Frequencies of ABCB1 and CYP gene polymorphisms. The *ABCB1*-3435-T allelic frequency was 0.35 in the whole cohort; however, the frequencies were less common in Black, Non-Hispanic (P = 0.006). Similarly, there were significant differences in the frequencies of *ABCB1*-G2677T (P < 0.001) and *ABCB1*-C1236T genotypes (P < 0.001) in Black, Non-Hispanic, compared to others. The *CYP2C19*-681-A allelic frequency was 0.19 in whole cohort and the frequencies were similar among race/ethnicity (P = 0.35) (**Table**). All patients had the *CYP2C19*-636-G/G genotype.

The ABCB1-3435C>T genotype is associated with NFV CL/F. NFV CL/F differed significantly among the *ABCB1*-3435C>T genotype (P < 0.001); children with the *ABCB1*-3435-C/C genotypes had higher median NFV CL/F [47.2 L/h/m^2, interquartile range (IQR): 32.7-68.7 L/h/m^2] compared to those with the C/T (36.1 L/h/m^2, IQR: 28.1-56.7 L/h/m^2) and the T/T genotype (35.4 L/h/m^2, IQR: 17.8-61.3 L/h/m^2). NFV CL/F did not differ among subjects with the other *ABCB1* genotype (P > 0.17). There was no difference in the association when examined by each race/ethnic group (P > 0.11).

The CYP2C19-681G>A polymorphism is associated with NFV CL/F. NFV CL/F differed significantly among the *CYP2C19*-681G>A genotypes (P < 0.001). When the data were analyzed in each race/ethnicity (**Figure**), a significant difference in NFV CL/F was observed in Black, non-Hispanics (P < 0.001). The same trends were also observed

for Hispanics (P = 0.12) and White, non-Hispanics (P = 0.25), but the differences did not achieve the level of significance.

Association between the CYP2C19-681G>A genotype and the M8: NFV ratio. Overall, the median M8: NFV ratio was associated with the *CYP2C19*-681G>A genotype (P < 0.001); the ratio was 0.45 (IQR: 0.22-0.96) in those with the -G/G genotype compared to 0.26 (IQR: 0.13-0.47) in -G/A or 0.02 (IQR: 0.01-0.08) for the -A/A genotype. The association between the *CYP2C9*-681G>A genotype was particularly strong for the Black, non Hispanic group (P < 0.001), but not for the Hispanic (P = 0.56), and White, non-Hispanic (P = 0.30) groups. No other genotypes were associated with the M8: NFV ratio (P = 0.29-0.87).

Virologic and immunologic responses during HAART in children with ABCB1 and CYP genotypes. The percentages in children who reached plasma HIV-RNA <400 copies/mL at week 12 did not differ by the *CYP2C19*-681G>A genotypes (P = 0.14-1.00). However, at week 24 the percentage of subjects among the *CYP2C19*-681G>A genotype who reached plasma HIV-RNA <400 copies/mL differed significantly (P = 0.01): 46% of subjects with the *CYP2C19*-681-G/G genotype achieved virologic suppression compared to 69% of those with the –G/A genotype, and 63% of those with the –A/A genotype. When examined by race/ethnicity, these differences were observed for the Black, non-

Hispanic group (P = 0.02) and the White, non-Hispanic group (P = 0.03), but not for Hispanics (P = 0.84). No differences were observed when the data were analyzed with the *ABCB1*-3435C>T genotype (P = 0.06), or the *CYP3A4*-392A>G genotype at week 24 (P = 0.26). Regarding immunologic recovery, changes in $CD4^+$ T-cell percentage from baseline to weeks 12 and 24 were not different among the three genotypes in *CYP2C19*-681G>A (P = 0.50, P = 0.44, respectively), or *ABCB1*-3435C>T (P = 0.08, P = 0.21, respectively).

Other factors contributing to NFV CL/F.

Concomitant antiretrovirals. Because nevirapine induces hepatic CYP3A and decreases the levels of PIs[12] and ritonavir acts as a potent pharmacokinetic enhancer for CYP substrates,[13] we evaluated the association between NFV CL/F and concomitant use of nevirapine or ritonavir. NFV CL/F was not different between subjects who received nevirapine and those who did not receive nevirapine (P = 0.70). In contrast, ritonavir use decreased NFV CL/F significantly (P = 0.002); the median NFV CL/F in patients who received ritonavir (35.8 L/h/m^2, IQR: 24.7-47.5 L/h/m^2) was lower compared to those who did not receive ritonavir (47.4 L/h/m^2, IQR: 32.5-70.6 L/h/m^2).

Association of race/ethnicity on NFV CL/F and clinical outcomes

Because race/ethnicity is an important determinant of these SNPs, we also analyzed the data based on their race/ethnicity. Black, non-Hispanics (43.4 L/h/m^2, IQR: 33.1-66.6 L/h/m^2) and Hispanics (45.2 L/h/m^2, IQR: 26.2-65.2 L/h/m^2) had higher median NFV CL/F compare to White, non-Hispanics (31.7 L/h/m^2, IQR: 27.3-53.3 L/h/m^2), but it did not reach a statistical significance (P = 0.09). M8: NFV ratio was not associated with race/ethnicity (P = 0.67). Furthermore, clinical outcomes including percentages in children who reached plasma HIV-RNA <400 copies/mL at week 12 (P = 0.54) or changes in $CD4^+$ T-cell percentage from baseline to weeks 12 (P = 0.89) was not associated with race/ethnicity.

A Multivariate analysis for predicting NFV CL/F. A multivariate analysis showed that the *CYP2C19*-681G>A variants (P < 0.001), concomitant use of ritonavir (P < 0.001), and age (P = 0.03) were independently associated with NFV CL/F. However, the *ABCB1*-3435 variants (P = 0.61), *CYP3A4*-392 homozygous variants (P = 0.42), and race/ethnicity (Black, non-Hispanics) (P = 0.07) were no longer statistically significant. Thus, the *CYP2C19*-681G>A genotype remains an important pharmacogenetic determinant of NFV CL/F even after controlling for other factors.

DISCUSSION

The data presented here demonstrate that *CYP2C19*-681G>A variants exert the greatest impact on NFV PK and virologic response. Controlling for various factors, only *CYP2C19*-681G>A genotype and concomitant PI usage continued to demonstrate a highly significant association with NFV CL/F.

Hepatic CYP2C19 is the critical enzyme responsible for conversion of NFV to its M8 metabolite.[14] Alteration in *CYP2C19*-681G>A in exon 5, which creates an aberrant splice site resulting in a truncated, non-functional protein,[15] is the SNP identified in *CYP2C19* most often associated with different clinical responses to pharmacologic agents for treating diseases (e.g. treatment of peptic ulcer disease using proton pump inhibitors).[16] Previous reports have described the impact of this genotype on the NFV: M8 ratio in HIV-1 infected adult populations.[5, 17, 18] Notably, Haas *et al.* reported a trend toward decreased virologic failure associated with the *CYP2C19*-681G>A genotype.[5] Our current data in children is in agreement with the Haas study, and provide further support for an important role of *CYP2C19*-681G>A variants in NFV PK and virologic response. We cannot rule out that other *CYP2C19* SNPs might also alter the PK of NFV or other PIs.[19] In addition, we only investigated seven SNPs which have been reported to be related to NFV PK. Furthermore, when we analyzed the data by each race/ethnicity, significant differences in NFV PK were only observed in Black, non-Hispanics, and

virologic response in Black, non-Hispanics and White, non-Hispanics. These apparent differences are likely related, in part, to the lower number of study participants in the White, non-Hispanic and Hispanic cohorts.

NFV was used extensively in years past, but is now rarely used in clinical practice for children in developed countries for a few reasons; NFV has been replaced by more potent PIs (e.g. lopinavir/ritonavir) with better PK profile and fewer incidences of adverse effects (e.g. diarrhea) and recent manufacture problem with a contamination of ethyl methylate.[20] However, NFV continues to be used in developing countries. The information learned in this current study may be helpful to improve the clinical outcomes of children who receive NFV.

In conclusion, the *CYP2C19*-681G>A, age, and concomitant ritonavir are significantly associated with NFV PK in HIV-1 infected children. In addition, favorable virologic response was observed in children with the *CYP2C19*-681G>A variants associated with lower oral NFV CL/F. These findings suggest that *CYP2C19*-681G>A is the most important pharmacogenetic determinant for NFV PK and virologic responses in children who receive HAART containing NFV.

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Table. Baseline characteristics of the 152 children in PACTG 366 and 377 with the *CYP2C19*-G681A genotypes

	All subjects*				CYP2C19-G681A			
		PACTG 366	PACTG 377		G/G	G/A	A/A	
	n = 152, (%)	n = 75 (49)	n = 77 (51)	P-value	n = 102, (%)	n = 42, (%)	n = 8, (%)	P-value
Sex, n (%)								
Male	79 (52)	46 (61)	33 (43)	0.01	53 (67)	22 (28)	4 (5)	0.99
Female	73 (48)	29 (39)	44 (57)		49 (67)	20 (27)	4 (6)	
Race/Ethnicity								
Black, non-Hispanic	92 ()	45 (60)	47 (61)	0.73	58 (63)	28 (30)	6 (7)	0.35
Hispanic	32 ()	15 (20)	17 (22)		24 (75)	8 (25)	0 (0)	
White, non-Hispanic	24 ()	12 (16)	12 (16)		18 (75)	5 (21)	1 (4)	
Others	4 ()	3 (4)	1 (1)		2 (50.0)	1 (25.0)	1 (25.0)	
Age (years) [median, (IQR)]	7.1 (3.8-9.6)	6.9 (3.2-10.6)	6.8 (4.4-9.2)	0.42	7.1 (3.5-10.1)	7.1 (3.9-9.4)	7.9 (5.9-10.7)	0.82
Concomitant PI or NNRTI								
No	23 (15)	0	23 (30)		12 (52)	9 (39)	2 (9)	
Ritonavir	40 (26)	40 (53)	0		29 (73)	9 (23)	2 (5)	0.63
Nevirapine	60 (40)	6 (8)	54 (70)		43 (72)	14 (23)	3 (5)	
Ritonavir + Nevirapine	29 (19)	29 (39)	0		18 (62)	10 (35)	1 (3)	
Baseline CD4+ (%) median, (IQR)	24 (16-32)	19 (10-28)	27 (21-35)	< 0.001	23 (16-32)	25 (19-31)	22 (6-40)	0.85
Mean HIV-1 RNA $\log_{10}$ copies/mL (SD)	4.56 (0.68)	4.71 (0.61)	4.41 (0.72)	0.003	4.54 (0.70)	4.62 (0.64)	4.44 (0.74)	0.65

* The subjects were selected from the whole study populations if they satisfied the following criteria: i) received NFV as a component of HAART for >24 weeks with reported excellent compliance to their treatment regimen; ii) virologic and immunologic data were available at baseline, weeks 12 and 24; and iii) PK data for NFV were available at week 4. *ABCB1*: ATP-binding cassette, sub-family B, member 1, CYP: cytochrome P450, IQR: interquartile range, PI: protease inhibitor, NNRTI: non-nucleoside reverse transcriptase inhibitor

Figure *CYP2C19*-681G>A

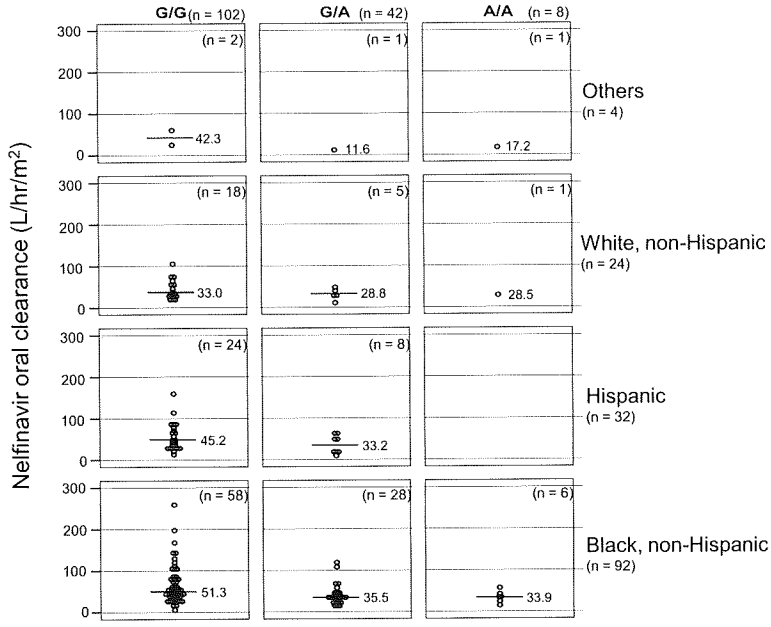

Figure. Oral clearance rate (CL/F, L/h/m^2) for nelfinavir in children with the *CYP2C19*-681G>A genotypes in each race/ethnicity. Each circle represents nelfinavir CL/F in each subject with the *CYP2C19*-681-G/G (left), -G/A (heterozygous, middle), and –A/A (homozygous, right) in each race/ethnicity. The lines in the middle represent the median of CL/F for nelfinavir.

A Human Immunodeficiency Virus Screening Algorithm to Address the High Rate of False-Positive Results in Pregnant Women in Japan

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Abstract

Background: Prenatal human immunodeficiency virus (HIV) testing is essential for the prevention of mother-to-child transmission. However, false-positive results of screening testing are a concern as they may cause unnecessary emotional stress to pregnant women waiting for confirmatory test results. In regions with an extremely low prevalence, the positive predictive values of screening are unacceptably low rate. Here, we propose a HIV screening algorithm consisting of serial two fourth-generation enzyme immunoassays to reduce the number of false-positive screening results.

Methodology/Principal Findings: When 6461 pregnant women presenting to two maternity hospitals located in the Tokyo metropolitan area of Japan from September, 2004 to January, 2006 were tested using Enzygnost HIV Integral as a first screening test, 27 showed positive reactions. When these positive reaction samples were tested using VIDAS HIV DUO Quick as a second screening test, only one of them had a positive reaction, and the remaining 26 were nonreactive. Confirmatory Western blots and nucleic acid amplification test also showed that one was positive and the remaining 26 were negative; the subject who was positive with the confirmatory tests was identical to the subject who was positive with the second screening test. Thus, by adding the second screening test, the false-positive rate was improved from 0.4% to 0%, and the positive predictive value from 3.7% to 100%, compared with the single screening test.

Conclusion: By applying our serial screening algorithm to HIV testing in maternity hospitals, many uninfected pregnant women would not need to receive confirmatory tests and be subjected to emotional turmoil while waiting for their confirmatory test results. This algorithm would be suitable for HIV testing of pregnant women living in low prevalence regions such as Japan.

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Introduction

The human immunodeficiency virus (HIV) epidemic in Japan is still at a low level compared with other developed countries, but the number of newly identified infections is increasing every year. For earlier detection and clinical and preventive services, much effort is made to implement voluntary HIV counseling and testing in a variety of health-care settings including public health centers, STD clinics, and outreach medical services. According to the report of the National AIDS Surveillance Committee, 13,894 persons with HIV/AIDS were reported between 1985 and 2007, and 1,500 new cases were reported in 2007 alone [1]. The HIV prevalence in Japan was estimated at 0.008% in 2007 [2]. Of all the HIV-infected persons reported in Japan, 71.0% were Japanese men; 11.4% were non-Japanese men; 6.2% were Japanese women; and 11.4% were non-Japanese women. Currently, about 70% of the Japanese men with HIV infection are men who have sex with men.

Although the HIV prevalence in women is very low in Japan (about 0.004%), universal HIV testing has been performed for pregnant women to prevent mother-to-child transmission since 1999

[3]. Nationwide questionnaire surveys on HIV testing in pregnant women are conducted every year. The HIV testing rate has gradually increased from 73.2% in 1999 to 97.2% in 2007. Over the 21 years between 1987 and 2007, mother-to-child transmission has occurred in only one in 219 (0.5%) HIV-infected pregnant women who received both antiretroviral therapy (ART) and a cesarean section, one in 17 (6%) women who had a cesarean section without ART, and 14 in 36 (39%) women who delivered vaginally [3].

Although prenatal HIV testing is essential for the prevention of mother-to-child transmission, there are concerns about false-positive results of screening tests [4,5]. Positive test results may cause anxiety of HIV infection and emotional stress in pregnant women waiting for confirmatory test results. Some severe cases were covered by the mass media in 2007, leading to an official notification on the frequent observation of HIV false-positive screening results from the Ministry of Health, Labour and Welfare of Japan [6].

There has been little study on the rate of false-positive results in HIV screening testing of pregnant women in Japan. Thus, we conducted a prospective study at two maternity hospitals in the Tokyo metropolitan area to evaluate the performance of screening test, including the prevalence, false-positive rate, and positive predictive value, and proposed a new HIV screening algorithm composed of two serial tests to enable a substantial reduction in the number of false-positive results at this stage.

Materials and Methods

Study Setting

The study was conducted from September, 2004 to January, 2006 in two maternity hospitals located in the Tokyo metropolitan area. Each of the hospitals conducts more than 1,000 deliveries each year.

HIV Testing

Blood samples were initially tested using Enzygnost HIV Integral (Siemens Healthcare Diagnostics, Deerfield, Illinois, USA), a fourth-generation enzyme-linked immunosorbent assay with the ability to detect HIV-1 gp41 antibody, HIV-2 gp36 antibody, and HIV-1 p24 antigen at a reference laboratory of the Health Science Research Institute Inc. (Yokohama, Japan). The Enzygnost HIV Integral can test 880 samples during each run lasting 240 min. The samples that tested positive in the initial screening were subjected to a secondary screening test and confirmatory tests, which were conducted at the Kanagawa Prefectural Institute of Public Health. The second screening test was performed using VIDAS HIV DUO Quick (bioMérieux, Marcy l'Etoile, France), a fourth-generation enzyme-linked fluorescent assay with the ability to detect HIV-1 gp160 antibody, HIV-2 gp36 antibody, and HIV-1 p24 antigen. The VIDAS HIV DUO Quick can test 60 samples during each run lasting 80 min. Confirmatory tests were performed using Western blot tests (Lab blot 1 and Lab blot 2; Bio-Rad Laboratories, Hercules, California, USA), and a nucleic acid amplification test (NAT), Amplicor HIV-1 Monitor test version 1.5 (Roche Molecular Systems, Branchburg, New Jersey, USA). HIV typing was performed using SERODIA•HIV-1/2 PA (Fujirebio, Tokyo, Japan). All the tests were conducted and interpreted as recommended by the manufacturers.

Samples for Evaluating the Sensitivity of the Screening Tests

Ten HIV-1 seroconversion panels (PRB 936, 937, 938, 939(E), 945, 951, 952, 953, 954, and 955) and two samples from HIV p24 Antigen Mixed Titer Performance panels (PRA 201-05 and 201-17) were obtained from SeraCare Life Sciences (formerly Boston Biomedica, West Bridgewater, Massachusetts, USA). Seroconversion panels were used to evaluate the sensitivity in the early phase of infection. Three samples (PRB 936-04, PRA 201-05, and PRA 201-17) were diluted twofold serially with HIV negative human pooled plasma and were used to evaluate the antigen detection sensitivity of the enzyme immunoassay (EIA).

Screening Algorithm

We proposed an algorithm (Fig. 1) to reduce the number of false-positive screening results in prenatal HIV testing. In our algorithm, each blood sample is tested serially with two EIA tests that should be highly sensitive and have different detection formats. The first screening test was performed on the day or next day of blood sampling during the first trimester, and the second screening test was done as soon as possible after the sample in the first test was found to be positive. If the result of the first screening test is negative, the test is reported as negative; if a result of the first screening test is positive, the same sample is tested using the second screening test. If the result of the second test is negative, the test is reported as negative; if the result of the second test is positive, confirmatory tests using Western blots and NAT are conducted.

Statistical Analysis

Specificity was calculated using a combination of Western blots and NAT as the gold standard. Confidence intervals (CIs) were estimated using approximation to the normal distribution.

Research Ethics

This study was jointly approved by the ethics committees at the two maternity hospitals and the Kanagawa Prefectural Institute of Public Health. The verbal informed consent for study participation including screening and confirmatory tests was obtained from study participants and recorded by the physician on a separate study-participation sheet. As blood samples used in this study had been collected as routine tests and thus no additional invasive action was required for participants, the committees approved this procedure according to the Ethical Guideline of the Ministry of Health, Labour and Welfare of Japan. All links between the test results and personal identifiers were removed and were known only to the physicians in charge of the subjects.

Results

Comparative Assays

The sensitivities of the Enzygnost HIV Integral and VIDAS HIV DUO Quick in the early phase of infection were compared using 10 HIV-1 seroconversion panels. HIV infection was detected with the VIDAS HIV DUO Quick earlier than with the Enzygnost HIV Integral in eight out of ten panels; the interval was an average of 4.5 days (Table 1). Next, the antigen detection sensitivities of the two tests were compared using serial twofold dilutions of three HIV-1 antigen samples (PRB936-04, PRA 201-05, and PRA201-17). The VIDAS HIV DUO Quick was 16–32 times more sensitive than the Enzygnost HIV Integral (Table 2).

Results of HIV Testing

Of the 6,461 study participants, 27 (0.42%) showed positive results for the first screening test performed using the Enzygnost HIV Integral. When the positive samples were tested with the second screening test performed using the VIDAS HIV DUO Quick, only one sample exhibited a positive reaction and the remaining 26 samples were nonreactive. When the samples that tested positive in the first screening were tested using confirmatory Western blots and NAT, only one sample was positive and the

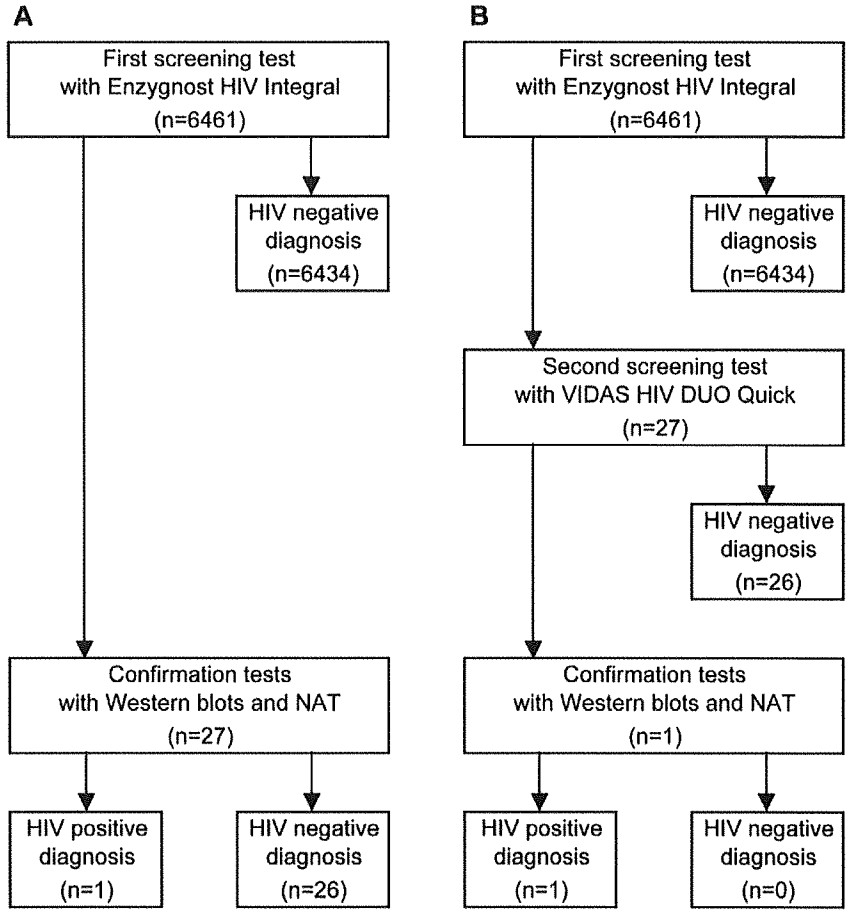

Figure 1. Comparison of the results obtained by two HIV testing algorithms. A, algorithm containing single test screening. **B**, algorithm containing serial two-test screening.
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Table 1. Test performance to detect bleed day with first positive result using seroconversion panel members.

Panel	Bleed day with first positive result		Difference in bleed days between the two tests
	Enzygnost HIV integral	VIDAS HIV DUO Quick	
PRB936	12	12	0
PRB937	21	14	7
PRB938	3	0	3
PRB939(E)	21	16	5
PRB945	13	13	0
PRB951	11	8	3
PRB952	17	10	7
PRB953	10	3	7
PRB954	21	17	4
PRB955	12	3	9
Average			4.5

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other 26 samples were negative (Fig. 1). The subject whose confirmatory test results were positive was the same as the one whose second screening test result was positive. The sample from this subject was positive with an HIV-1 Western blot, indeterminate with an HIV-2 Western blot, and HIV-1 positive on HIV typing. As for the signal-to-cutoff (S/CO) ratio on the Enzygnost HIV Integral used as the first screening test, the one positive sample was 6.47; of 26 false-positive samples, two were $\geq$6.0, four were 2.0–6.0, and 20 were $<$2.0. Western blots of the 26 negative samples showed that one was indeterminate with both HIV-1 and HIV-2 Western blots (S/CO ratio, 1.24), two were indeterminate with only HIV-1 Western blots (0.92 and 5.87), and one was indeterminate with only HIV-2 Western blots (6.34): all were negative on HIV typing.

In the standard protocol using a single screening test, the false-positive rate was 0.40% (95% CI, 0.25–0.56%), and the positive predictive value was 3.7%. However, when an additional screening test was introduced, the overall specificity of the screening was improved dramatically, and the above values were changed to 0% and 100%, respectively.

Discussion

According to the guideline for prevention of mother-to-child transmission of HIV in Japan [7], women who are found to be pregnant at hospital are generally tested for HIV during the first

Table 2. Antigen detection limits by antigen-antibody combined detection tests using 3 antigen positive specimens in the panels.

Panel No.	Antigen-antibody combined detection test		1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
PRB936-04	VIDAS HIV DUO Quick	TV[1]	9.84	5.55	3.01	1.53	0.89	0.47	0.32	0.21
		Result	POS	POS	POS	POS	POS	POS	POS	NEG
	Enzygnost HIV Integral	S/CO[2]	4.52	2.32	0.90	0.64	0.38	0.21	NT[3]	NT
		Result	POS	POS	IND	NEG	NEG	NEG	NT	NT
PRA201-05	VIDAS HIV DUO Quick	TV	3.27	1.69	0.98	0.56	0.31	0.23	0.15	0.13
		Result	POS	POS	POS	POS	POS	NEG	NEG	NEG
	Enzygnost HIV Integral	S/CO	1.58	0.63	0.34	0.19	NT	NT	NT	NT
		Result	POS	NEG	NEG	NEG	NT	NT	NT	NT
PRA201-17	VIDAS HIV DUO Quick	TV	2.98	1.58	0.84	0.45	0.28	0.22	0.17	NT
		Result	POS	POS	POS	POS	POS	NEG	NEG	NT
	Enzygnost HIV Integral	S/CO	1.77	0.78	0.36	0.19	NT	NT	NT	NT
		Result	POS	NEG	NEG	NEG	NT	NT	NT	NT

POS, positive; NEG, negative; IND, indeterminate.
[1]TV, test value. TV$<$0.25 was judged as negative, and TV$\geq$0.25 was judged as positive.
[2]S/CO, signal-to-cutoff ratio.
[3]NT, not tested.
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trimester, and HIV-positive women are treated with antiretroviral therapy from the second trimester and intravenous administration of zidovudine during labor. Babies are treated with zidovudine syrup for 6 weeks after birth. Testing of women in labor is performed with a rapid antibody test, and positive women are regarded as infected with HIV, and zidovudine administration was initiated to the woman and a newborn baby.

Routine HIV testing for pregnant women has been underway since 1999 in Japan. A questionnaire survey conducted in 2003 reported that only 7 out of 82,290 pregnant women were diagnosed as being HIV-1 positive [8]; thus, the prevalence was 0.009%, which is extremely low compared with 0.15%–5% in the United States [9]. This survey also reported that the false-positive rate of screening tests was 0.094%, and that its positive predictive value was 8.3%. These values agree with those obtained in the present study. Since about one million pregnant women are tested each year in Japan, about 1,000 women are probably notified of false-positive results after screening tests.

Identifying such false-positive results using confirmatory testing is not easy. With the introduction of fourth-generation EIA tests for screening, the confirmatory test sequence has become very complicated. Because these EIA tests can detect antibodies against HIV-1 and HIV-2 as well as HIV-1 antigen, the confirmation of positive results requires an HIV-1 Western blot, HIV-2 Western blot, and NAT for HIV-1 RNA. Western blots result in a high percentage of indeterminate results [10]. Furthermore, even if the result of HIV-2 Western blot is negative, HIV-2 infection cannot be denied because the sensitivity of HIV-2 Western blots is lower than those of EIA tests for the detection of HIV-2 antibody. Therefore, even if samples are shown to be negative with any one of the three tests, the subjects should be retested one month later. Consequently, once a pregnant woman is assigned a positive screening test result by a false-positive reaction, she must undergo two rounds of confirmatory tests in one month.

HIV-tested pregnant women have been reported to encounter various problems associated with false-positive screening results [3,9–13]. In Japan, pregnant women who were notified of positive screening results felt strong anxiety and depression while waiting for the results of the confirmatory tests; some of the women became suspicious of their partners, and some considered abortion or divorce [3]. One woman was notified of an HIV positive result by her clinician without receiving sufficient explanation about the screening testing; later, when confirmatory testing showed that she was HIV negative, she became upset and untrusting of medical services [3]. Such emotional disturbances have been reported in other countries [9,10]. However, the situation in Japan may be somewhat different from those in most of developed countries because the HIV prevalence (0.009%) is extremely low. Most obstetricians have never treated an HIV-infected individual and thus have little chance of learning HIV infection and its diagnosis. Therefore, it is the most important to help obstetricians to understand the nature of HIV testing and to provide clients with counseling and information, including the high frequency of false-positive results in screening testing and the necessity of confirmatory testing to obtain a decisive result.

As an alternative approach to resolving these problems from a technical point of view, we proposed a screening algorithm consisting of serial two fourth-generation enzyme immunoassays to reduce the number of false-positive test results. When this algorithm was applied to the 6,461 pregnant women who participated in this study, the specificity of screening was improved from 99.6% to 100% and the positive predictive value was improved from 3.7% to 100%, compared with the standard protocol. Although the two screening tests were conducted at separate reference laboratories in this clinical trial, these tests can be sequentially done at the same place. By applying this algorithm to clinical settings, many uninfected clients would not need to receive confirmatory tests and thus would not be subjected to emotional turmoil while waiting for their confirmatory test results. In addition, because extensive confirmatory tests and repeat visits are not required, it results in cost savings. Although the number of participants in this study is limited, the increase in specificity and positive predictive value can likely be extrapolated to a larger population of pregnant women.

It has been suggested that false positives may be caused by alloantibodies resulting from pregnancy, transfusions, or trans-

plantation [10]. We did not collect other medical conditions of the study participants, which may influence testing results. The false-positive rate of Enzygnost HIV Integral in this study was 0.40%, which is within a range (0.3%–0.8%) of previously reported false-positive rate of this kit [14–16]. Therefore, it is unlikely that the false-positive rate observed in the first screening was influenced by medical conditions including pregnancy and testing factors such as quality control and performance in the reference laboratory.

The proposed protocol is characterized by a specificity-optimized serial two-test algorithm. When the specificity is optimized, a serial testing algorithm and a parallel testing algorithm are equally sensitive and specific. A parallel testing algorithm is time-saving for diagnosis of HIV infection; a serial testing algorithm is cost-effective and less laborious. Because the HIV prevalence was very low in Japan, most of positive results are due to false positives of the tests. Furthermore, simultaneous two screening tests are not covered the public medical insurance. We think that a serial testing is more acceptable in our country.

The order of the two tests could be determined based on several factors including throughput, cost, labor intensity, sensitivity, and specificity. In this study, the VIDAS HIV DUO Quick was used as the second screening test because it is less suitable for large-scale testing and has been shown to be more sensitive in the early phase of infection than the Enzygnost HIV Integral. The latter characteristic may help to reduce the number of false-negative results in the second screening. However, it should be noted that the overall sensitivity and specificity of a serial screening algorithm are determined only by the combination of the two tests and their order is irrelevant.

Inevitably, the sensitivity of the serial screening algorithm is lower than those of the individual tests employed therein, and the specificity is higher. However, if the two tests employed are highly sensitive, the decrease in sensitivity is expected to be marginal and less than the difference among currently available screening tests [17]. On the other hand, the adoption of the algorithm improves the specificity dramatically. Theoretically, the sensitivity of the first test should be as high as possible to ensure the detection of the largest possible number of HIV-positive samples [17,18]. However, recent EIA tests have undergone dramatic improvements, and most fourth-generation tests have achieved nearly 100% sensitivity on HIV-1/2 reference positive samples and are capable of detecting early infection two to seven days after NAT [15,19,20]. The ranking of these tests on seroconversion panels varies among individual panels [15,19,20], and is probably indecisive for field samples. Therefore, as long as two highly sensitive fourth-generation tests are used, which test is more sensitive may not be the primary determinant of the test order.

We should be cautious in applying the proposed screening algorithm to other health-care settings. In clinical settings specializing in HIV infection, clinicians are likely to diagnose their patients with a high sensitivity and specificity. The HIV-1 prevalence in this setting would likely be relatively high, and the clinicians would be well trained with regard to providing counseling and information regarding HIV testing. In such a setting, the present algorithm may not be required. Meanwhile, voluntary HIV counseling and testing have been implemented chiefly in public health centers on a free-of-charge basis, and rapid antibody tests are widely used for screening testing. The sensitivity and specificity of third-generation rapid test were shown to be lower than those of fourth-generation EIA tests [16,17]. In these cases, the introduction of a fourth-generation EIA test as the second screening would not miss true positive samples and would enable a great reduction in false-positive screening samples.

As the performance of HIV diagnostic tools evolve, the diagnostic algorithms should also be changed to be accurate as well as beneficial to the clients, and they need to be developed specifically for individual health-care settings. The screening algorithm presented in this study provides improved specificity and positive predictive value, and cost savings, which is suitable and beneficial for HIV testing in low prevalence settings such as for maternity hospitals in Japan.

Author Contributions

Conceived and designed the experiments: TSS KK SK MI. Performed the experiments: TSS KS KS MK. Analyzed the data: TSS RY YT NI SK MI. Contributed reagents/materials/analysis tools: TSS HH HS YT NI. Wrote the paper: TSS SK MI. Coordinated with the patients, physicians, laboratory stuffs and data analyzer: RWH.

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Progressive Renal Tubular Dysfunction Associated with Long-Term Use of Tenofovir DF

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Abstract

It became evident that tenofovir DF (TDF) causes a modest and gradual decline in GFR, however, the impact of long-term use of TDF on tubular function has not been fully evaluated. In 40 patients treated with TDF and 23 patients treated with other NRTIs, urine β_2-microglobulin (U-BMG), percentage tubular reabsorption of phosphate (%TRP), alkaline phosphatase (ALP), serum creatinine, and calculated GFR were prospectively measured for 96 weeks. In patients receiving TDF, median U-BMG rose from 188 μg/liter at baseline to 555 μg/liter at week 96 ($p=0.02$), median %TRP declined from 94% at baseline to 90% at week 96 ($p=0.002$), median ALP ratio compared with baseline persistently increased from 1 to 1.278 at week 96 ($p=0.001$), and serum creatinine showed significant but minimal change from 0.64 mg/dl to 0.74 mg/dl at week 96 ($p=0.02$). The GFR level declined minimally but significantly in TDF-receiving patients (–17 ml/min/1.73 m^2), whereas it did not change in other NRTI-receiving patients [+3 ml/min/1.73 m^2; mixed models analysis of variance (MMANOVA) $p=0.03$ for overall change from baseline to week 96]. U-BMG, %TRP, ALP, or serum creatinine did not change significantly in other NRTI-receiving patients during the observation period. In five patients with marked changes in U-BMG (>10,000 μg/liter) and %TRP (<80%), both U-BMG and %TRP immediately recovered in all patients after discontinuing TDF, whereas GFR levels did not fully recover for 6 months in three patients. Prolonged treatment with TDF caused progressive renal tubular dysfunction as well as a modest decline in GFR. If U-BMG levels >10,000 μg/liter and %TRP values <80% are observed, discontinuing TDF may be beneficial.

Introduction

Tenofovir disoproxil fumarate (TDF), a nucleotide analogue of adenosine 5′-monophosphate, is one of the most widely used antiretroviral agents for HIV-1-infected patients. Although clinical trials have concluded that TDF-associated renal toxicity is rare and reversible,[1–3] it is evident that long-term administration of TDF causes a gradual decrease in glomerular filtration rate (GFR).[4–7] Furthermore, a growing number of case reports suggested that TDF-associated renal toxicity is mainly caused by proximal tubular injury.[8–11] TDF is excreted via renal proximal tubular transporters.[12,13] Adefovir and cidofovir, both nucleotide analogues, have been reported to cause human renal toxicity via mitochondrial injury in renal tubular epithelial cells.[14] Nevertheless, the impact of the long-term use of TDF on proximal tubular function has not been fully evaluated.

Materials and Methods

This study was conducted prospectively from May 2004 to May 2007. Among 164 HIV-1-infected patients who were registered in Ogikubo Hospital, 110 patients were treated with antiretroviral drugs. Of 110 treated patients, 63 patients who could come to Ogikubo Hospital regularly to have regular blood and urine sampling with informed consents were enrolled in this study. Exclusion criteria were a moderately low level of calculated GFR (<80 ml/min/1.73 m^2). Of 63 enrolled patients, 40 patients were treated with a TDF-based regimen and 23 patients were treated with another NRTI-based regimen. The characteristics of the sample population are shown in Table 1. Of the 40 patients who received TDF, 32 ART-experienced patients simply switched from d4T to TDF to avoid future risk of lipodystrophy or other d4T-related adverse effects. In TDF-receiving patients ($n=40$), combined NRTIs were as follows: 34 patients with lamivudine (3TC) or emtricitabine (FTC), 4 patients with abacavir (ABC), and 2 patients with didanosine (ddI). In other NRTI-receiving patients ($p=23$), combinations of two NRTIs were as follows: 10 patients with zidovudine (ZDV) + 3TC, 10 patients with stavudine (d4T) + 3TC, 2 patients with d4T + ddI, and 1 patient with d4T + ABC. Informed consent was obtained from all enrolled patients.

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TABLE 1. CHARACTERISTICS OF SAMPLE POPULATION

	TDF	*Other NRTI*
Number of patients	40	23
Sex male	40 (100%)	23 (100%)
Median of age (range)	35 (27–66)	32 (22–68)
Median of CD4 (cell/mm^3)	376 (69–1243)	224 (12–748)
Median of HIV RNA (copies/ml)	33 (<50–100,000)	18,000 (<50–100,000)
History of HAART	Naive 8 Experienced 32	Naive 9 Experienced 14
Underlying antiretrovirals		
Efavirenz	14 (35%)	6 (26%)
Nevirapine	3 (8%)	0 (0%)
Atazanavir/ritonavir	16 (40%)	6 (26%)
Lopinavir/ritonavir	5 (13%)	3 (13%)
Nelfinavir	2 (5%)	6 (26%)
Dual therapy	0 (0%)	2 (9%)
Route of HIV-1 infection		
Contaminated blood products	36 (90%)	17 (74%)
Sexual transmission	4 (10%)	6 (26%)
Underlying disease		
Diabetes mellitus	6 (15%)	2 (9%)
Indinavir-associated renal atrophy	2 (5%)	0 (0%)
Pretreatment with indinavir	7 (18%)	7 (0%)

Laboratory testing

Urine β_2-microglobulin (U-BMG), %TRP, alkaline phosphatase (ALP), serum phosphorus, serum uric acid, serum creatinine, and GFR were prospectively measured along with a urinalysis performed every 4–12 weeks, from baseline to 96 weeks, in 40 patients treated with TDF. In 23 patients treated with other NRTIs, serum creatinine, GFR, and ALP were prospectively measured every 3 months, while U-BMG and %TRP were measured every 12 months for 2 years in 17 patients during the same period. U-BMG was determined using a spot urine sample. %TRP was calculated using the following formula: %TRP = [1– (urine phosphorus/serum phosphorus × serum creatinine/urine creatinine)] × 100. Urine phosphorus and urine creatinine were measured on the spot urine sample and serum creatinine and serum phosphorus levels were obtained from blood samples on the same day. GFR was calculated based on the simplified modification of diet in renal disease (MDRD) equation, which is described in the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative.[15] In urinalysis, urine glucose and urine protein were evaluated in accordance with the color chart of the dipsticks (Labsticks; Bayer Medical Corp., CA). Renal tubular epithelial cells were counted with high-power fields (HPF; 400× objective), and granular casts were counted with low-power fields (LPF; 100× objective) from urine sediments.

Statistical analyses

Changes over time within groups were assessed using the Wilcoxon signed rank test, and levels of renal parameters between two groups at week 96 were compared using the Mann–Whitney *U* test. Moreover, mixed models analysis of variance (MMANOVA) was used to assess the overall pattern of changes in GFR from baseline to week 96 in the total sample population.[16] MMANOVA allowing for the influence of TDF/other NRTI assignment, naive/experienced assignment, baseline U-BMG level, and a potential interaction between TDF/other NRTI assignment and naive/experienced assignment was applied to adjust the significance level for the change on GFR. All analyses were performed using SAS release 8.02 (SAS Institute, Cary, NC).

Results

Renal parameters

In TDF-receiving patients, 3/40 (8%) patients discontinued TDF before week 96 due to a progressive decline of GFR and tubular dysfunction, whereas there was no patient who discontinued antiretrovirals in other NRTI-receiving patients.

The median [interquartile range (IQR)] of U-BMG was significantly elevated from 188 (134–359) μg/liter at baseline to 555 (229–1425) μg/liter at week 96 ($p = 0.02$) in TDF-receiving patients (Fig. 1a). Highly elevated U-BMG (>10,000 μg/liter) was observed in 3 of 40 (8%) patients by week 96, and moderately elevated U-BMG (1000–10,000 μg/liter) was observed in 15 of 40 (38%) patients by week 96. In contrast, in other NRTI-receiving patients ($n = 17$), U-BMG did not change significantly during the study period [from 170 (96–217) μg/liter at the initial point of the study to 150 (81–307) μg/liter at the end of the study; $p = 0.37$], and there was no patient with a moderate or marked elevation of U-BMG (≥1000 μg/liter).

The median (IQR) of %TRP showed a significant decline from 94 (92–96)% at baseline to 90 (89–95)% at week 96 in TDF-receiving patients ($p = 0.04$; Fig. 1a), whereas there was no significant decline of %TRP in the other NRTI group [$n = 17$; from 96 (95–97)% at the initial point to 94 (91–96)% at week 96 ($p = 0.33$)]. A marked decline in %TRP (<80%) was observed in 3/40 (8%) patients, and a moderate decline (80–90%) was observed in 18/40 (45%) patients at week 96, whereas there was no patient with a moderate or marked decline (%TRP <90%) in the other NRTI group. Comparing the patients who had mildly decreased %TRP (%TRP <90% on two occasions)

with those with normal %TRP (%TRP ≥90%), the former had significantly higher levels of median serum creatinine [0.79 (0.74–0.91) mg/dl vs. 0.63 (0.56–0.70) mg/dl, $p=0.002$] and significantly lower levels of median GFR [118 (106–136) ml/min/1.73 m^2 vs. 160 (139–177) ml/min/1.73 m^2, $p=0.001$] at week 96.

The median (IQR) of MDRD-GFR declined gradually from 150 (126–165) ml/min/1.73 m^2 at baseline to 136 (116–157) ml/min/1.73 m^2 at week 96 ($p=0.02$) in TDF-receiving patients, whereas it did not change in other NRTI-receiving patients ($n=23$) [from 129 (112–138) ml/min/1.73 m^2 at baseline to 136 (124–145) ml/min/1.73 m^2 at week 96 ($p=0.39$); Fig. 1b]. In using the Cockcroft-Gault equation, the median (IQR) of GFR also declined from 138 (112–155) ml/min/1.73 m^2 at baseline to 127 (105–148) ml/min/1.73 m^2 at week 96 ($p=0.02$), whereas it did not significantly change in other NRTI-receiving patients [from 129 (117–143) ml/min/1.73 m^2 at baseline to 135 (116–155) ml/min/1.73 m^2 at week 96 ($p=0.65$)]. The change in MDRD-GFR over time was reassessed using MMANOVA. In using MMANOVA, GFR declined significantly in TDF-receiving patients (–17 ml/min/1.73 m^2, $p=0.04$), whereas it did not change in other NRTI-receiving patients (+3 ml/min/1.73 m^2, $p=0.43$). The overall difference between the two treatment group was statistically significant (MMANOVA, $p=0.03$). GFR change was not significantly influenced by previous administration of HAART (MMANOVA, $p=0.07$) or baseline U-BMG levels (MMANOVA, $p=0.28$). There was no significant interaction between TDF/other NRTI assignment and naive/experience assignment (MMAOVA, $p=0.73$). The median (IQR) of serum creatinine increased from 0.64 (0.59–0.75) mg/dl at baseline to 0.74 (0.64–0.80) mg/dl at week 96 ($p=0.02$), whereas it did not change in other NRTI-receiving patients [$n=23$; from 0.73 (0.68–0.83) mg/dl at baseline to 0.70 (0.66–0.78) mg/dl at week 96 ($p=0.14$), respectively].

The median (IQR) of ALP persistently and significantly rose during the study period in the TDF group [from 289 (261–382) IU/liter at baseline to 355 (280–421) IU/liter at week 96 ($p=0.001$)], whereas it did not change significantly in other NRTI groups [from 172 (138–250) IU/liter at baseline to 180 (148–247) IU/liter at week 96 ($p=0.98$)]. In comparing the ALP ratio (relative to baseline), the median (IQR) ALP ratio in patients receiving TDF was significantly higher than in patients receiving other NRTIs [1.278 (1.059–1.354) vs. 1.003 (0.876–1.098) ($p=0.02$); Fig. 1c]. Even in patients receiving TDF, serum phosphorus and serum uric acid were not significantly decreased during the study period. The median (IQR) serum phosphorus level was 3.4 (2.9–3.6) mg/dl at baseline and 3.0 (2.7–3.4) mg/dl at week 96 ($p=0.20$), and serum uric acid was 6.1 (5.0–7.0) mg/dl at baseline and 5.5 (4.9–6.6) mg/dl at week 96 ($p=0.08$).

In TDF-receiving patients, a reduction in GFR level was associated with U-BMG levels. GFR significantly decreased in patients with higher U-BMG (≥1000 μg/liter) in two or more occasions from 132 (124–159) ml/min/1.73 m^2 at baseline to 118 (104–151) ml/min/1.73 m^2 at week 96 ($p=0.01$), whereas it did not decrease in the other patients [from 155 (134–172) mg/dl at baseline to 143 (133–164) mg/dl ($p=0.59$)]. In comparing GFR levels at week 96 between the patients with higher U-BMG (≥1000 μg/liter) on two or more occasions and those with lower U-BMG (<1000 μg/liter), the former level was significantly lower than the latter [118 (104–151) ml/min/1.73 m^2 vs. 143 (133–164) ml/min/1.73 m^2 ($p=0.04$)].

In urinalysis, the ratio of the patients with positive urine protein did not significantly increase in both TDF-receiving patients and other NRTI-receiving patients [19% at baseline and 26% at week 96 ($p=0.84$) in TDF-receiving patients and 5% at baseline and 5% at week 96 in other NRTI-receiving patients ($p=0.86$), respectively]. There was no statistical difference in the ratio of positive urine protein at week 96 between the two groups. A ratio of the patients with positive urine glucose did not significantly change in both TDF-receiving patients and other NRTI-receiving patients [19% at baseline and 26% at week 96 in TDF-receiving patients ($p=0.84$) and 5% at baseline and 5% at week 96 in other NRTI-receiving patients, respectively]. Granular cast was observed in 5% at baseline and 6% at week 96 in TDF-receiving patients, and 0% at baseline and 0% at week 96 in other NRTI-receiving patients. There was no significant difference between the two groups at baseline or week 96 ($p=0.77$ and 0.85, respectively). Renal tubular epithelial cells were observed in 17% at baseline and 8% at week 96 in TDF-receiving patients ($p=0.46$), and 5% at baseline and 9% at week 96 in other NRTI-receiving patients. There was no significant difference between the two groups at baseline or week 96 ($p=0.29$ and 0.43, respectively). Among the five TDF-receiving patients with rapid deterioration of U-BMG and %TRP, granular casts were observed in only two patients and renal tubular epithelial cells were observed in three patients.

Severe TDF-associated renal toxicity and its recovery after discontinuation of TDF

In this study, severe renal toxicity was observed in five TDF-receiving patients (Table 2), whereas neither reduction of GFR nor tubular dysfunction was observed in other NRTI-receiving patients. Among these five patients, three patients (Patients 1–3 in Table 2) showed TDF-associated renal toxicity during the study period, and they discontinued TDF. The other two patients had acute renal failure after the study period (Patients 4 and 5). An extremely abnormal value of U-BMG (>10,000 μg/liter) and %TRP (<80%) were observed in all five patients, but both of them recovered to baseline levels immediately after TDF was discontinued in all cases. In three of five patients, GFR levels rapidly declined from a normal level (>90 ml/min/1.73 m^2) to a mildly decreased level (60–89 ml/min/1.73 m^2), and in the other two patients, it declined from normal to a moderately decreased level (30–59 ml/min/1.73 m^2). In three patients (Patient 1, 2, and 4), the GFR level did not fully recover for 6 months after discontinuation of TDF (Table 2).

No association between TDF-associated renal toxicity and low CD4 cell count

Among TDF-receiving patients, urine-β_2-microglobulin, %TRP, ALP, GFR, and serum creatinine were compared between patients with low CD4 cell counts at baseline (<200/μl; $n=11$) and patients with normal CD4 cell counts at baseline (≥200/μl; $n=29$). In the 11 patients with low CD4 cell count <200, U-BMG at baseline and week 96 was 307 (235–455) μg/liter and 411 (262–711) μg/liter; %TRP was 94

(92–95)% and 90 (88–93)%; GFR was 159 (146–174) ml/min/1.73 m^2 and 147 (134–163) ml/min/1.73 m^2; and serum creatinine was 0.62 (0.57–0.66) mg/dl and 0,67 (0.62–0.74) mg/dl. In contrast, in the 29 patients with normal CD4 cell count >200, U-BMG at baseline and week 96 was 154 (113–194) μg/liter and 499 (208–1790) μg/liter; %TRP was 94 (92–96)% and 91 (85–93)%; GFR was 151 (130–163) mlmin/1.73 m^2 and 130 (116–157) ml/min/1.73 m^2; and serum creatinine was 0.64 (0.59–0.75) mg/dl and 0.74 (0.64–0.80) mg/dl.

Discussion

Several large clinical studies revealed that long-term use of TDF caused a gradual reduction of GFR, whereas tubular dysfunction has not been fully evaluated. Although increased urinary loss of BMG has already been observed in the patients treated with TDF both in adults[17] and children,[18,19] it has not been determined whether long-term use of TDF causes tubular dysfunction, or whether TDF-associated tubular dysfunction is persistent/progressive or transient. This study first showed that long-term use of TDF caused progressive tubular dysfunction, whereas a decline in GFR was significant but minimal. Three of five patients with severe proximal tubular dysfunction (U-BMG > 10,000 μg/liter and %TRP < 80%) did not show a marked low level of estimated GFR (<60 ml/min/1.73 m^2), although they presented with a rapid reduction of estimated GFR. Also, in a previously reported case with typical Fanconi's syndrome,[20] maximum serum creatinine was 1.06 mg/dl and minimum calculated GFR was 82 ml/min/1.73 m^2. Measuring tubular function is useful to detect progressive tubular dysfunction, which causes Fanconi's syndrome.

Some analyses suggested a potential association of progressive tubular dysfunction and a gradual decline of GFR in TDF-associated renal impairment. In TDF-receiving patients, GFR levels in patients with high U-BMG (≥1000 μg/liter) on two or more occasion were significantly lower than that in patients with lower U-BMG (<1000 μg/liter). In analyses using MMANOVA for the total sample population, maximum U-BMG levels were significantly associated with the

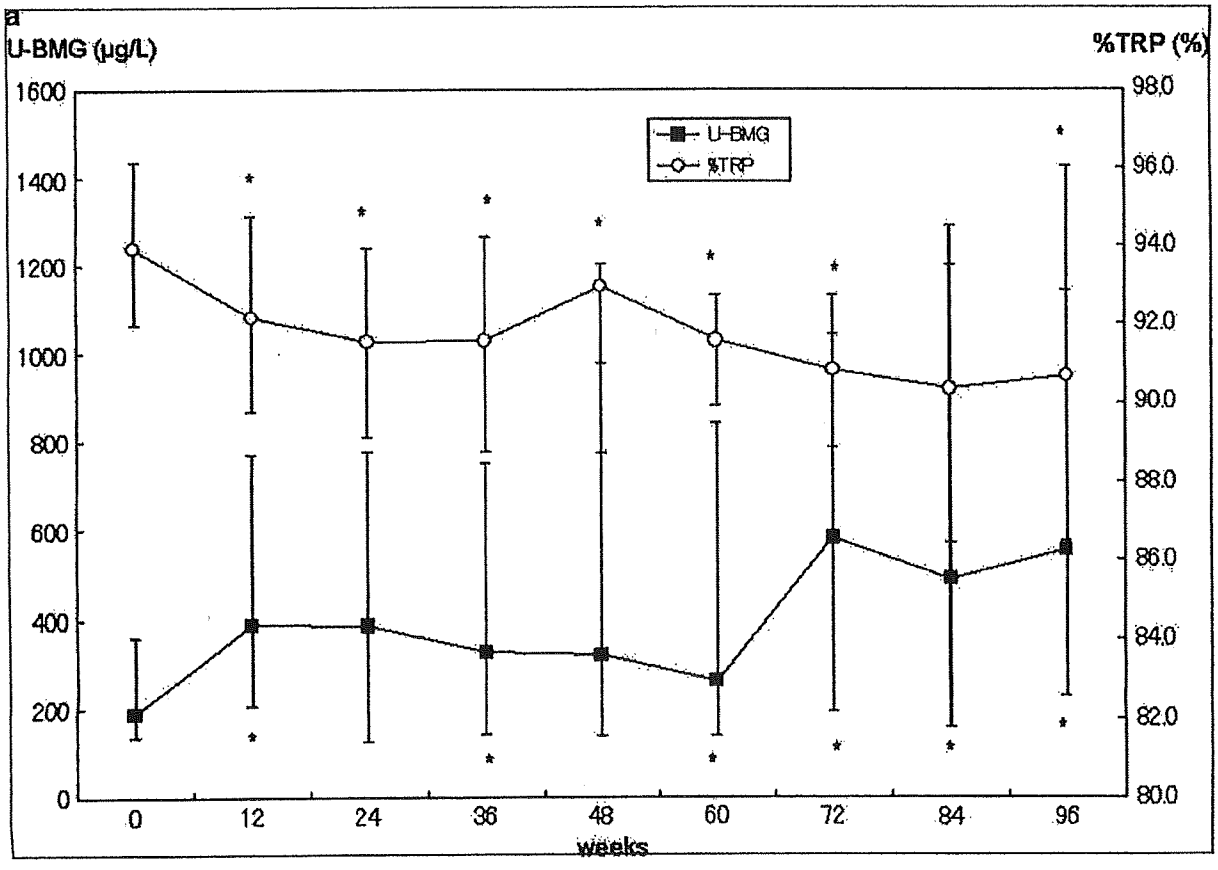
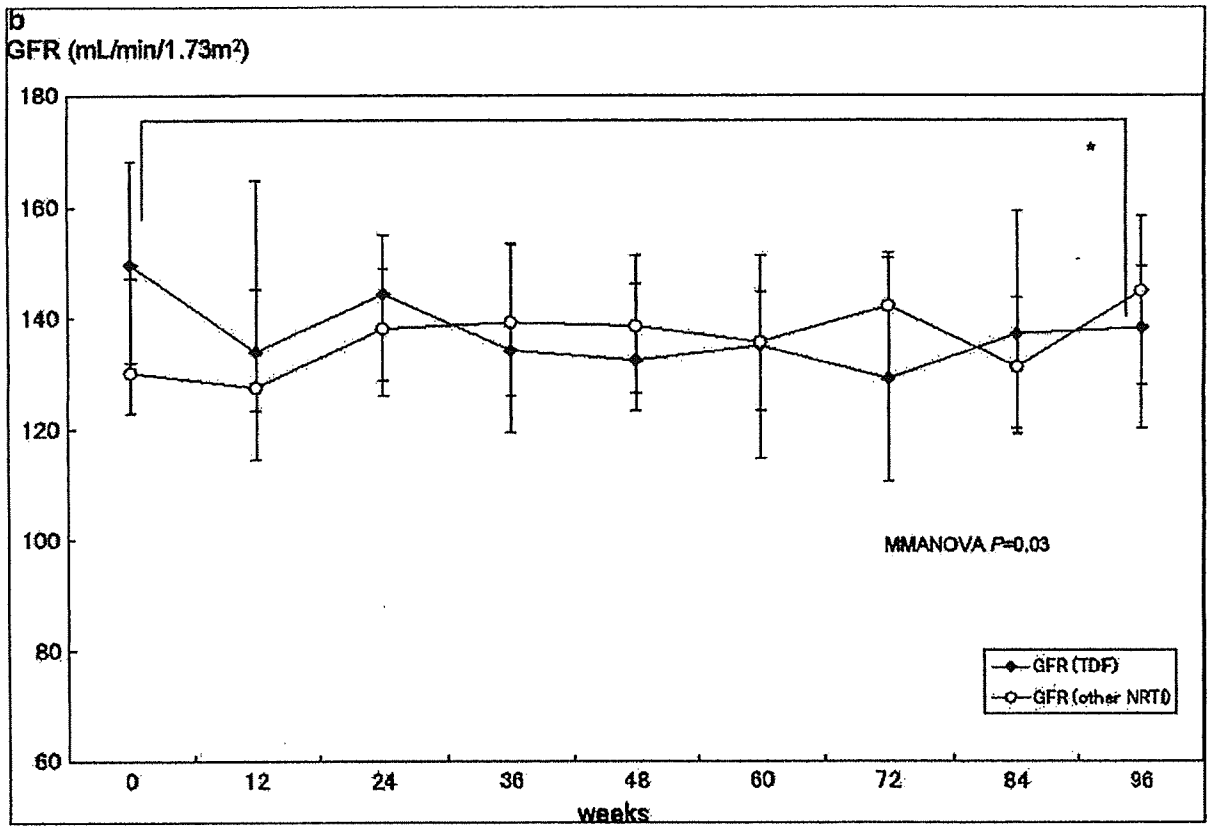


FIG. 1. Urine β_2-microglobulin (U-BMG) and percentage tubular reabsorption of phosphate (%TRP), calculated glomerular filtration rate (GFR), and alkaline phosphatase. (a) The U-BMG level in patients receiving TDF (■) and %TRP in patients receiving TDF (○). (b) The glomerular filtration rate (GFR) in patients receiving TDF (■) and in patients receiving other NRTIs (○). GFR was calculated using the simplified modification of diet in renal disease (MDRD) formula. (c) The ratio of alkaline phosphatase compared with baseline in patients receiving TDF (■) and patients receiving other NRTIs (○). Data are shown as median (IQR). $^*p < 0.05$, $^{**}p < 0.001$ using the Wilcoxon signed rank test.



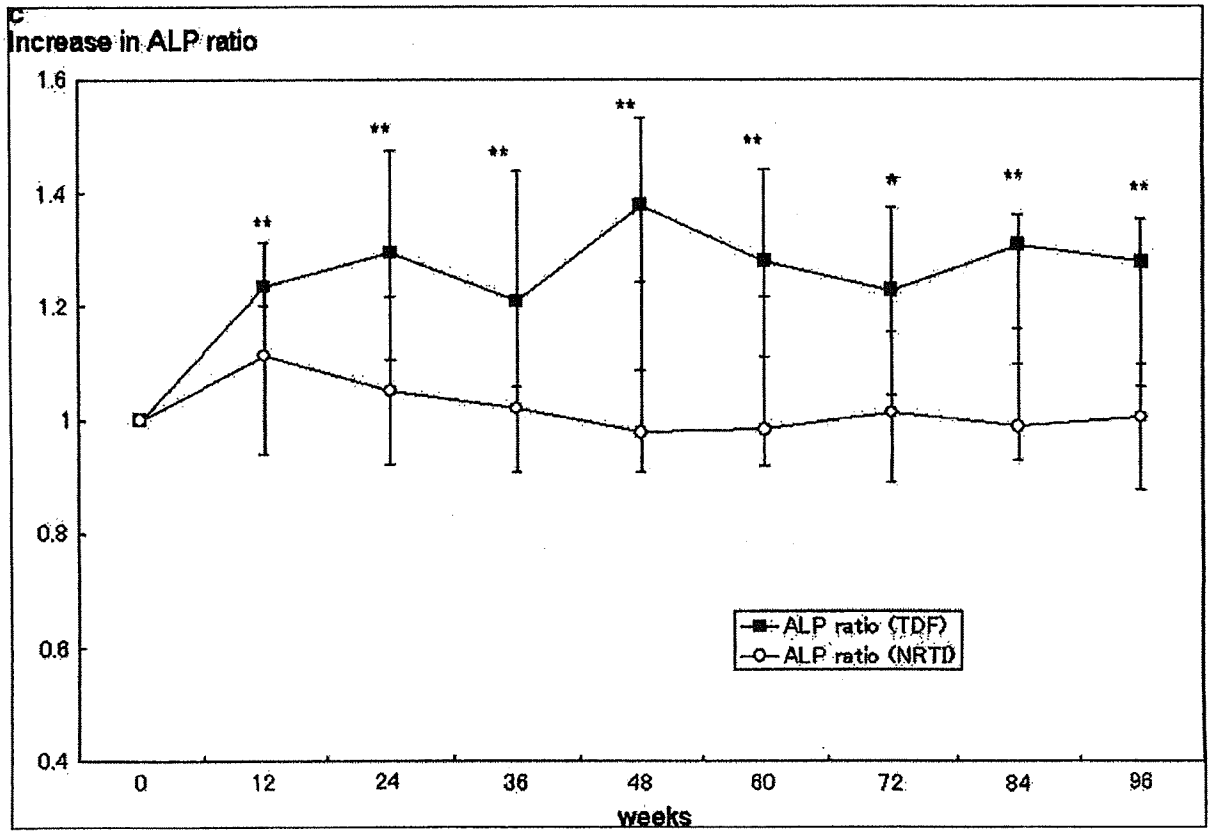


FIG. 1. (*Continued*).

TABLE 2. COMPARISON OF GFR, SERUM CREATININE, URINE-β_2MG, AND TRP(%) AT BASELINE, WORST LEVEL DURING TDF-RECEIVING, AND RECOVERY LEVEL AFTER DISCONTINUATION OF TDF IN FIVE PATIENTS WITH SEVERE RENAL TOXICITY[a]

Patient No.	Key drug	TDF duration (weeks)	Risk factors	GFR (ml/min/1.73 m^2)			Urine-β_2MG (μg/liter)			TRP (%)		
				BL	Minimum	Recovery	BL	Maximum	Recovery	BL	Minimum	Recovery
1	NNRTI	12	DM	101	62	82	406	55,100	329	87	42	91
2	NNRTI	12	DM	92	45	65	354	79,900	1150	78	19	79
3	PI	1	IDV-related renal atrophy	124	108	102	86	49,900	110	99	74	89
4	PI	106	DM, IDV-related renal atrophy, VCM, NSAIDs	126	48	68	260	22,800	537	96	50	85
5	NNRTI	194	TMP-SMX	159	70	109	711	11500	984	93	52	82

[a]BL, baseline; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; DM, diabetes mellitus; IDV, indinavir; TMP-SMX, trimethoprim-sulfamethoxazole; VCM; vancomycin; NSAIDs: nonsteroidal anti-inflammatory drugs.

GFR level at week 96 (MMANOVA, $p = 0.0002$). Additionally, in 12/40 (30%) TDF-receiving patients with a moderate decrease in GFR (<70% of baseline) by week 96, a significantly higher level of U-BMG [2750 (2050–12800) μg/liter vs. 263 (185–578) μg/liter at week 96, $p = 0.008$] and a relatively lower %TRP [88 (84–91)% vs. 91 (86–95)% at week 96, $p = 0.15$] were observed. Of interest, among these 12 patients, elevated U-BMG (maximum U-BMG >1000 μg/liter) was observed in 56% by week 12, in 67% by week 24, and in 100% at week 48, whereas the decrease in GFR (minimum GFR <70% of baseline) was observed in only 18% of patients by week 12, in 36% by week 24, and in 55% by week 48. Early elevation of U-BMG (>1000 μg/liter) seemed to be one of the useful predictors of a modest decline in GFR. However, it could not be statistically determined which level of U-BMG and which week of measurement would be the best predictors of a decline in GFR.

It has not been determined yet whether TDF-associated renal dysfunction is reversible. Some case reports also suggested that rapidly deteriorating renal function can result in irreversible or fatal renal failure.[8–11] In this study, the GFR did not fully recover for 6 months in three of five patients. In a case with severe renal dysfunction, a longer observation period (>6 months) may be required to determine whether the GFR truly recovers. Moreover, all of the above five patients had some risk factors for renal impairment, such as diabetes mellitus or IDV-associated renal atrophy, although their GFR levels at baseline were above 90 ml/min/1.73 m^2. Therefore, it is still uncertain whether the GFR level will recover in the patients without risk factors after discontinuing TDF. In contrast, and importantly, quite severe tubular dysfunction immediately recovered to the baseline level after discontinuing TDF in all of the five cases and in the previously reported one case[20] with severe renal toxicity, regardless of any risk factors. It may be safer to decide to continue or discontinue TDF in accordance with the U-BMG level or %TRP level, if possible.

This study was conducted prospectively and available patients at Ogikubo Hospital were enrolled without any selection. Although the number of patients in each sample group was small and different ($n = 40$ in TDF-receiving patients vs. $n = 23$ in other NRTI-receiving patients) due to a small available sample population, there was no significant difference in patient characteristics between TDF-receiving patients and NRTI-receiving patients. Moreover, there were more risk factors for renal impairment in those receiving other NRTIs—the median CD4 cell count was lower, the median HIV RNA was higher, and the percentage of the patients pretreated with IDV was higher.

Despite these findings, none of the other NRTI-receiving patients showed deterioration of any renal parameters. Additionally, characteristics of the sample population did not greatly deviate from those of the general population, since the change of renal function of TDF-receiving patients in this study was consistent with other large observational studies. The ratio of the elevation in the serum creatinine level is comparable to other observational studies.[21,22]

On the other hand, it is uncertain whether the observed overall change in GFR in this study was applicable to general populations. The absolute decline in GFR by –17 ml/min/1.73 m^2 in and the proportion of 12/40 (30%) patients with a modest decrease in GFR (<70% of baseline) in TDF-receiving patients after 2 years was comparable to large observational studies.[4,23] However, another observational study showed a lower ratio of the decline in GFR.[5] This may provide a limitation to the generalization of this conclusion.

There are some limitations to the use of U-BMG testing in the routine monitoring of TDF-treated patients. Although the U-BMG level is a specific marker of proximal tubular dysfunction, it sometimes becomes elevated in progressive HIV infection and often varies according to serum β_2-microglobulin level.[24] However, because markedly elevated U-BMG (>10,000 μg/liter) is quite rare even in progressive HIV infection,[24] the criterion of discontinuing TDF (10,000 μg/liter) may be reasonable.

The percentage TRP, which directly reflects the urinary loss of phosphorus, has been shown to be quite sensitive in detecting tubular dysfunction. ALP, a marker of osteoblastic activity, showed persistent increases in patients receiving TDF, whereas the serum phosphorus level did not decrease. Tubular dysfunction does not cause an immediate decrease in serum phosphorus levels because the level is maintained by bone mineralization.[24] An indicator of urinary loss of phosphorus is not the serum phosphorus level but %TRP and ALP level. However, the ALP level can be affected by other factors including liver disease, therefore, an increased ALP ratio compared with baseline is a better measure of bone mineralization.