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-naive 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-naivety, 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 m^2, 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 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 m^2 [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 m^2 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 m^2 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 m^2 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/κ, 1)$^{\alpha}$ $\times$ max(SCr/κ, 1)$^{-1.209}$ $\times$ 0.993$^{\mathrm{Age}}$ $\times$ 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.
[a]Median (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/m^2 (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 m^2 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 m^2 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 m^2 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 m^2 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 m^2 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 m^2 at 1 year of TDF to −23.7 ml/min per 1.73 m^2 at 5 years of TDF exposure, and in the control, from −8.0 ml/min per 1.73 m^2 at 1 year to −13.5 ml/min per 1.73 m^2 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 m^2 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 m^2, at 2 years −3.6 ml/min per 1.73 m^2, at 3 years −5.5 ml/min per 1.73 m^2, at 4 years −6.6 ml/min per 1.73 m^2, and at 5 years −10.3 ml/min per 1.73 m^2 (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 m^2 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 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 m^2 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 m^2 at 1 year to 15.0 ml/min per 1.73 m^2 at 5 years of TDF exposure, and in the control, from −4.1 ml/min per 1.73 m^2 at 1 year to −8.3 ml/min per 1.73 m^2 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 m^2, respectively, which indicated that the loss in eGFR relative to control increased over time ($P<0.001$).

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.

Discussion

In this 10 years observational cohort of treatment-naive 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 m^2 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 m^2. 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 m^2 at 1 year of TDF exposure to −5.5 ml/min per 1.73 m^2 at 3 years, and to −10.3 ml/min per 1.73 m^2 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 m^2; 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.

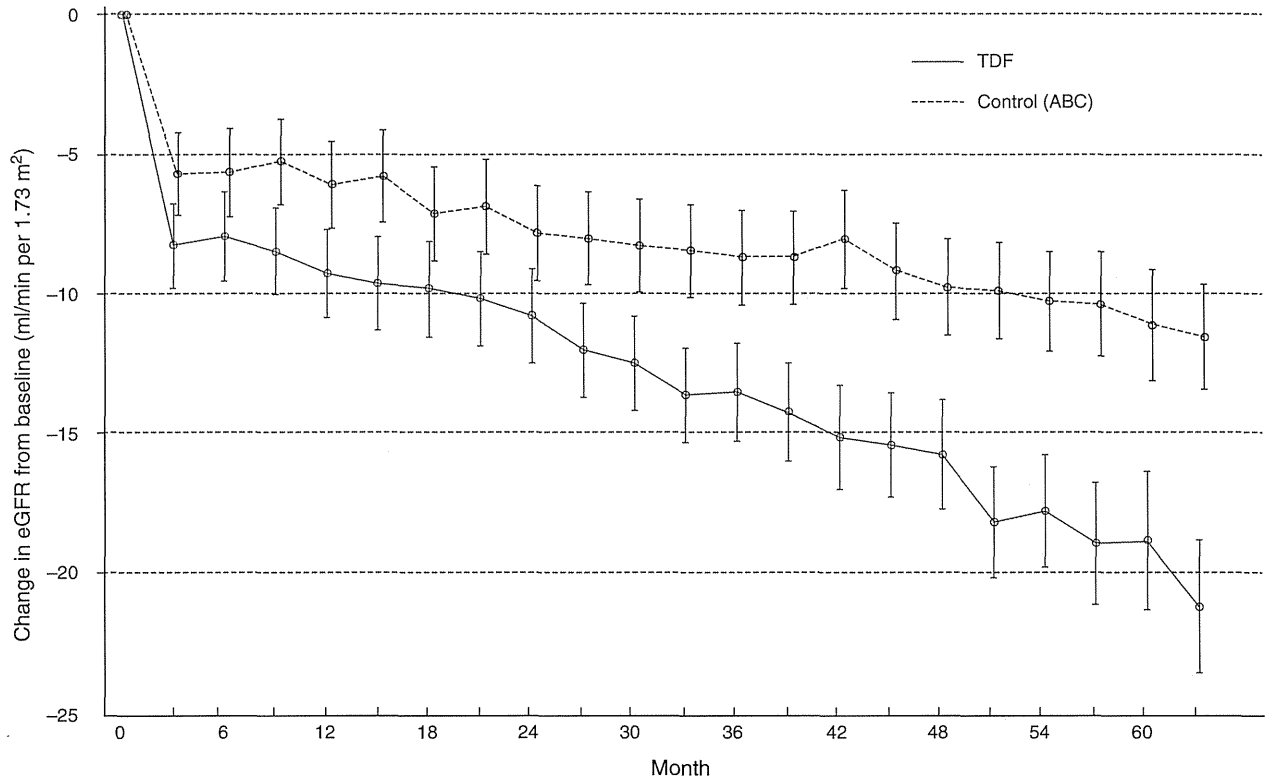

Fig. 1. Adjusted mean change in estimated glomerular filtration rate from baseline to 5 years in treatment-naive patients treated with tenofovir disoproxil fumarate-containing antiretroviral therapy (red line) and controls (patients treated with abacavir-containing ART) (black line). Least-square means 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 m^2 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 m^2, 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 m^2 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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Diagnostic Utility of Quantitative Plasma Cytomegalovirus DNA PCR for Cytomegalovirus End-Organ Diseases in Patients With HIV-1 Infection

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Objective: To investigate the diagnostic value of quantitative plasma cytomegalovirus (CMV)-DNA polymerase chain reaction (PCR) for CMV end-organ diseases (CMV-EOD) in patients with HIV-1 infection.

Design: Single-center cross-sectional study.

Methods: The study subjects were HIV-1–infected patients with CD4 ≤200 per microliter, who had undergone ophthalmologic examination with plasma CMV-DNA PCR measured within 7 days. CMV retinitis and other CMV-EOD were diagnosed according to the ACTG criteria. PCR value was converted into the WHO international standard.

Results: CMV retinitis and all CMV-EOD were diagnosed in 23 (5%) and 37 (8%) of the 461 study patients, respectively. CMV-DNA was undetectable (<185 IU/mL) in 2 patients with CMV retinitis and 1 with encephalitis. The area under the receiver operating characteristic curve of CMV-DNA for CMV retinitis and all CMV-EOD were 0.80 [95% confidence interval (CI): 0.71 to 0.89] and 0.82 (0.75 to 0.89), respectively. The sensitivity, specificity, positive predictive value, and negative predictive value for each cutoff value of CMV-DNA were as follows: for CMV retinitis, ≥10,086 IU/mL: 26.1%, 94.1%, 18.8%, 96%; ≥2946 IU/mL; 56.5%, 86.8%, 18.3%, 97.4%; ≥959 IU/mL; 60.9%, 78.1%, 12.7%, 97.4%; detectable CMV-DNA (≥185 IU/mL): 91.3%, 48.2%, 8.5%, 99.1%; for all CMV-EOD: ≥10,086 IU/mL: 32.4%, 95.3%, 37.5%, 94.2%; ≥2946 IU/mL; 54.1%, 88%, 28.2%, 95.6%; ≥959 IU/mL; 62.2%, 79.5%, 20.9%, 96%; detectable CMV-DNA; 91.9%, 49.5%, 13.7%, 98.6%.

Conclusions: Plasma CMV-DNA PCR has a high diagnostic value for both CMV retinitis and all CMV-EOD in patients with advanced HIV-1 infection. A cutoff value of CMV-DNA ≥10,086 IU/mL and ≥2946 IU/mL yields high specificity, whereas undetectable CMV-DNA load (<185 IU/mL) likely rules out CMV-EOD.

Key Words: cytomegalovirus infection, CMV-DNA PCR, HIV-1 infection, CMV retinitis, CMV end-organ diseases

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INTRODUCTION

Although antiretroviral therapy (ART) has substantially improved the prognosis of patients with HIV-1 infection, a large number of patients are still diagnosed with HIV-1 infection at a late stage, often with concurrent opportunistic infections.[1,2] Cytomegalovirus end-organ disease (CMV-EOD) is a major debilitating opportunistic infection in patients with advanced HIV-1 infection.[3,4] Among CMV end-organ diseases, retinitis is the most common clinical manifestation, which can cause total blindness.[5] Other manifestations include colitis, pneumonitis, esophagitis, and various neurological diseases.[3,6] Although the wide availability of ART has substantially reduced the incidence of CMV-EOD,[7] CMV-EOD is associated with increased mortality even in the ART era.[8]

In HIV-1–infected patients, blood tests to detect CMV by polymerase chain reaction (PCR) is not recommended for the diagnosis of CMV-EOD by the American Adult and Adolescent Opportunistic Infection Guidelines,[3] in contrast with the management of solid-organ transplantation where real-time quantitative PCR is the standard of care for the diagnosis of CMV-EOD.[9,10] One major problem related to the assessment of the diagnostic utility of quantitative PCR for CMV-EOD is that there is often poor interinstitutional correlation of quantitative PCR tests,[11] which curtails the establishment of cutoff values for clinical decision-making. In this regard, the WHO International Standard, which attempts to establish reproducibility in quantitative CMV load across laboratories, has only become available in 2010.[12,13] Hence, only a few studies in the field of HIV-1 infection have investigated this issue.[14–17] Another issue in diagnosis of CMV-EOD is that definitive diagnosis of CMV colitis is sometimes difficult in a small number of patients because tissue biopsy can only be obtained through colonoscopy. The latter is not always feasible, especially in patients with poor general condition, thus resulting in possible underdiagnosis of

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CMV-EOD. However, diagnosis of CMV retinitis is relatively easy with ophthalmologic examination including dilated retinal examination using indirect ophthalmoscopy by experienced ophthalmologist.

This study was designed to assess the diagnostic value of quantitative plasma CMV-DNA PCR for CMV-EOD in patients with advanced HIV-1 infection, with a special effort to tackle the abovementioned 2 obstacles by (1) using a PCR method that is traceable to the WHO international standard and (2) only enrolling patient who underwent ophthalmologic examination to avoid underdiagnosis of CMV retinitis, the most prevalent CMV-EOD, and calculating the diagnostic utilities of CMV PCR separately for CMV retinitis and all CMV-EOD.

METHODS

Study Design, Setting, and Participants

We conducted a single-center cross-sectional study to investigate the usefulness of quantitative plasma CMV-DNA PCR for the diagnosis of CMV-EOD among patients with advanced HIV-1 infection at AIDS Clinical Center, National Center for Global Health and Medicine (NCGM), Tokyo, Japan. This center is the largest referral center for HIV-1 infection in Japan.[18] The following criteria were applied for enrollment: inclusion criteria—(1) HIV-1–infected patients aged ≥18 years who first visited our clinic between January 2004 and December 2013 and underwent full ophthalmologic examination, (2) patients with CD4 count ≤200 per microliter, and (3) plasma CMV-DNA PCR was measured within 7 days from the day of the ophthalmologic examination; and exclusion criterion—patients who had already been diagnosed with CMV retinitis or other CMV-EOD at the time of referral to our clinic, because it is often difficult to confirm retinal photography or pathology, which are required for the diagnosis of CMV-EOD according to the standard ACTG criteria for such cases.[19] At our clinic, ophthalmologic examination including dilated retinal examination using indirect ophthalmoscopy by experienced ophthalmologist is routinely conducted on first visit to our clinic. When the diagnosis of CMV retinitis was uncertain, the examination was repeated within 1–4 weeks and then confirmed by at least 2 ophthalmologists. For patients with suspected CMV encephalitis, CMV-DNA PCR of cerebrospinal fluid was routinely assessed, and gastroscopy or colonoscopy was performed with biopsy for those with suspected CMV esophagitis/colitis. Plasma CMV-DNA PCR was also routinely conducted for HIV-1-infected patients with CD4 ≤200 per microliter.

The study was approved by the Human Research Ethics Committee of NCGM. 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

The results of the first ophthalmologic examination for each patient were extracted from the medical charts, together with plasma CMV-DNA PCR value determined within 7 days of the examination. The diagnosis of CMV retinitis was based on the standard ACTG criteria of "confirmed CMV retinitis," which include diagnosis by an experienced ophthalmologist and documentation of CMV retinitis by retinal photography.[19] Data on other CMV-EOD were also extracted from the medical records; the diagnosis of other CMV-EOD was based on the standardized ACTG criteria and confirmed within 4 weeks of ophthalmologic examination.[19] Baseline characteristics [age, sex, ethnicity, history of AIDS, route of HIV-1 transmission, and treatment status for HIV-1 infection (either treatment naive or experienced)], CD4 count, and HIV-1 viral load were also collected. For CD4 count and HIV-1 viral load, the data closest to and preceding the day of the first ophthalmologic examination were used. Systemic steroid use, anti-CMV treatment, and chemotherapy were also recorded. They were defined as therapies administered either orally or intravenously within 1 month preceding the ophthalmologic examination.

Measurement of Quantitative CMV-DNA PCR

Throughout the study period, CMV PCR tests were conducted within 24 hours after sample blood collection using the geniQ CMV Real-Time PCR assay. The tests were performed at KITASATO-OTSUKA Biomedical Assay Laboratories Co. (KOBAL; Sagamihara, Japan), which is accredited by ISO15189. Details of the procedures, probes, and primers for the assay were reported previously[20] and run on the ABI 7900HT system (Applied Biosystems, Foster City, CA). The assay used had a CMV-DNA limit of detection of 200 copies per milliliter. The geniQ CMV correlates well with COBAS AmpliPrep/COBAS TaqMan CMV Test (Roche Molecular System, Branchburg, NJ); [R^2 = 0.9763, y = 0.9784x + 0.0427, where y = $\log_{10}$(geniQ CMV copies/mL) and x = $\log_{10}$(COBAS AmpliPrep/COBAS TaqMan CMV Test), n = 59, unpublished data]. Because COBAS AmpliPrep/COBAS TaqMan CMV Test is traceable to the WHO First International Standard with coefficient of 1.1,[12] the geniQ CMV was converted to WHO international unit (IU) using following formula: = 0.91 × 10 raised to the power of {[$\log_{10}$(geniQ CMV copies/mL) − 0.0427]/0.9784}.

Statistical Analysis

Baseline characteristics were compared between patients with and without CMV retinitis, and those with and without CMV-EOD, using the Student *t* test and χ^2 test (Fisher exact test) for continuous and categorical variables. The univariate logistic regression model was applied to estimate the effects of different CMV-DNA cutoff values [CMV-DNA PCR ≥10,000 copies/mL (10,086 IU/mL), ≥3000 copies/mL (2946 IU/mL), and ≥1000 copies/mL (959 IU/mL), and detectable CMV load [≥200 copies/mL (185 IU/mL)] and other variables on the occurrence of CMV retinitis and all CMV-EOD. Undetectable CMV-DNA [<200 copies/mL (185 IU/mL)] was treated as 10 IU/mL (1 $\log_{10}$ IU/mL) in logarithmic calculations. Multivariate logistic regression model was applied to estimate the effects of each CMV-DNA cutoff values on the development of CMV retinitis and all CMV-EOD. The model for CMV retinitis was adjusted for age and CD4 count, because low CD4

count is an established risk factor for CMV retinitis[21] and also for variables with P value of <0.05 in univariate analysis (other CMV diseases). The model for all CMV-EOD was adjusted for age, CD4 count, and anti-CMV treatment. Sex was not added to the models because all patients with retinitis and all except 1 CMV-EOD patient were males. Receiver operating characteristic (ROC) curves were constructed, and the area under the curve (AUC) was estimated with 95% confidence interval (CI) to quantify the accuracy of CMV-DNA PCR. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and adjusted odds ratio for the diagnosis of CMV retinitis and all CMV-EOD were calculated for abovementioned different cutoff values. Additional analyses were conducted for data of patients with CD4 count of $\leq$100 and those with CD4 count of $\leq$50 per microliter. Statistical significance was defined as 2-sided P values <0.05. We used odds ratios (ORs) with 95% CIs. All statistical analyses were performed with the Statistical Package for Social Sciences version 21.0 (SPSS, Chicago, IL).

RESULTS

As shown in Figure 1, 1635 patients visited our clinic for the first time during the study period and underwent ophthalmologic examination. Of the 764 patients who had CD4 <200 per microliter, 461 (60%) patients underwent CMV-DNA PCR within 7 days of the examination and were included as the study patients. They were mostly Asian males and treatment naive for HIV-1 infection (Table 1). The median CD4 count and HIV-1 load were 42 per microliter [interquartile range (IQR), 18–78/μL] and 5.23 $\log_{10}$copies per milliliter (IQR, 4.85–5.68 $\log_{10}$copies/mL), respectively. CMV-DNA was detected in 248 (54%), 218 (58%), and 164 (62%) patients of the total study population (CD4 $\leq$200/μL), patients with CD4 $\leq$100 per microliter, and those with CD4 $\leq$50 per microliter, respectively.

CMV retinitis was diagnosed in 23 (5.0%) patients. Furthermore, 8 CMV colitis, 5 encephalitis, and 4 esophagitis cases were diagnosed. All encephalitis cases had documented detection of viral nucleic acids in cerebrospinal fluid, and all colitis and esophagitis had documented pathological evidence of CMV infection.[19] Because 3 patients had more than 1 CMV-EOD, 37 (8.0%) patients were diagnosed with CMV-EOD. The median CD4 counts of patients with CMV retinitis and those with any CMV-EOD were 31/μL (IQR, 16-74/μL; range, 7–158/μL) and 25/μL (IQR, 10–57/μL; range, 3–158/μL), respectively (Table 1).

Patients with CMV retinitis or CMV-EOD had higher CMV load and were more likely to have CMV load of $\geq$10,086 IU/mL, $\geq$2946 IU/mL, $\geq$959 IU/mL, and detectable load than patients without retinitis or CMV-EOD, respectively (Table 1). CMV-DNA was undetectable in 2 patients with retinitis and 1 patient with encephalitis. None of these 3 patients had received anti-CMV treatment within 1 month preceding the day of PCR examination. Patients with CMV retinitis and those with CMV-EOD tended to be on anti-CMV treatment compared with those free of these diseases (P = 0.095 and P = 0.018, respectively). There was no difference in CD4 count between patients with CMV retinitis and without retinitis, whereas CD4 count of the patients with CMV-EOD was marginally lower than that of those free of CMV-EOD (P = 0.053). There was no difference

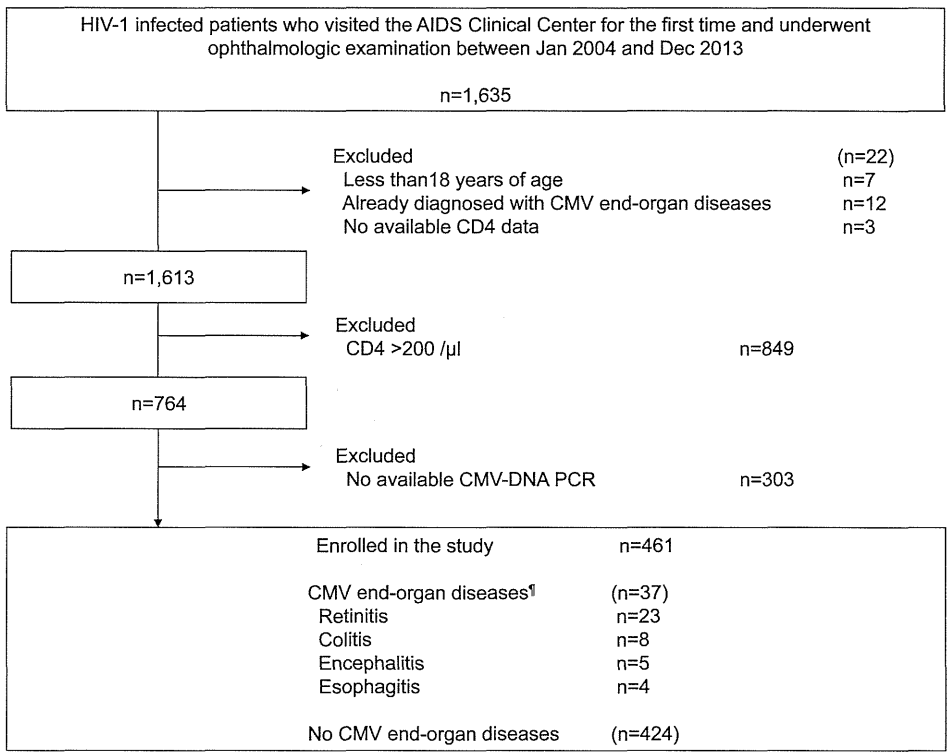

FIGURE 1. Patient enrollment process. ¶Three patients had more than 1 CMV-EOD.

TABLE 1. Baseline Characteristics of HIV-1-Infected Patients With and Without Cytomegalovirus Retinitis and CMV-EOD

		CMV Retinitis			All CMV-EOD		
	All Study Patients (n = 461)	CMV Retinitis (n = 23)	No CMV Retinitis (n = 438)	*P*	All CMV-EOD (n = 37)	No CMV-EOD (n = 424)	*P*
Sex (male), n (%)	433 (94)	23 (100)	410 (94)	0.39	36 (97)	397 (94)	0.72
Age*	39 (33–48)	42 (33–53)	39 (33–47)	0.23	41 (34–50)	39 (33–47)	0.24
CMV load (log_{10} IU/mL)*	2.27 (1.00–2.91)	3.47 (2.67–4.00)	2.27 (1.00–2.82)	<0.001	3.47 (2.67–4.31)	2.27 (1.00–2.75)	<0.001
≥10,086 IU/mL, n (%)	32 (7)	6 (26)	26 (6)	0.003	12 (32)	20 (5)	<0.001
≥2946 IU/mL, n (%)	71 (15)	13 (57)	58 (13)	<0.001	20 (54)	51 (12)	<0.001
≥959 IU/mL, n (%)	110 (24)	14 (61)	96 (22)	<0.001	23 (62)	87 (21)	<0.001
Detectable (≥185 IU/mL), n (%)	248 (54)	21 (91)	227 (52)	<0.001	34 (92)	214 (51)	<0.001
CMV diseases other than retinitis, n (%)	17 (4)	3 (13)	14 (3)	0.047	NA	NA	NA
CD4 count (/μL)*	42 (18–78)	31 (16–74)	43 (18–78)	0.55	25 (10–57)	43 (19–81)	0.053
HIV RNA viral load (log_{10} copies/mL)*	5.23 (4.85–5.68)	5.32 (4.57–5.84)	5.23 (4.85–5.67)	0.52	5.51 (4.89–5.72)	5.20 (4.84–5.67)	0.89
ART, n (%)	40 (9)	3 (13)	37 (8)	0.44	3 (8)	37 (9)	1.00
Systemic steroid use, n (%)	134 (29)	5 (22)	129 (30)	0.49	13 (35)	121 (29)	0.45
Anti-CMV treatment, n (%)	36 (8)	4 (17)	32 (7)	0.095	7 (19)	29 (7)	0.018
Chemotherapy, n (%)	10 (2)	1 (4)	9 (2)	0.40	1 (3)	9 (2)	0.57
History of AIDS, n (%)	339 (74)	23 (100)	316 (72)	0.001	37 (100)	302 (71)	<0.001
Homosexual contact, n (%)	365 (79)	17 (74)	348 (80)	0.60	29 (78)	336 (79)	0.84
Diabetes mellitus, n (%)	15 (3)	1 (4)	14 (3)	0.54	2 (5)	13 (3)	0.34

*Median (IQR).
NA, not applicable.

in age, HIV RNA viral load, and the percentage of patients on ART, on systemic steroid use, on chemotherapy, and with diabetes mellitus, between patients with and without CMV retinitis and patients with and without CMV-EOD (Table 1).

Univariate analysis showed that CMV-DNA ≥10,086, ≥2946, and ≥959 IU/mL were all associated with CMV retinitis, whereas undetectable load (<185 IU/mL) was inversely associated with CMV retinitis (OR, 0.1; 95% CI: 0.02 to 0.44; $P = 0.002$) (Table 2). Concurrent CMV diseases other than retinitis were also associated with retinitis and on anti-CMV therapy were marginally associated with retinitis. Similarly, CMV-DNA ≥10,086, ≥2946, and ≥959 IU/mL were all associated with CMV-EOD, whereas undetectable CMV load was inversely associated with CMV-EOD (OR, 0.1; 95% CI: 0.03 to 0.30; $P < 0.001$). The use of anti-CMV treatment was also associated with CMV-EOD.

ROC-AUC of CMV-DNA PCR was 0.80 (95% CI: 0.71 to 0.89) for CMV retinitis and 0.82 (95% CI: 0.75 to 0.89) for all CMV-EOD. The sensitivity, specificity, PPV, NPV, and the result of multivariate analysis for each cutoff value of CMV-DNA for the diagnosis of CMV retinitis are shown in (Table 3). CMV-DNA PCR of ≥10,086 and of ≥2946 IU/mL had 94.1% and 86.8% specificity, respectively, whereas detectable PCR had 91.3% sensitivity (NPV 99.1%). For all CMV-EOD, CMV-DNA of ≥10,086 and of ≥2946 IU/mL had 95.3% and 88% specificity, respectively, whereas detectable PCR had 91.9% sensitivity (NPV 98.6%) (Table 4).

Among patients with CD4 count of ≤100/μL (n = 376) and ≤50/μL (n = 265), 20 (5.3%) and 14 (5.3%) patients, respectively, were diagnosed with CMV retinitis and 33 (8.8%) and 26 (9.8%) patients, respectively, were diagnosed with any CMV-EOD. For patients with CD4 ≤100/μL, ROC-AUC of the CMV-DNA PCR for the diagnosis of CMV retinitis was 0.77 (95% CI: 0.67 to 0.87), and for the diagnosis of all CMV-EOD, it was 0.79 (95% CI: 0.71 to 0.87). For those with CD4 ≤50/μL, ROC-AUC for CMV retinitis was 0.73 (95% CI: 0.62 to 0.84) and for CMV-EOD, it was 0.76 (95% CI: 0.67 to 0.85).

The sensitivity, specificity, PPV, and NPV for each cutoff value for the diagnosis of CMV retinitis in patient with CD4 counts ≤100/μL and ≤50/μL are shown in (Table 3). For patients with CD4 ≤100/μL, CMV-DNA PCR of ≥10,086 and ≥2946 IU/mL yielded 93.3% and 84.8% specificity, respectively, whereas detectable CMV-DNA had 90% sensitivity (NPV 98.7%). Similarly, for patients with CD4 ≤50/μL, CMV-DNA of ≥10,086 and ≥2946 IU/mL had 92% and 82.1% specificity, respectively, and detectable CMV-DNA had 92.9% sensitivity (NPV 99%). These parameters for the diagnosis of all CMV-EOD in patients with CD4 count ≤100/μL and ≤50/μL are shown in (Table 4). For patients with CD4 ≤100/μL, CMV-DNA PCR of ≥10,086 and ≥2946 IU/mL had 94.5% and 86% specificity, respectively, whereas detectable CMV-DNA had 90.9% sensitivity (NPV 98.1%). Similarly, for patients with CD4 ≤50/μL, CMV-DNA of ≥10,086 and ≥2946 IU/mL had 93.7% and 83.7% specificity, respectively, and detectable CMV-DNA had 92.3% sensitivity (NPV 98%).

DISCUSSION

This cross-sectional study showed that quantitative plasma CMV-DNA PCR test is a useful surrogate marker for the diagnosis of both retinitis and all CMV-EOD in

TABLE 2. Results of Univariate Analysis to Estimate the Association of Each Variable With CMV Retinitis and All CMV-EOD

	CMV Retinitis			All CMV-EOD		
	OR	95% CI	*P*	OR	95% CI	*P*
CMV load ≥10,086 IU/mL	5.6	2.03 to 15.4	0.001	9.7	4.26 to 22.1	<0.001
CMV load ≥2946 IU/mL	8.5	3.57 to 20.3	<0.001	8.6	4.23 to 17.5	<0.001
CMV load ≥959 IU/mL	5.5	2.33 to 13.2	<0.001	6.4	3.15 to 12.9	<0.001
Undetectable CMV load (<185 IU/mL)	0.1	0.02 to 0.44	0.002	0.1	0.03 to 0.30	<0.001
Age per 1 yr increment	1.0	0.99 to 1.06	0.24	1.0	0.99 to 1.05	0.24
CD4 per 1/μL increment	1.0	0.99 to 1.01	0.55	1.0	0.98 to 1.00	0.057
HIV-1 RNA load per 1 $\log_{10}$ copies/mL increment	0.9	0.57 to 1.32	0.52	1.0	0.71 to 1.49	0.89
ART	1.6	0.46 to 5.73	0.45	0.9	0.27 to 3.15	0.90
CMV diseases other than retinitis	4.5	1.21 to 17.1	0.025	NA	NA	NA
Anti-CMV treatment	2.7	0.86 to 8.32	0.090	3.2	1.29 to 7.86	0.012
Chemotherapy	2.2	0.26 to 17.9	0.47	1.3	0.16 to 10.4	0.82
Systemic steroid use	0.7	0.24 to 1.83	0.43	1.4	0.67 to 2.75	0.40

HIV-1–infected patients with CD4 count ≤200/μL. The cutoff value of ≥10,086 IU/mL yielded 94.1% specificity for CMV retinitis and 95.3% specificity for all CMV-EOD, and the cutoff of ≥2946 IU/mL had 86.8% specificity for retinitis and 88% specificity for all CMV-EOD. Undetectable load (<185 IU/mL) can likely rule out CMV retinitis and EOD, since undetectable load showed 91.3% and 91.9% sensitivity (99.1% and 98.6% NPV) for retinitis and all EOD, respectively. In subgroup analysis of patients with CD4 count of ≤100/μL and ≤50/μL, the results were also similar. Especially, the result that undetectable CMV load can rule out any CMV-EOD with >90% sensitivity (NPV >98%) should help clinical decision making as a surrogate marker.

This study has 2 major strengths. First, to the best of our knowledge, this is the first study that has investigated the diagnostic value of quantitative CMV-DNA PCR for CMV-EOD in patients with HIV-1 infection, with the results converted to the WHO international unit to allow comparison of the cutoff values with those obtained by other laboratories. Another important dimension of the study was the processing of the PCR test within 24 hours after blood sample collection. In this regard, the stability of CMV viral load in blood and plasma samples stored over a long period of time has not been well validated.[22,23]

Second, we only included patients who underwent full ophthalmologic examination to avoid underdiagnosis of retinitis to appropriately evaluate the diagnostic value of plasma CMV-DNA PCR. For other CMV diseases, such as esophagitis and colitis, underdiagnosis is possible to some extent because in clinical practice, not all patients with difficulty in swallowing or abdominal pain can undergo endoscopy and pathological examination, which are required for the diagnosis of CMV gastrointestinal diseases.[24,25] However, it is relatively easy for experienced ophthalmologists to make a definitive diagnosis for CMV retinitis. Furthermore, CMV retinitis forms the largest proportion of

TABLE 3. Diagnostic Accuracy of CMV-DNA PCR for CMV Retinitis Using Different Cutoff Values for the Entire Study Population (CD4 ≤200/μL), patients With CD4 ≤100/μL, and those With ≤50/μL

	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Adjusted OR OR (95% CI)	*P*
Study patients (N = 461)						
CMV-DNA PCR ≥10,086 IU/mL	26.1	94.1	18.8	96.0	4.2 (1.39 to 12.9)	0.011
CMV-DNA PCR ≥2946 IU/mL	56.5	86.8	18.3	97.4	7.9 (3.12 to 20.1)	<0.001
CMV-DNA PCR ≥959 IU/mL	60.9	78.1	12.7	97.4	5.0 (2.02 to 12.6)	0.001
Detectable CMV-DNA PCR (≥185 IU/mL)	91.3	48.2	8.5	99.1	9.0 (2.02 to 40.0)	0.004
Patients with CD4 ≤100 (n = 376)						
CMV-DNA PCR ≥10,086 IU/mL	20	93.3	14.3	95.4	2.8 (0.75 to 10.2)	0.13
CMV-DNA PCR ≥2946 IU/mL	55	84.8	16.9	97.1	6.7 (2.53 to 18.0)	<0.001
CMV-DNA PCR ≥959 IU/mL	60	75.3	12	97.1	4.3 (1.65 to 11.4)	0.003
Detectable CMV-DNA PCR (≥185 IU/mL)	90	43.8	8.3	98.7	6.6 (1.48 to 29.7)	0.013
Patients with CD4 ≤50 (n = 265)						
CMV-DNA PCR ≥10,086 IU/mL	21.4	92	13	95.5	2.3 (0.49 to 11.0)	0.29
CMV-DNA PCR ≥2946 IU/mL	42.9	82.1	11.8	96.3	2.9 (0.90 to 9.37)	0.075
CMV-DNA PCR ≥959 IU/mL	50	72.5	9.2	96.3	2.4 (0.76 to 7.43)	0.14
Detectable CMV-DNA PCR (≥185 IU/mL)	92.9	39.8	7.9	99	7.5 (0.95 to 58.8)	0.057

TABLE 4. Diagnostic Accuracy of CMV-DNA PCR for All CMV Organ Diseases Using Different Cutoff Values for the Entire Study Population (CD4 ≤200/μL), Patients With CD4 ≤100/μL, and Those With ≤50/μL

	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Adjusted OR OR (95% CI)	P
Study patients (n = 461)						
CMV-DNA PCR ≥10,086 IU/mL	32.4	95.3	37.5	94.2	7.5 (3.16 to 18.0)	<0.001
CMV-DNA PCR ≥2946 IU/mL	54.1	88	28.2	95.6	7.2 (3.47 to 15.2)	<0.001
CMV-DNA PCR ≥959 IU/mL	62.2	79.5	20.9	96	5.4 (2.59 to 11.1)	<0.001
Detectable CMV-DNA PCR (≥185 IU/mL)	91.9	49.5	13.7	98.6	9.7 (2.91 to 32.5)	<0.001
Patients with CD4 ≤100 (n = 376)						
CMV-DNA PCR ≥10,086 IU/mL	27.3	94.5	32.1	93.1	5.1 (1.95 to 13.3)	0.001
CMV-DNA PCR ≥2946 IU/mL	51.5	86	26.2	94.9	5.8 (2.67 to 12.5)	<0.001
CMV-DNA PCR ≥959 IU/mL	60.6	76.7	20	95.3	4.5 (2.09 to 9.56)	<0.001
Detectable CMV-DNA PCR (≥185 IU/mL)	90.9	45.2	13.8	98.1	7.6 (2.27 to 25.7)	0.001
Patients with CD4 ≤50 (n = 265)						
CMV-DNA PCR ≥10,086 IU/mL	30.8	93.7	34.8	92.6	5.8 (1.95 to 17.4)	0.002
CMV-DNA PCR ≥2946 IU/mL	46.2	83.7	23.5	93.5	3.8 (1.58 to 9.08)	0.003
CMV-DNA PCR ≥959 IU/mL	53.8	74.1	18.4	93.7	3.0 (1.27 to 6.99)	0.012
Detectable CMV-DNA PCR (≥185 IU/mL)	92.3	41.4	14.6	98	7.9 (1.81 to 34.3)	0.006

CMV-EOD in patients with HIV-1 infection.[3,6] These were the reasons for setting up the abovementioned inclusion criteria for study patients and for separately analyzing the diagnostic value of CMV load for CMV retinitis and all CMV-EOD. The results for CMV retinitis and all CMV-EOD were very similar.

Our results that quantitative plasma CMV-DNA PCR test is useful for the diagnosis of CMV-EOD, especially in ruling out CMV-EOD, are in conflict with the American Adult and Adolescent Opportunistic Infection Guidelines, which do not recommend the use of blood tests to detect CMV by PCR for the diagnosis of CMV-EOD.[3] However, it needs to be noted that, to the best of our knowledge, only 3 studies have previously investigated the utility of blood CMV load for the diagnosis of CMV-EOD with cross-sectional study design,[14–16] although many other studies either longitudinally investigated the utility of CMV load for the prediction or progression of CMV-EOD or death during the follow-up period,[26–34] or examined the effectiveness of preemptive therapy for CMV-EOD based on positive CMV load.[19,35–38] The major limitations of the abovementioned 3 studies that investigated the diagnostic utility of blood CMV load for CMV-EOD included small sample size (n = 70 for Yoshida et al,[14] n = 58 for Pellegrin et al,[16] and n = 53 for Brantsaeter et al[15]), and importantly, their results were not convertible to the WHO international unit. In comparison, this study included a far larger number of study population of 461 patients, and the results were convertible to the WHO international unit. These 2 features probably explain the reasons why the results of this study are in conflict with the recommendations made by the abovementioned American Guidelines.[3]

Apart from the abovementioned strengths of this study, we need to acknowledge some study limitations. First, although all study patients underwent full ophthalmologic examination for the screening of CMV retinitis, due to the nature of observational study, not all patients necessarily underwent the appropriate procedures (eg, endoscopy or biopsy) required to establish the diagnosis of CMV-EOD. Thus, underdiagnosis of CMV-EOD other than retinitis is possible in this study. However, as explained above, the majority of CMD-EOD cases had retinitis (23 of 37), consistent with previous studies,[3,6] and the diagnostic parameters for each cutoff value of CMV load was similar for CMV retinitis and all CMV-EOD, suggesting that substantial underdiagnosis of CMV-EOD other than retinitis was unlikely. Second, this study did not exclude patients who have received anti-CMV treatment, although the treatment could have affected the value of CMV-DNA PCR. This is because it is not sometimes easy to judge whether the patient had received anti-CMV treatment or not, and the number of patients who received either oral or intravenous anti-CMV treatment within 1 month preceding the ophthalmologic examination was relatively small; 36 (8%) of all study patients, 4 (17%) of patients with CMV retinitis, and 7 (19%) of those with any CMV-EOD. Furthermore, exclusion of patients with anti-CMV treatment did not alter the results; the cutoff value of ≥10,086 IU/mL yielded 95.3% and 96.2% specificity for CMV retinitis and for all CMV-EOD, respectively, and undetectable load had 89.5% and 90% sensitivity (99% and 98.5 NPV) for retinitis and all EOD, respectively.

In conclusion, quantitative plasma CMV-DNA PCR using WHO international unit was a useful surrogate diagnostic marker for CMV-EOD in HIV-1–infected patients with CD4 count ≤200/μL. The cutoff value of ≥10,086 IU/mL yielded 94.1% specificity for retinitis and 95.3% specificity for all CMV-EOD, whereas undetectable load (<185 IU/mL) had 91.3% and 91.9% sensitivity (99.1% and 98.6% NPV) for retinitis and all CMV-EOD, respectively. Especially, the result that undetectable load could rule out any CMV-EOD with >90% sensitivity (>98% NPV) can be helpful in clinical practice for the screening of CMV-EOD in patients with HIV-1 infection.

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Case report

Exacerbation of microcytic anemia associated with cessation of anti-retroviral therapy in an HIV-1-infected patient with beta thalassemia

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ABSTRACT

We report a patient with Japanese minor β thalassemia and HIV-1 infection.

The patient showed prolonged anemia, which was originally attributed to chronic parvovirus B19 infection. Twelve years later, the patient presented with exacerbation of microcytic anemia following cessation of anti-retroviral therapy; the exacerbation resolved when anti-retroviral therapy was resumed. Sequencing of the β globin gene revealed heterozygosity for a four-nucleotides deletion at codon 41/42 and minor β thalassemia was confirmed.

Because HIV-1-infected patients frequently show anemia due to nutritional deficiencies, opportunistic infections, AIDS-related malignancies, drug treatment and a direct effect of HIV-1 on the bone marrow, it is likely to overlook other causes of anemia.

Thalassemia should be considered in the differential diagnosis of anemia even in HIV-1 infected patients, when microcytic anemia without iron deficiency is observed.

Our case suggested that active HIV infection may have worsened β thalassemia, and early introduction of anti-retroviral therapy is beneficial for the recovery of anemia.

1. Introduction

HIV-1 infected patient frequently manifest anemia [1]. Anemia prior to anti-retroviral therapy (ART) is often caused by amebic or cytomegalovirus colitis, parvovirus B19 infection, and HIV-1 infection itself [2]. After anti-retroviral therapy, anemia is mainly due to ART therapy itself, especially using Zidovudine (ZDV or AZT), which resulting in macrocytic changes.

In this paper, we present an HIV-1-infected β-thalassemia patient who showed exacerbation of microcytic anemia along with the cessation of ART, and the anemia resolved when ART was resumed. Hemoglobinopathy should be considered in the differential diagnosis of anemia even in HIV-1 infected patient, especially where there is microcytic anemia without iron deficiency. Early re-introduction of anti-retroviral therapy is beneficial for the recovery of anemia in β-thalassemia patient with HIV infection.

2. Case report

In March 2000, the patient, in his early forties, was admitted to the Kyushu Medical Center with Pneumocystis pneumonia. Since two months before the admission, severe anemia had continued. The HIV-1 RNA copy number in the plasma was 90,000 copies/ml (Fig. 1) and CD4 positive T cell count was 70/μl. Acquired immune deficiency syndrome (AIDS) was diagnosed. At the time of admission (March 2000), the hemoglobin concentration [Hb] was 5.8 g/dl and mean corpuscular volume (MCV) was 72.4 fl. On April 7,

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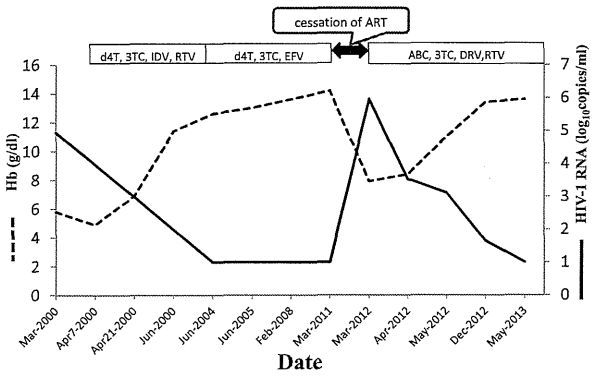

Fig. 1. Clinical course of Hemoglobin (Hb) and HIV RNA copy number along with the cessation of anti-retrovirus therapy (ART) and re-administration of ART. Correlation between HIV-1 viremia and exacerbation of anemia was observed. d4T sanilvudine, 3TC lamivudine, IDV indinavir, RTV ritonavir, EFV efavirenz, ABC abacavir, DRV darunavir.

following the administration of antibiotics, [Hb] declined to 4.7 g/dl without any hemorrhagic lesion, and the white blood cell (WBC) count declined to 900/μl. On April 13, bone marrow aspiration showed hypoplasia, and antiretroviral therapy [Sanilvudine (d4T), Lamivudine (3TC), Indinavir (IDV), and Ritonavir (RTV); later switched to d4T, 3TC, and Efavirenz (EFV)] was started on the same day. PCR of the bone marrow fluid revealed parvovirus B19 infection which suggested that the pancytopenia was caused by the bone marrow suppression due to antibiotics administration, or by HIV-1 infection itself, and parvovirus B19 infection-accelerated severe anemia. On April 21, HIV RNA was reduced to 1000 copies/ml and [Hb] was 7.6 g/dl. He discharged on May 2000 and his [Hb] continued to recover. On July 2000, his [Hb] was 12.5 g/dl: however, MCV was 82.7 fl and remained microcytic. The cause of continued anemia of the patient was attributed to chronic parvovirus B19 infection at that time and was reported elsewhere [3].

Five years later in June 2005, he attended Kagoshima University Hospital. At that time, the CD4 positive T cell count was 465/μl and the HIV-1 RNA copy number in the plasma was undetectable (<50 copies/ml). RBC was 5 million/μl, [Hb] was 13 g/dl, MCV was 79 fl and the reticulocyte count was 15‰. For three years he continued the same antiretroviral therapy (d4T, 3TC, EFV) during which the HIV-1 RNA copy number was always undetectable and the CD4 count ranged between 441 and 790/μl. At that time, his [Hb] level ranged between 11.4 and 13.9 g/dl and MCV ranged between 77 and 79 fl.

In Feb 2008, he moved to another prefecture. Four years later, in March 2012, he again visited Kagoshima University Hospital due to job re-relocation, and complained of easy fatigue. He had discontinued antiretroviral therapy of his own will eight months prior to this visit. His HIV-1 RNA copy number in the plasma was 920000 copies/ml, CD4 count was 101/μl, [Hb] was 7.9 g/dl, MCV was 64 fl and reticulocyte count was 19‰. He showed no evident opportunistic infection at this time. Four days later, antiretroviral therapy [3TC, Abacavir (ABC), Darunavir (DRV), RTV] was resumed. Ten days after re-administration of ART, his [Hb] was 7 g/dl, but 24 days after re-administration of ART (April 2012), his [Hb] increased to 8.4 g/dl and MCV was 68 fl, HIV-1 RNA copy number decreased to 3300 copies/ml and CD4 count recovered to 308/μl. In May 2012 (52 days after re-administration of ART), his [Hb] increased to 11 g/dl, HIV-1 RNA copy number decreased to 1300 copies/ml and CD4 count recovered to 422/μl. One year later in May 2013, his [Hb] increased to 13.6 g/dl along with the complete inhibition of HIV RNA copy number in the plasma (Fig. 1). The serum iron in March 2012 was 37 μg/dl (normal range 44–192) and UIBC was 161 μg/dl (normal range 111–255). However, 24 days after re-administration of ART, serum iron was 92 μg/dl and UIBC was 112 μg/dl without iron administration, which suggested his microcytic anemia was not from iron deficiency. Because the [Hb] in March 2012 was so low with microcytic change and there was no hemorrhagic lesion or opportunistic infection, another reason for the anemia was suspected.

Target cells were observed in the peripheral blood (Fig. 2). Hemoglobin analysis revealed a HbA2 of 9% and HbF of 4%, which suggested the existence of a hemoglobinopathy. Further tests for hemoglobinopathies showed a prolongation of the glycerol lysis time (107 s, compared to the normal control of 22–55sec) which implies elevated osmotic resistance. Finally, DNA sequencing revealed heterozygosity in the β globin gene, with the deletion of 4 nucleotides at codon 41/42 (TTCTTT to TT) in one allele (Fig. 3), and β-thalassemia minor was diagnosed.

3. Discussion

Anemia is a common clinical finding in HIV-1-infected patients. Many factors may contribute to the development of anemia in HIV-1-infected patients including nutritional deficiencies, opportunistic infections, AIDS-related malignancies, drug treatment and a direct effect of HIV-1 on the bone marrow [2].

Our case showed severe anemia ([Hb] 5.8 g/dl) when AIDS was first diagnosed, when he had a high HIV-1 RNA in the plasma. The patient's anemia improved after anti-retroviral therapy, but mild anemia continued. At this time the anemia was attributed to chronic parvovirus B19 infection [3,4]. However, even after the recovery of the CD4+ cell count, mild anemia with microcytic change continued for years. Because his anemia was mild ([Hb] 13 g/dl), it was not investigated further at that time. Twelve years later, when he ceased ART, he again showed moderate microcytic anemia (Hb 7.9 g/dl), and this anemia resolved when ART was resumed. Because there was no hemorrhagic lesion, or opportunistic infection, we sought another cause of anemia. First, the hemoglobin fraction was measured, and both Hb-A2 and Hb-F were elevated, suggesting a hemoglobinopathy. Finally, sequencing of the β globin gene revealed a four-nucleotide deletion at codon 41/42 in one allele of the β globin gene, leading to the diagnosis of β thalassemia minor.

Even in non-thalassemic HIV-1 carriers, higher values of Hb-A2 have been observed during ART, especially with Zidovudine (ZDV) [5–7]: that is increased HbA2 alone is not a sufficient reason to suspect thalassemia in HIV-1 patients receiving ART. However, treatment with anti-retroviral drugs such as ZDV often results in

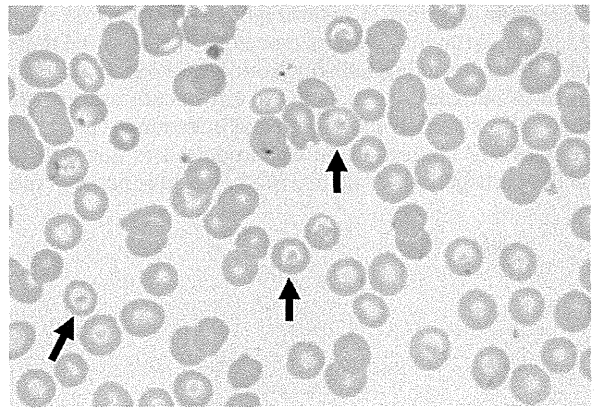

Fig. 2. Target cells were observed in the blood film (arrows).

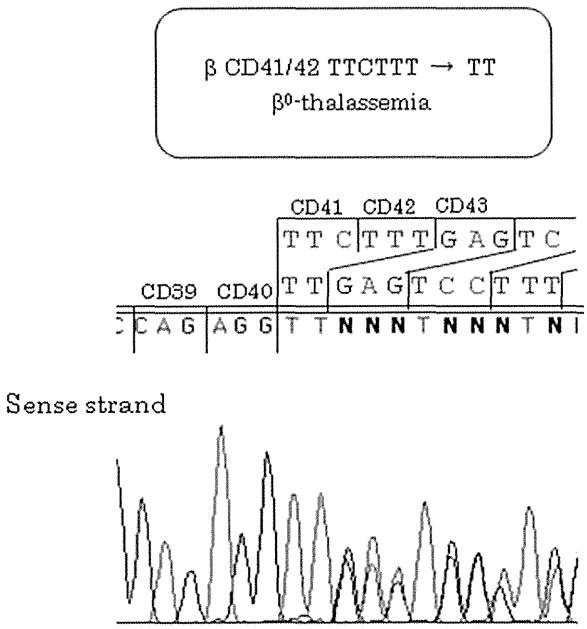

Fig. 3. DNA sequencing revealed four-nucleotide deletion at codon 41/42 in one allele of the β-globin gene.

macrocytosis [8]. It has also been reported that mean corpuscular volume (MCV) of HIV-1 patients with thalassemia after ART increased from microcytic levels to normocytic levels, and ART did not worsen anemia in patients with thalassemia [9,10]. Therefore, HIV-1-infected patients with non-iron-deficient microcytic anemia, in whom a hemoglobinopathy is suspected from abnormal hemoglobin fractions should be subjected to gene analysis to make a concrete diagnosis of thalassemia.

Thalassemia is relatively rare in Japan, where malaria is uncommon. The frequency of β-thalassemia in Japan is one in 600 to 1000 of the general population [11].

Most β-thalassemia patients in Japan are heterozygote and present with thalassemia minor. They are prone to be misdiagnosed as having iron deficiency anemia.

The four-nucleotide deletion at codon 41/42 in β globin gene found in this patient is the fourth most frequent mutation found in Japanese β thalassemia patients [11]. It is not known whether active HIV-1-infection (i.e. not controlled by ART) exacerbates all types of β thalassemia, but in the present case there was a strong temporal association between exacerbation of microcytic anemia when HIV-1 infection worsened following cessation of ART, and resolution of the anemia when ART was resumed (Fig. 1). Although most β-thalassemia in Japanese is heterozygous and shows no overt hemolysis but mild anemia with macrocytosis, it is reported that some of the mutant including four-nucleotide deletion at codon 41/42 observed in our case occasionally do have acute exacerbation by acquired factors such as pregnancy and infection [11].

Effect of HIV replication on erythropoiesis is not well understood. The pathogenesis of anemia in HIV-positive patients could be multifactorial [2]. Dysfunction of erythroid differentiation related to bone marrow (BM) microenvironment damage and stromal cell impairment by HIV-1 infection is reported [12]. It is also reported that IL-1β, IFN-γ, TGFβ1 and TNFα, which are elevated in BM as a result of chronic inflammation that may be associated with HIV-1 viremia, suppress the growth of progenitor cell *in vitro* and may play an important role in the induction of HIV-associated anemia [13]. Moreover, unbalanced hemoglobin chain synthesis during HIV-1 infection has been reported [14]. These multiple factors may be involved in the temporal correlation between HIV-1 viremia and exacerbation of microcytic anemia observed in the present case.

In conclusion, thalassemia should be considered in the differential diagnosis in an HIV-1-infected patient who presents with microcytic anemia without iron deficiency.

And early introduction of anti-retroviral therapy is beneficial for the recovery of anemia in β thalassemia.

Conflict of interest

The authors declare that there have no conflict of interest.

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研究ノート

HIV母子感染児の告知支援に関する解析と対策の評価

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目的：HIV母子感染児の病名告知では，心理的問題，家族関係や学校生活への影響など多様な問題が発生する。本研究では，医療者向けの総論的パンフレットを作成し，感染児への病名告知における支援環境の整備を図る。

対象および方法：①HIV母子感染の現状，②中学・高校生におけるHIV感染症に関する認識の調査・分析を行い，感染児，家族，医療関係者に対応可能なパンフレットの項目の抽出と検討を行った。作成したパンフレットは，感染児支援や研究にあたる医療関係者等に評価を求め妥当性を検証した。

結果：医療関係者や家族では，子どもの告知に対する迷いや見解の多様性が認められた。HIV感染児の告知後反応は，落ち着いた反応から抑うつ的なものまで多様であった。中学・高校生のアンケート結果では，HIV感染症の認識にてネガティブな見解や知識の混乱が見られた。HIV母子感染に関する研究者や医療者による告知支援パンフレット（『この子の明日の健康のために子どものHIV感染について告知と支援を考える』）の評価は8割以上が肯定的であり，自由記述では具体的な事例や文言を含めた資材作成の要望が見られた。

結論：今後はモデル事例と具体的チェック項目を記載した実践編（事例編）の作成が必要と思われる。中学・高校生のアンケート結果から，感染児の生活環境調整・心理社会的基盤作りとしても，子どもへのHIV感染症の予防啓発活動は重要であると考える。

キーワード：HIV母子感染，感染児，告知支援，パンフレット

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序　　文

日本では，平成23年12月までに妊娠転帰が明らかなHIV感染妊娠は777例で出生児は518例である。このうち母子感染報告は52例であり，近年でも少数例ではあるが報告が続いている[1)]。一方，HIVに感染した妊婦から出生し，感染が判明している子ども（以下感染児）の最多年齢は16歳（9人/52人）であり，全感染児の75％が11～19歳の10代が占めている[1)]。また，感染児の服薬状況は，cARTの副作用が20例中6例認められ，アドヒアランス維持が困難な例は中断例を含めると45％に上っている[2)]。

アドヒアランス維持はHIV診療において最も重要な課題のひとつである。WHOはその手段として「GUIDELINE ON HIV DISCLOUSURE COUNSELLING FOR CHILDREN UP TO 12 YEARS OF AGE」において子どもの理解力に応じて段階的に病名を説明し，学齢期（6～12歳）にはHIVという病名を告知することを推奨している[3)]。しかし日本における感染児への病名告知は，平成19年度の調査報告によると追跡可能な感染児23例中7例の実施に留まっている[2)]。また，日本には感染児の告知に関する独自のガイドラインが存在しないため，感染児への病名告知では医療関係者や家族が病気や感染経路の説明，治療アドヒアランスの確保，家族関係への影響の緩和や心理的反応への支援等といった問題に手探りで個別に対応している現状がある。感染児の最多年齢である16歳は，第二次性徴，性活動も活発な時期と重なるため，アドヒアランス維持と感染拡大防止の観点から告知の必要性が増している。しかし，この年代は心身の成長の個別性が大きく，情緒的に不安定になりやすい時期であるため，実際の告知では，医療者や保護者が告知のタイミングや対応に悩んでいる[2)]。また，HIV感染症に関する子どもの学習は，新学習指導要領において中学3年生からの取扱いであり[4)]，提供される授業時間数は限られ，正しい知識を得る機会は少ない。そのような状況で告知を行ったケースのなかでは，感染児に抑うつ状態，内向的・逃避的状態など予期せぬ反応が見られた。告知した教育施設からは受け入れ困難に加え登校制限

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を受けたという報告があり，問題は山積している[2]。未告知の場合，感染児の飲み疲れやアドヒアランス不良に関して感染児と医療者や保護者が服薬および治療の意義を共有しにくいため，その支援は容易ではない[2]。そのため，告知経験のある医療関係者等からは，思春期の子どもの心理的特徴を考慮し，子ども自身の傾向や生活環境のアセスメント，家族・学校関係者・地域の支援者を含めた支援体制の構築が求められている[2,5]。

本研究では，感染児への告知支援として，1）医療関係者等が活用できるパンフレット（『この子の明日の健康のために子どものHIV感染について告知と支援を考える』）を作成し，パンフレットの有用性に関して専門家に評価を求め，検討を加えることを目的とする。

方　　法

パンフレットの内容の決定と作成したパンフレットの評価を以下の手順で行った。

1. パンフレットの項目の抽出

1-1. HIV母子感染の現状

HIV母子感染の疫学ならびに告知の現状について，日本エイズ学会誌，厚生労働科学研究費補助金エイズ対策研究事業（母子・周産期領域）の報告書の分析を行った。さらに，HIV母子感染に関する診療または研究経験がある医療関係者等を対象に，平成16年～24年，断続的に感染児に対する病名告知に関する聞き取り調査を行い，HIV母子感染児への病名告知の実態把握を行った。

1-2. 中学・高校生世代のHIV感染症に対する認識の現状

感染児に直接HIV感染症に関する認識を尋ねることは，その行為が病気を伝える可能性と成り得ることを否定できず，その後の告知時に，子どもが事前の質問をどのように受け取るか不明なため実施は難しい。そのため，感染児の最多年齢世代である中学・高校生に対してHIV/AIDSに関する認識調査を行い，その世代の疾患に対する認識を基にパンフレット項目を抽出する方法をとった。

分析対象：2004年1月～2008年12月に実施した性教育講演会の受講者943名（中学3年生188名，高校1年生～3年生758名）を対象に，無記名自記式質問用紙を用いた集団アンケート（図1）。有効回答数は912（有効回答率96.7%）。統計手法は，χ^2検定を用い，$p<0.05$を統計的に有意であると判断した。

2. パンフレットの評価

調査対象者にパンフレットを配布し，自記式アンケートを行い，結果を分析した。

(1) 調査対象：当研究班協力経験がある医療関係者，感染妊婦への対応経験がある医療関係者（産科・内科等）20名。

(2) 調査内容：①HIV感染児への病名告知（支援）経験有無，②パンフレットの評価（評価項目）見やすさ，わかりやすさ，分量・サイズ，有用性，情報の適切さ，参考度，③自由記載。

(3) 調査期間：平成24年7～11月。

結　　果

1. パンフレットの項目の抽出

1-1. HIV母子感染の現状

先行研究のうち，HIV母子感染児の現状の詳細が報告されていたのは平成19年度HIV母子感染全国調査研究報告書[2]と尾崎ら(2008)[5]の報告であった。報告[2,5]では，小児科調査研究班に報告された感染児は累積44例であり，そのうち17例が追跡不可能（11例死亡，6例不明・帰国），27例が追跡可能症例と考えられた。このうち調査の同意が得られたのは23例であった。この時点の感染児の臨床区分は無症状が20例，CD4値500/μL以上13例，Log_{10} HIV-RNAコピー/mLが2未満13例，3未満6例であり，半数以上がウイルス学的にコントロールされていた。HAARTについては，17例がアドヒアランス良好であったが，6例に副作用が出現しており，5例に耐性が発生していた。アドヒアランスは3例で90%以下が確認され，4例に治療中断が見られた。理由としては飲み疲れ（2例），親の非協力（1例），本人の反発（1例）があげられた。病名告知は，7例実施されており，7～16歳の幅があった。感染児の反応としては，「ショックが大きかった」，「抑うつ状態になった」，「落ち着いていた」とさまざまであった。低年齢では「理解できていないようであった」との報告も見られた。感染児の周囲への病名告知例が6例あり，学校以外の施設では保健所，児童相談所，教育委員会があげられていた。告知を受けた施設の反応について4例から回答が得られていた。告知を受けた施設の反応は，3例が問題なし，あるいは協力的，1例が動揺していた。動揺していた1例では，病名告知後の教育機関側の受容が困難となり，登校制限が起こったことが報告された。周囲に対する告知が適切であったかどうかは，1例が適切で，2例はどちらともいえない，1例は早かった（不適切）との回答であった。主治医が考える告知時期は思春期（中学・高校生）が多く，HAART導入時期や家族が希望した時期もあげられていた。支援体制では，カウンセリングは14例に実施されており，利用されている社会資源としてカウンセラー（臨床心理士）以外にも保健師，児童相談所，MSW，通訳，ピアカウンセラーなど多岐にわたる支援体制が活用されていた。

一方，平成16年以降実施している医療関係者（厚生労働科学研究費補助金エイズ対策研究事業研究班協力者で感染児の告知に関与経験がある者）への告知に関する聞き取

エイズ教育講演事前アンケート

学校　　1年　2年　3年　　男子　女子

◆ 以下の質問について、当てはまるものに○をつけて下さい ◆

1. 「HIV/エイズ」という病気を知っていますか
①詳しく知っている
②だいたい知っている
③少しだけ知っている
④知らない

2. 「HIV/エイズ」の情報は何で知りましたか？
当てはまるもの全てに○をつけて下さい。
①学校の授業
②テレビ
③漫画や雑誌
④健康や医学に関する本
⑤先輩や友達
⑥家族
⑦その他

3. 「エイズ」と「HIV」の違いがわかりますか
①わかる
②わからない

4. 「エイズ/HIV」の検査はどこでできるか知っていますか？
①知っている → どこ？（　　）
②知らない

5. 「エイズ/HIV」はどのような場合にうつると思いますか？当てはまるもの全てに○をつけて下さい。
①同じ風呂に入る
②血液を輸血する
③（コンドームなしで）性行為をする
④コップの回しのみをする
⑤握手をする
⑥針（注射）のまわし打ち
⑦同じ蚊にさされる

6. 今あなたの周りにエイズ患者（HIV感染者）がいたら、どのように接しますか？
①自然に普通に接すると思う
②あまり親しくは付き合わないようにする
③その人と話をしないようにする
④近寄らないようにする
⑤全く付き合いを止める
⑥その他（　　）

7. 学校で「エイズ」の学習をすることは必要だと思いますか？
①絶対に必要だと思う
②内容によっては必要だと思う（例：　　）
③必要ではないと思う
④わからない

8. 日本では今「エイズ患者（HIV感染者）」は？
①増えていると思う
②減っていると思う

9. 「エイズ」や「HIV」はあなたにとって
①関係があると思う
②関係がないと思う
③わからない

10. 「エイズ」「HIV」について講演で聞いてみたいこと、知りたい事があったら書いて下さい

図 1　アンケート用紙

りでは，以下に示す告知前後の準備や工夫，保護者が治療に与える影響が報告された。

1)　告知以前に保護者が治療に与える影響

・子どもは親の影響を受けやすい印象。親は病気や服薬の意義を理解する際のモデル。

・保護者のアドヒアランスがよいと子どものアドヒアランスがよい。

・ART中断例では，保護者がその重要性を理解せず，感染児に適切な医療情報を提供しなかったため，感染児が飲む意義がわからなくなり，治療中断に至った。

・保護者が治療の重要性を認識しアドヒアランス遵守を厳しくしても，感染を知らない他の身内が理解できず，対応に一貫性を欠き，保護者も感染児もそれぞれに悩んでいた。

2)　告知に関する医療者・保護者の不安

・ARTは始めている。いずれ告知するときにどう対応したらいいのか不安である。

・告知をするときに亡くなった親の説明や感染経路の説