virus-infected patients. *J Clin Endocrinol Metab* 87: 4845-4856.
20. Bernasconi E, Boubaker K, Junghans C, Flepp M, Furrer HJ, Haensel A, Hirschel B, Boggian K, Chave JP, Opravil M, Weber R, Rickenbach M, Telenti A (2002) Abnormalities of body fat distribution in HIV-infected persons treated with antiretroviral drugs: The Swiss HIV Cohort Study. *J Acquir Immune Defic Syndr* 31: 50-55.
21. Miller J, Carr A, Emery S, Law M, Mallal S, Baker D, Smith D, Kaldor J, Cooper DA (2003) HIV lipodystrophy: prevalence, severity and correlates of risk in Australia. *HIV Med* 4: 293-301.
22. Lichtenstein KA, Ward DJ, Moorman AC, Delaney KM, Young B, Palella FJ, Jr., Rhodes PH, Wood KC, Holmberg SD (2001) Clinical assessment of HIV-associated lipodystrophy in an ambulatory population. *Aids* 15: 1389-1398.
23. Pere D, Ignacio SL, Ramon T, Fernando L, Alberto T, Pompeyo V, Juan G, G MJ, Paloma G, Antonio V, Jaime C, Esteban R, Bernardino R, GAML, Trinitario S, Ferran T, Juan Ramon L, Myriam G (2008) Dyslipidemia and Cardiovascular Disease Risk Factor Management in HIV-1-Infected Subjects Treated with HAART in the Spanish VACH Cohort. *Open AIDS J* 2: 26-38.
24. Nguyen A, Calmy A, Schiffer V, Bernasconi E, Battegay M, Opravil M, Evison JM, Tarr PE, Schmid P, Perneger T, Hirschel B (2008) Lipodystrophy and weight changes: data from the Swiss HIV Cohort Study, 2000-2006. *HIV Med* 9: 142-150.
25. Mallal SA, John M, Moore CB, James IR, McKinnon EJ (2000) Contribution of nucleoside analogue reverse transcriptase inhibitors to subcutaneous fat wasting in patients with HIV infection. *Aids* 14: 1309-1316.
26. Lichtenstein KA (2005) Redefining lipodystrophy syndrome: risks and impact on clinical decision making. *J Acquir Immune Defic Syndr* 39: 395-400.
27. Haubrich RH, Riddler SA, DiRienzo AG, Komarow L, Powderly WG, Klingman K, Garren KW, Butcher DL, Rooney JF, Haas DW, Mellors JW, Havlir DV (2009) Metabolic outcomes in a randomized trial of nucleoside, nonnucleoside and protease inhibitor-sparing regimens for initial HIV treatment. *Aids* 23: 1109-1118.
28. Haugaard SB (2006) Toxic metabolic syndrome associated with HAART. *Expert Opin Drug Metab Toxicol* 2: 429-445.
29. Perez-Molina JA, Domingo P, Martinez E, Moreno S (2008) The role of efavirenz compared with protease inhibitors in the body fat changes associated with highly active antiretroviral therapy. *J Antimicrob Chemother* 62: 234-245.
30. Dube MP, Komarow L, Mulligan K, Grinspoon SK, Parker RA, Robbins GK, Roubenoff R, Tebas P (2007) Long-term body fat outcomes in antiretroviral-naive participants randomized to nelfinavir or efavirenz or both plus dual nucleosides. Dual X-ray absorptiometry results from A5005s, a substudy of Adult Clinical Trials Group 384. *J Acquir Immune Defic Syndr* 45: 508-514.
31. Jemsek JG, Arathoon E, Arlotti M, Perez C, Sosa N, Pokrovskiy V, Thiry A, Soccodato M, Noor MA, Giordano M (2006) Body fat and other metabolic effects of atazanavir and efavirenz, each administered in combination with zidovudine plus lamivudine, in antiretroviral-naive HIV-infected patients. *Clin Infect Dis* 42: 273-280.
32. Cameron DW, da Silva BA, Arribas JR, Myers RA, Bellos NC, Gilmore N, King MS, Bernstein BM, Brun SC, Hanna GJ (2008) A 96-week comparison of lopinavir-ritonavir combination therapy followed by lopinavir-ritonavir monotherapy versus efavirenz combination therapy. *J Infect Dis* 198: 234-240.
33. Srasuebkul P, Calmy A, Zhou J, Kumarasamy N, Law M, Lim PL (2007) Impact of drug classes and treatment availability on the rate of antiretroviral treatment change in the TREAT Asia HIV Observational Database (TAHOD). *AIDS Res Ther* 4: 18.

Appendix

The members of the TREAT Asia HIV Observational Database (TAHOD)

CV Mean, V Saphonn* and K Vohith, National Center for HIV/AIDS, Dermatology & STDs, Phnom Penh, Cambodia; FJ Zhang*, HX Zhao and N Han, Beijing Ditan Hospital, Beijing, China; PCK Li*† and MP Lee, Queen Elizabeth Hospital, Hong Kong, China; N Kumarasamy* and S Saghayam, YRG Centre for AIDS Research and Education, Chennai, India; S Pujari* and K Joshi, Institute of Infectious Diseases, Pune, India; TP Merati* and F Yuliana, Faculty of Medicine Udayana University & Sanglah Hospital, Bali, Indonesia; E Yunihastuti* and O Ramadian, Working Group on AIDS Faculty of Medicine, University of Indonesia/

Ciptomangunkusumo Hospital, Jakarta, Indonesia; S Oka* and M Honda, National Center for Global Health and Medicine, Tokyo, Japan; JY Choi* and SH Han, Division of Infectious Diseases, Dept. of Internal Medicine, Yonsei University College of Medicine, Seoul, South Korea; C KC Lee* and R David, Hospital Sungai Buloh, Kuala Lumpur, Malaysia; A Kamarulzaman* and A Kajindran, University of Malaya Medical Centre, Kuala Lumpur, Malaysia; G Tau*, Port Moresby General Hospital, Port Moresby, Papua New Guinea; R Ditangco* and R Capistrano, Research Institute for Tropical Medicine, Manila, Philippines; YMA Chen*, WW Wong and YW Yang, Taipei Veterans General Hospital and AIDS Prevention and Research Centre, National Yang-Ming University, Taipei, Taiwan; PL Lim*, OT Ng and E Foo, Tan Tock Seng Hospital, Singapore; P Phanuphak*, and M Khongphattanayothin, HIV-NAT/Thai Red Cross AIDS Research Centre, Bangkok, Thailand; S Sungkanuparph* Sasisopin Kiertiburanakul and B Piyavong, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; T Sirisanthana*‡ and W Kotarathititum, Research Institute for Health Sciences, Chiang Mai, Thailand; AH Sohn*, L Messerschmidt* and B Petersen, The Foundation for AIDS Research, Bangkok, Thailand; J Chuah*, Gold Coast Sexual Health Clinic, Miami, Queensland, Australia; DA Cooper, MG Law*, J Zhou*, and A Jiamsakul, National Centre in HIV Epidemiology and Clinical Research, The University of New South Wales, Sydney, Australia.

*TAHOD Steering Committee member; †Steering Committee Chair; ‡Co-Chair.

OPEN ACCESS Freely available online

PLoS NEGLECTED TROPICAL DISEASES

Amebiasis in HIV-1-Infected Japanese Men: Clinical Features and Response to Therapy

Koji Watanabe[1,2], Hiroyuki Gatanaga[1,2]*, Aleyla Escueta-de Cadiz[3], Junko Tanuma[1], Tomoyoshi Nozaki[3], Shinichi Oka[1,2]

1 AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan, **2** Center for AIDS Research, Kumamoto University, Kumamoto, Japan, **3** Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan

Abstract

Invasive amebic diseases caused by *Entamoeba histolytica* are increasing among men who have sex with men and co-infection of ameba and HIV-1 is an emerging problem in developed East Asian countries. To characterize the clinical and epidemiological features of invasive amebiasis in HIV-1 patients, the medical records of 170 co-infected cases were analyzed retrospectively, and *E. histolytica* genotype was assayed in 14 cases. In this series of HIV-1-infected patients, clinical presentation of invasive amebiasis was similar to that described in the normal host. High fever, leukocytosis and high CRP were associated with extraluminal amebic diseases. Two cases died from amebic colitis (resulting in intestinal perforation in one and gastrointestinal bleeding in one), and three cases died from causes unrelated to amebiasis. Treatment with metronidazole or tinidazole was successful in the other 165 cases. Luminal treatment was provided to 83 patients following metronidazole or tinidazole treatment. However, amebiasis recurred in 6 of these, a frequency similar to that seen in patients who did not receive luminal treatment. Recurrence was more frequent in HCV-antibody positive individuals and those who acquired syphilis during the follow-up period. Various genotypes of *E. histolytica* were identified in 14 patients but there was no correlation between genotype and clinical features. The outcome of metronidazole and tinidazole treatment of uncomplicated amebiasis was excellent even in HIV-1-infected individuals. Luminal treatment following metronidazole or tinidazole treatment does not reduce recurrence of amebiasis in high risk populations probably due to amebic re-infection.

Citation: Watanabe K, Gatanaga H, Cadiz AE-d, Tanuma J, Nozaki T, et al. (2011) Amebiasis in HIV-1-Infected Japanese Men: Clinical Features and Response to Therapy. PLoS Negl Trop Dis 5(9): e1318. doi:10.1371/journal.pntd.0001318

Editor: Judd L. Walson, University of Washington, United States of America

Received May 10, 2011; **Accepted** August 1, 2011; **Published** September 13, 2011

Funding: This study was supported by a grant from the National Center for Global Health and Medicine. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: higatana@acc.ncgm.go.jp

Introduction

Invasive amebiasis (IA) caused by *Entamoeba histolytica* is the second most common cause of mortality associated with parasitic infections worldwide, accounting for 40,000 to 100,000 deaths annually [1]. Amebiasis is transmitted by ingestion of food or water containing the cyst form of *E. histolytica*, which is prevalent in developing countries in Central and South America, Asia, and Africa. In the developed countries, most cases arise in travelers and immigrants from such endemic areas [2]. Recently, however, three developed East Asian countries (Japan, Taiwan, and South Korea) reported increased risk for amebiasis among men who have sex with men (MSM) due to oral-anal sexual contact [3–12]. The annual incidence of human immunodeficiency virus type 1 (HIV-1) infection is also increasing among MSM in these countries [13-17], resulting in growing concern on IA in HIV-1-infected MSM [6,9–12,18]. The recommended treatment for IA is metronidazole (750 mg t. i. d. for 10 days) or tinidazole (2 g q. d. for 3 days), followed by a luminal agent (paromomycin 500 mg t. i. d. for 10 days or diloxanide furoate 500 mg t. i. d. for 10 days) to eliminate intestinal colonization [18,19]. A previous report described no difference in the response to metronidazole or tinidazole treatment between HIV-1-positive and –negative IA patients [20]. However, the efficacy of luminal treatment in preventing recurrence, which can arise by relapse or re-infection, has not yet been assessed rigorously. In this study, we retrospectively analyzed 170 HIV-1-infected Japanese patients with IA, together with genomic typing of *E. histolytica* in 14 of these patients, and delineated the clinical features of IA in HIV-1-infected individuals and the efficacy of metronidazole, tinidazole and luminal treatment.

Methods

Ethics statement

The Institutional Review Board of National Center for Global Health and Medicine (Tokyo, Japan) approved this study. All patients who provided clinical samples for genotyping of *E. histolytica* gave written informed consent.

Case review

The medical records of HIV-1-infected cases diagnosed with IA at the AIDS Clinical Center, National Center for Global Health and Medicine, between April 1997 and March 2010, were reviewed. The diagnosis of IA was made when one of the following criteria was satisfied; 1) identification of and/or positive PCR (methods; see below) in clinical specimens (stool or punctuate-exudate) for erythrophagocytic trophozoites in patients with IA-